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RESEARCH ARTICLE

Dietary arsenic consumption and urine arsenic in an endemic population: response to improvement of drinking water quality in a 2-year consecutive study

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Abstract We assessed the association between arsenic intake through water and diet, and arsenic levels in first morning-void urine under variable conditions of water contamination. This was done in a 2-year consecutive study in an endemic population. Exposure of arsenic through water and diet was assessed for participants using arsenic-contaminated water (≥50 μg L^{-1}) in a first year (group I) and for participants using water lower in arsenic (<50 μg L^{-1}) in the next year (group II). Participants with and without arsenical skin lesions were considered in the statistical analysis. Median dose of arsenic intake through drinking water in groups I and II males was 7.44 and 0.85 μg kg body $wt.^{-1}$ day^{-1} (p <0.0001). In females, it was 5.3 and 0.63 μg kg body $wt.^{-1}$ day^{-1} (p <0.0001) for groups I and II, respectively. Arsenic dose through diet was 3.3 and 2.6 μg kg body $wt.^{-1}$ day^{-1} (p=0.088) in males and 2.6 and 1.9 μg kg body $wt.^{-1}$ day^{-1} (p=0.0081) in females. Median arsenic levels in urine of groups I and II males were 124 and 61 μg L^{-1} (p=0.052) and in females 130 and 52 μg L^{-1} (p=0.0001), respectively. When arsenic levels in the water were reduced to below 50 μg L^{-1} (Indian permissible limit), total arsenic intake and arsenic intake through the water significantly decreased, but arsenic uptake through the diet was found to be not significantly affected. Moreover, it was found that drinking water mainly contributed to variations in urine arsenic concentrations. However, differences between male and female participants also indicate that not only arsenic uptake, but also many physiological factors affect arsenic behavior in the body and its excretion. As total median arsenic exposure still often exceeded 3.0 μg kg body $wt.^{-1}$ day^{-1} (the permissible lower limit established by the Joint Expert Committee on Food Additives) after installation of the drinking water filters, it can be concluded that supplying the filtered water only may not be sufficient to minimize arsenic availability for an already endemic population.

Keywords Arsenic · Drinking water · Cooking water · Diet · Dose · Urine

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Introduction

Arsenic pollution in groundwater, used for drinking purposes, is considered as a problem of global concern. Among many possible pathways of arsenic exposure, drinking water is considered as the most significant. Epidemiological data collected during many years relied mainly on the concentration of arsenic in the drinking water as proxy for human exposure (Guha Mazumder et al. 1988; Smith et al. 2000, 2006). However, chronic arsenic-toxicity symptoms recorded in Bangladesh and West Bengal (India) may reflect also pathways other than the

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consumption of water (Huq and Naidu 2003). Soil–crop–food transfer as well as cooking with arsenic-enriched water has been suggested as additional major exposure pathways (Alam, et al. 2003). There is a lot of evidence on the presence of elevated arsenic levels in rice grains in regions of West Bengal and Bangladesh where paddy fields are irrigated with arsenic-rich water (Duxbury et al. 2003; Meharg and Rahman 2003; Williams et al. 2005; Rahman et al. 2006). Arsenic contamination of vegetables grown in soils irrigated with arsenic-contaminated water has also been reported earlier (Roychowdhury et al. 2002; Alam et al. 2003). 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 (Roychowdhury et al. 2002; Kile et al. 2007b; Ohno et al. 2007). Variations in arsenic content of rice depends on the rice variety (different genotypes) (Meharg and Rahman 2003) and cooking methods (Bae et al. 2002; Sengupta et al. 2006). Cooking rice with arsenic-enriched water leads to an increase in arsenic content of the rice (Bae et al. 2002; Rahman et al. 2006; Ohno et al. 2007; Signes et al. 2008). On the other hand, discarding of gruel may also reduce arsenic load from cooked rice (Sengupta et al. 2006; Ohno et al. 2007; Pal et al. 2009). Thus, risk assessments should ideally consider exposure from cooked rice rather than from raw rice (Bae et al. 2002). An early report from West Bengal demonstrated that despite having safe water for drinking and cooking, arsenic levels in urine may be high, which could be due to unavoidable intake of arsenic through edible crops grown in contaminated water, food materials contaminated through washing, and occasional drinking of contaminated water (Mandal et al. 1998).

Arsenic levels in urine, hair, and nails are important biomarkers of arsenic intake. Arsenic concentrations in urine are used as biomarkers of recent arsenic exposure, while levels in hair and nails are biomarkers of chronic exposure (NRC National Research Council 1999; WHO 2005). A study in Bangladesh showed that arsenic in toenails can be attributed for 69, 14, and 17 % to drinking water exposure that occurred 3, 6, and 9 months before toenail collection (Kile et al. 2005). Urinary excretion is the major pathway for the elimination of arsenic from the human body (Crecelius 1977). Following ingestion, inorganic arsenic is primarily excreted in the urine as dimethylarsinic acid and to a lesser extent, monomethylarsonic acid (Styblo et al. 2000). However, less toxic organic species, such as arsenobetaine occurring primarily in fish and seafood, may also be excreted without prior transformations (Molin et al. 2012). Thus, variations in arsenic species occurrence in dietary components may also alter the presence of different species in the urine (Cascio et al. 2011). Significant relationships have previously been noted between arsenic exposure in drinking water and arsenic excretion in urine (Marchiset-Ferlay et al. 2012 and references cited herein). Nowadays, inorganic arsenic species, methylarsonic acid, and dimethylarsinic acid are often measured individually as these urinary species are better indicators of toxicological effects and exposure through drinking water without interference to dietary intakes (Marchiset-Ferlay et al. 2012; Spayd et al. 2012). However, total arsenic concentrations in urine are still measured, especially when relations between arsenic in urine and arsenic intake through both drinking water and different dietary compounds are studied.

Limited information is available on the correlation between total arsenic intake through diet and water, and arsenic levels in urine under variable conditions of water contamination (before and after installation of filters removing arsenic from the water). Therefore, we assessed the relative contribution of arsenic intake through water and diet to total arsenic concentrations in urine in people from an endemic population being exposed to different levels of water contamination.

Experimental

Study design

For this study, 208 participants were recruited from six villages of the Chakdaha Block in the Nadia District, West Bengal, India. These participants belonged to 212 households identified previously in another cross-sectional study conducted by Guha Mazumder et al. (2010, 2013). To assess total individual arsenic exposure, total arsenic level in drinking water and diet samples collected during 24 h was determined for each participant in two consecutive years (2008–2009: prior to installation of filter for arsenic removal and 2009–2010: after installation of filter for arsenic removal). Water, diet, and urine samples could be collected from 161 participants (out of the initially recruited 208 participants) using arsenic-contaminated (≥50 μg L^{-1}) and uncontaminated (<50 μg L^{-1}) water in the first year and from 117 participants using arsenic-poor water (<50 μg L^{-1}) in the second year. In the first year, 69 out of the 161 participants were using arsenic-contaminated (≥50 μg L^{-1}) water. The same water was always used for drinking as well as cooking purposes. Further analysis was concentrated on the 69 participants using contaminated water in the first year (group I) and the 117 participants using arsenic-poor water (<50 μg L^{-1}) in the second year (group II) (Table 1). In the present study, the limit between arsenic-poor water and arsenic-contaminated water was set at 50 μg L^{-1}, as this is the maximum allowable limit in India. Twenty-seven participants of group I also occurred in group II (after installation of a filter to remove arsenic from their water). They were marked as group IA and group IIA. In groups I and II, some individuals had skin lesions which are typically attributed to arsenic exposure. Skin lesions were identified by two physicians (D. N. Guha Mazumder and Aloke Ghose; co-authors of the manuscript) following proper methodology (Guha Mazumder et al. 1998; WHO 2005). The

Table 1 Participants of groups I and II

	Group I (n=69)		Group II (n=117)		p Value
	Median	Range	Median	Range	
Male	n=45 n=10 (+) Skin lesion n=35 (−) Skin lesion		n=66 n=35 (+) Skin lesion n=31 (−) Skin lesion		
Age (years)	45	17–68	42	18–61	0.81
Height (cm)	160	147–170	161	145-175	0.91
Weight (kg)	54	40–85	51	37–79	0.19
BMI (kg m^{-2})	20.4	15.6–30.5	19.0	14.2–36.7	0.17
Female	n=24 n=7 (+) Skin lesion n=17 (−) Skin lesion		n=51 n=29 (+) Skin lesion n=22 (−) Skin lesion		
Age (Years)	42	28–55	41	18–61	0.93
Height (cm)	152	140-204	151	135–172	0.23
Weight (kg)	49	39–76	48	23–74	0.17
BMI (kg m^{-2})	20.9	16.9–29.2	20.8	12.6–30.0	0.61

Values are rounded up to avoid integers as per applicable

(+) participants with arsenical skin lesion, (−) participants without arsenical skin lesion

physicians had several years of experience in diagnosing arsenic-caused skin lesions in West Bengal. Drinking water and diet were considered as intake routes and urine (biomarker of current arsenic exposure) as main excretion path for each participant. Individual participants gave written consent for their participation. The study protocol was approved by the Ethical Committee of the DNGM Research Foundation, upon fulfilling Helsinki criteria and recommendation of the Indian Council of Medical Research, Government of India.

Field study

From each recruit, information on demographic and social characteristics and occupation (broadly grouped into sedentary and moderate workers) was collected (Gopalan et al. 2010). Weight and height were measured, and body mass index (BMI) was calculated (weight in kg/height in meter square).

Water and urine samples

Water samples were collected from present drinking and cooking water sources of each family and also from previous water sources when they were still available. These samples were collected in certified arsenic-free polyethylene containers. One drop of concentrated nitric acid/water (1:1) per 10 ml of water sample was added as preservative. For group II, total daily water consumption by each participant was calculated from the number of glasses (250 ml capacity) of water the participant consumed in a 24-h period, written down in self-reports. For group I, the average water intake amounts reported by Guha Mazumder et al. (2013) for each gender of this group, i.e., 2.62 L day^{-1} for females and 3.86 L day^{-1} for males, were used when processing the data.

Timed urine sampling (i.e., 24-h urine collection) is the gold standard for measuring biomarkers in urine, but this was not feasible in the current study due to the large number of participants. Therefore, first morning-void urine samples were collected always. The samples were collected in certified arsenic-free polyethylene containers. Both the water and urine samples along with field-blank samples were kept in ice boxes during transport from the field to the laboratory, and properly stored at −20 °C till further analysis in laboratory.

Diet samples

Food samples were collected by duplicate portion sampling method (Ohno et al. 2007; Deb et al. 2012). Food (raw and cooked rice, cooked and dry cereals, cooked pulses, cooked vegetables, chapatti, and cooked animal protein) intake was ascertained by a detailed questionnaire based primarily on 24-h recall. The "senior" woman (mother or eldest daughter-in-law of the family) involved in preparation of food for the family was interviewed. The participating woman was questioned about each meal, from the previous day's afternoon meal to the lunch on the following sampling day. The quantity of each food item administered in each meal to each participant by the serving woman was recorded. To estimate the amount of cooked food consumed, a portable weighing machine (SIKA, Mettler Toledo) and bowls of different volumes (standard amounts listed by the National Institute of Nutrition, Hyderabad) were used. All

wet cooked food items of the meals were categorized and collected in certified arsenic-free polyethylene containers, stored in ice buckets in the field, and further stored at −20 °C until analysis in the laboratory. Participants drinking water from multiple sources were excluded.

Arsenic analysis

Total arsenic concentrations in water samples were measured using an Atomic Absorption Spectrophotometer attached to a Flow Injection Hydride Generation System (Das et al. 1995) (FI-HG-AAS, Perkin Elmer A Analyst 200, FIAS 100). Lower limit of detection was 0.03 μg L^{-1}.

In case of mixed cooked food categories, the samples were wet-weighted (dry weight in case of raw food) and oven-dried until constant weight at 60 °C and the moisture percentage was calculated. For total arsenic analysis, the dry samples were crushed. Part of each homogenized powdered sample (1.00 g) was digested in a 100-ml digestion flask following the procedure of Datta et al. (2010). Quality assurance included analysis of National Institute of Standards and Technology (NIST) rice flour SRM 1568a with each sample batch. Recovery percentages varied from 96 to 98 %.

For analysis of total arsenic in urine samples, the samples were acid-digested (Das et al. 1995) and arsenic analysis was performed using FI-HG-AAS (PerkinElmer A Analyst 200). It was decided not to adjust measured arsenic concentrations for hydration status of the participant using creatinine as this approach has certain limitations, which were reported by Marchiset-Ferlay et al. (2012). Quality assurance included analysis of NIST urine sample SRM-2670 with each sample batch. Recovery percentages varied from 95 to 97 %.

Statistical methods

The following formulas were used to calculate total, dose, and rate values of arsenic intake through different sources:

Daily arsenic intake from drinking water (in microgram per day) = arsenic concentration (in microgram per liter) of current drinking source × water consumption rate (liter per day).

Daily arsenic intake from each cooked food category (in microgram per day) = arsenic concentration in each cooked food category (in microgram per kilogram wet weight) × consumption rate (in kilogram wet weight per day) of that food category.

Daily total arsenic intake = daily arsenic intake from drinking water + total daily arsenic intake from diet (= sum of the different food categories).

Daily arsenic dose (in microgram per kilogram body weight per day) = participant's daily arsenic intake/body weight determined.

Since responses of participants belonging to the same family can be correlated, generalized estimating equations (GEE) based regression model with the independent working correlation matrix was used to analyze the data (Hardin and Hilbe 2002). This approach is similar to the conventional regression approach, but differs in the way the standard errors are estimated and hence how the p values are calculated. In contrast to conventional regression, the GEE approach remains valid if responses are not independent. Note that by accounting for the family dependency, we automatically account for the fact that some participants occurred both in groups I and II. To model the arsenic level in urine as a function of the arsenic intake through diet and water, we start with the model which includes the arsenic intake through diet and the arsenic intake through water as main effects as well as their interaction, and we also include the age, sex, and BMI of the participant since they can be potential confounders. Since GEE regression is only valid if the sample size is large enough, for sample sizes smaller than 15, we use the conventional regression technique (assuming normality and constant variance of the error). Residual plots were considered to assess the goodness-of-fit of the model. A backwards stepwise procedure was carried out which removed the non-significant effects at the 5 % level of significance. All hypothesis tests are performed at the 5 % level of significance and effects associated with a p value less than 0.01 are referred to as strongly significant.

Since 27 participants occurred both in groups I and II, the effect of arsenic-poor water can be examined more accurately by comparing the individuals over time. The Wilcoxon-signed-rank test was used to analyze these paired observations.

Results

Age, height, body weight, body mass index, and basic characters of selected participants are described in Table 1. No significant differences were observed between groups I and II.

No significant differences were found in intake of the major cooked dietary items between male participants of both groups, e.g., raw rice (p=0.77), cooked rice (p=0.96), and cooked vegetables (p=0.33) (Table 2). Female participants of group II consumed a slightly smaller amount of raw rice (p=0.044) and cooked rice (p=0.056), but almost the same amount of cooked vegetables (p=0.20) compared to group I (Table 2). Arsenic concentration of cooked dietary items were found to be slightly lower after use of arsenic-poor water in case of cooked rice (p=0.012), and chapati (p=0.018). Arsenic contents were found to significantly increase for cooked chicken/meat samples (p <0.0001), but this could perhaps be attributed to the fact that only five samples could be included (Table 3). Surprisingly, we also observed an increase of arsenic content in cooked fish (p=0.012) and dry cereals (p=0.0052) from groups I to II (Table 3). For cooked fish, this can perhaps be explained by the relatively low number of samples and variation in arsenic

Table 2 Daily consumption rate of various food categories and water by participants of groups I and II (kg day^{-1})

	Group I (n=45)			Group II (n=66)			p Value
Male	n	Median	Range	n	Median	Range	
Raw rice	45	0.5	0.125–0.806	66	0.441	0.13–0.965	0.77
Cooked rice	45	1.4	0.35–2.41	66	1.345	0.4–2.9	0.96
Cooked vegetables[1]	45	0.265	0.03–0.602	66	0.273	0.05–0.73	0.33
Cooked pulses[2]	14	0.06	0.005–0.25	15	0.1	0.02–0.25	0.52
Chapati[3]	11	0.2	0.04–0.36	12	0.15	0.08–0.62	0.90
Dry cereals[4]	17	0.015	0.005–0.055	17	0.03	0.005–0.15	0.0085
Cooked fish[5]	25	0.05	0.014–0.12	25	0.05	0.005–0.10	0.21
Cooked egg[6]	9	0.04	0.014–0.067	12	0.022	0.01–0.05	0.0099
Cooked chicken/meat	8	0.091	0.04–0.15	9	0.15	0.07–0.3	0.017
Female	Group I (n=24)			Group II (n=51)			
Raw rice	24	0.407	0.126–0.75	51	0.362	0.05–0.73	0.044
Cooked rice	24	1.2	0.45–2	51	1.06	0.15–2.11	0.056
Cooked vegetables[1]	24	0.29	0.14–0.712	51	0.26	0.04–0.6	0.20
Cooked pulses[2]	5	0.1	0.016–0.24	8	0.1	0.05–0.3	0.85
Chapati[3]	8	0.21	0.08–0.4	10	0.11	0.03–0.35	0.053
Dry cereals[4]	9	0.02	0.015–0.04	13	0.03	0.01–0.06	0.14
Cooked fish[5]	14	0.026	0.014–0.12	20	0.035	0.008–0.09	0.37
Cooked egg[6]	2	0.042	0.04–0.045	8	0.022	0.01–0.05	0.011
Cooked chicken/meat	3	0.082	0.062–0.15	4	0.11	0.1–0.25	0.30

[1] Vegetables: potato, carrot, radish, sweet potato, colocasia, oal, thor, onion, cabbage, cauliflower, spinach, sajna sag, note sag, pumpkin sag, lau sag, kochu sag, tomato, onion stalk, brinjal, papaya, sajne data, parwar, cluster beans, beans, jhinga, pumpkin, bitter gourd, bottle gourd, ladies finger, plantain green, kakrol, chal kumra, kochu lati, mocha, pumpkin flower, chichinga, green jack fruit

[2] Cooked pulses: lentil, mug, matar, Bengal gram, kalai, green peas, soya bean nugget, bari (made from pulses) etc.

[3] Chapati: made from wheat flour, bread etc.

[4] Dry cereals: puffed rice, flaked rice, biscuits etc.

[5] Fish: rohu, mrigel, hilsa, puti, pona, bata

[6] Egg: hen, duck, and poultry

contents in different types of fish. For dry cereals, no relation between arsenic in water and arsenic in the food is expected as no water is used to prepare the cereals prior to consumption. Arsenic content (median value) of the drinking and cooking water was 97 μg L^{-1} (range: 63 to 150 μg L^{-1}) and 103 μg L^{-1} (range: 63 to 150 μg L^{-1}) for male and female participants in group I, and 16 μg L^{-1} (range: <0.03 to 50 μg L^{-1}) for both the male and female participants in group II (Table 4).

Daily arsenic intake through water by male participants of group II (41 μg day^{-1}) is significantly lower (p <0.0001) than the intake by male participants of group I (374 μg day^{-1}) (Table 4). Diet of the group I male participants is a source of 174 μg arsenic day^{-1}, whereas group II male participants intake 132 μg arsenic day^{-1} through their diet, a difference which is not significant (p=0.058) (Table 4). Median dose of arsenic intake through drinking water was 7.44 and 0.85 μg kg body wt.$^{-1}$ day^{-1} for groups I and II males and 5.30 and 0.63 μg kg body wt.$^{-1}$ day^{-1} for females, respectively (Table 4, Fig. 1), while for the diet, it was 3.3 and 2.6 μg kg body wt.$^{-1}$ day^{-1} for males, and 2.6 and 1.9 μg kg body wt.$^{-1}$ day^{-1} for females, respectively (Table 4, Fig. 2). Differences in intake through drinking water were highly significant for both males and females. They were not significant for intake through diet by males. Median total dose of arsenic intake through water and diet together was 11.39 and 3.75 μg kg body wt.$^{-1}$ day^{-1} (p <0.0001) for groups I and II male participants, respectively (Fig. 3), while it was 8.80 and 2.84 μg kg body wt.$^{-1}$ day^{-1} (p <0.0001) for females of groups I and II participants, respectively (Table 4, Fig. 3). Thus, if the population is supplied with water containing less arsenic, there is a highly significant decrease of arsenic intake through the water, but the decrease of arsenic intake through the diet is less pronounced. However, the median total dose of arsenic exposure was still found to be higher than 3 μg kg body wt.$^{-1}$ day^{-1}, which is the permissible lower limit, established by the Joint Expert Committee on Food Additives (FAO and WHO 2011), for 100 and 62 % of the male participants and 100 and 48 % of the female participants belonging to groups I and II, respectively. As total median arsenic exposure still often exceeded the standard of 3.0 μg kg body wt.$^{-1}$ day^{-1} after installation of the drinking water filters,

Table 3 Arsenic content (μg kg^{-1}) of various cooked dietary items in groups I and II

Dietary item	Group I			Group II			*p* Value
	n	Median	Range	*n*	Median	Range	
Raw rice	37	311	46–874	73	369	102–871	0.31
Cooked rice	37	108	43–211	73	88	20–173	0.012
Cooked vegetables	37	79	26–199	72	68	12–506	0.29
Cooked pulses	10	34	24–70	9	52	5–68	0.67
Chapati	11	171	131–279	13	120	35–338	0.018
Dry cereals	14	185	89–442	18	304	53–494	0.0052
Cooked fish	21	96	72–154	18	124	48–210	0.012
Cooked egg	5	7	7–150	9	89	27–132	0.056
Cooked chicken/meat	5	47	29–122	5	227	177–252	<0.0001

it can be concluded that only supplying the filtered water may not be sufficient to minimize arsenic availability for an already endemic population.

Median arsenic concentration in urine of groups I and II male participants was 124 and 61 μg L^{-1} (p=0.052) and for female participants this was 130 and 52 μg L^{-1} (p=0.0001), respectively, for groups I and II (Fig. 4). So, it seems that male participants experience a lower decrease of urinary arsenic release compared to female participants upon using arsenic-poor water.

When looking at total arsenic intake dose (irrespective of gender) for participants having arsenical skin lesions, a strongly significant (p <0.0001) lower dose is observed in group II (median: 3.06 μg kg body $wt.^{-1}$ day^{-1}, n=64) compared to group I (median: 8.63 μg kg body $wt.^{-1}$ day^{-1}, n=17). Similarly, for participants having no skin lesions, total arsenic intake dose is significantly lower (p <0.001) in group II (median: 3.73 μg kg body $wt.^{-1}$ day^{-1}, n=53) compared to group I (median: 10.10 μg kg body $wt.^{-1}$ day^{-1}, n=52), irrespective of gender. Also, the level of urinary arsenic release is significantly lower (p=0.0021) in group II participants having arsenical skin lesions (median: 48 μg L^{-1}) compared to group I participants having skin lesions (median: 124 μg L^{-1}). Similarly, urinary arsenic release is significantly lower (p=0.018) in group II (median: 67 μg L^{-1}) compared to group I (133 μg L^{-1}) for participants having no skin lesions. Differences in urinary arsenic concentrations between participants having skin lesions and participants having no skin lesions were not observed within group I (median

Table 4 Comparative evaluation of principal arsenic intake by the participants

	Group I		Group II		*p* Value
	Median	Range	Median	Range	
Male	*n*=45		*n*=66		
Arsenic in drinking and cooking water (μg L^{-1})	97	63–150	16	<0.03–50	<0.0001
Daily arsenic intake through water (μg day^{-1})	374	243–579	41	<0.03–210	<0.0001
Arsenic dose through water (μg kg body wt^{-1} day^{-1})	7.44	3.80–14.48	0.85	0–3.82	<0.0001
Daily arsenic intake through diet (μg day^{-1})	174	65–339	132	36–388	0.058
Arsenic dose through diet (μg kg body $wt.^{-1}$ day^{-1})	3.3	1.0–8.3	2.6	0.5–7.4	0.088
Daily arsenic intake through water and diet (μg day^{-1})	562	328–891	188	55–507	<0.0001
Arsenic dose through water and diet (μg kg body $wt.^{-1}$ day^{-})	11.39	5.86–21.46	3.75	1.05–9.05	<0.0001
Female	*n*=24		*n*=51		
Arsenic in drinking and cooking water (μg L^{-1})	103	63–150	16	<0.03–50	<0.0001
Daily arsenic intake through water (μg day^{-1})	270	165–393	30	<0.03–156	<0.0001
Arsenic dose through water (μg kg body $wt.^{-1}$ day^{-1})	5.30	2.23–9.83	0.63	0–2.77	<0.0001
Daily arsenic intake through diet only (μg day^{-1})	148	51–255	95	11–209	0.0012
Arsenic dose through diet (μg kg body $wt.^{-1}$ day^{-1})	2.6	0.8–6.5	1.9	0.5–4.8	0.0081
Daily arsenic intake through water and diet (μg day^{-1})	456	234–648	139	36–256	<0.0001
Arsenic dose through water and diet (μg kg body $wt.^{-1}$ day^{-1})	8.80	3.54–15.32	2.84	0.64–5.27	<0.0001

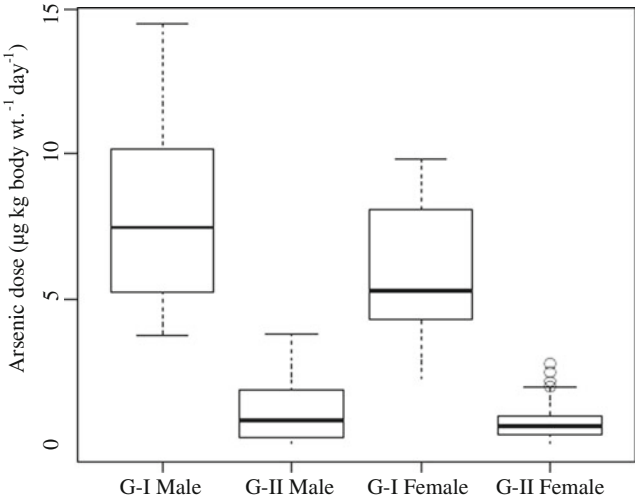

Fig. 1 Arsenic dose taken in through drinking water

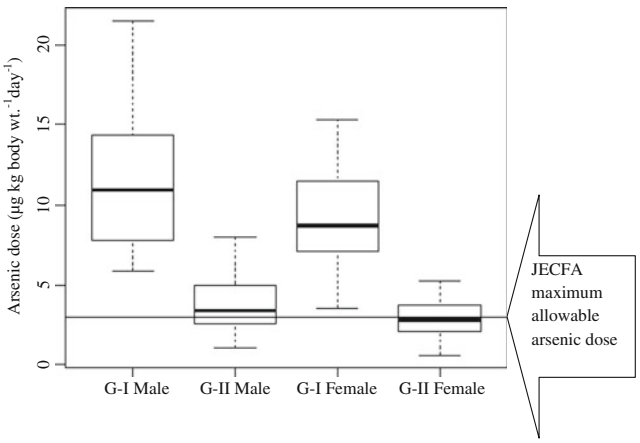

Fig. 3 Arsenic dose taken in through drinking water and diet

124 and 133 μg L^{-1}, respectively; p=0.41) and within group II (median 48 and 67 μg L^{-1}, respectively; p=0.19).

Table 5 shows the estimates and p values of the regression model with urine arsenic concentration as response and arsenic dose through diet as well as arsenic dose through water as predictors. As mentioned in the Statistical Methods section, we corrected for possible confounding factors such as sex, age, and BMI. It can be concluded that there is a significant effect of arsenic intake through water on the mean arsenic concentration of urine in group II (p=0.0099). However, for group I, this effect was not significant (p=0.11). For both groups, the arsenic intake from the diet did not significantly affect the concentration of arsenic in the urine on average. The estimated effect of arsenic intake through water is 2.7 times larger in group II compared to group I (Table 5). Table 6, showing the results when the data were split according to gender and skin lesion, only reports the results related to main effects of arsenic dose from diet and water, and, if significant, the associated interaction effect. We conclude that, in group I, there is a significant interaction effect of arsenic dose from diet and water for men without skin lesion: the significant interaction indicates that the effect of arsenic dose from water on the mean arsenic concentration in urine depends significantly on the arsenic dose from the diet. For the women in group I, there is only a significant effect of the arsenic intake from water in the absence of skin lesion. In the case of group II, there is a significant interaction between the arsenic intake from diet and water for men with skin lesion. For women, there is a significant effect of the arsenic intake from water, both in the presence and absence of skin lesion.

As discussed earlier, 27 participants are included in both groups I and II, i.e., they are included in the study before and after installation of filters to remove arsenic from their water. They were marked as groups IA and IIA, respectively. For all 27 participants occurring in both groups, arsenic concentration in drinking and cooking water was lower in 2009–2010 (median 26 μg L^{-1}) compared to 2008–2009 (median 96 μg L^{-1}). Accordingly, arsenic intake through water (Fig. 5-middle) showed a strong significant decrease for all 27 participants

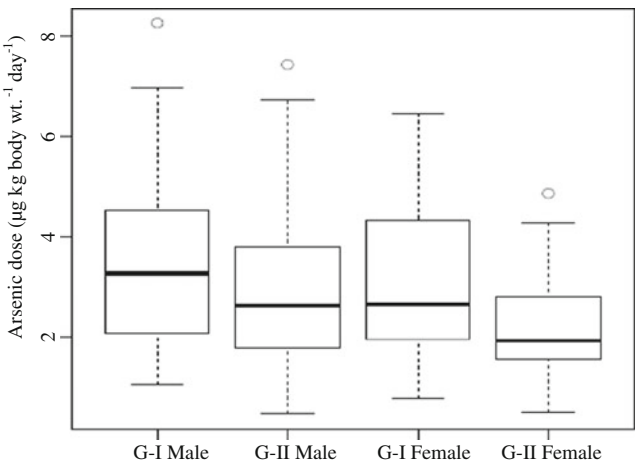

Fig. 2 Arsenic dose taken in through diet

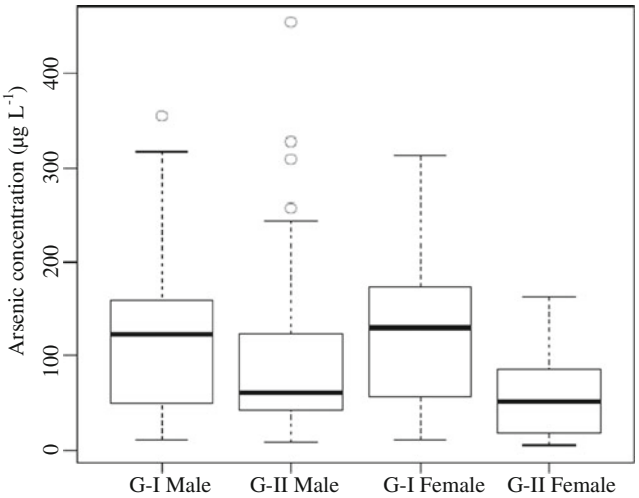

Fig. 4 Arsenic release in urine

Table 5 Linear regression analysis of urine arsenic concentration with arsenic exposure (dose) through diet and water as exposure routes. Estimate refers to estimated effect of the corresponding predictor

Parameters	Group I		Group II	
	Estimate	*p* Value	Estimate	*p* Value
Arsenic through water only (μg day^{-1})	0.097	0.11	0.26	0.0099
Arsenic through diet only (μg day^{-1})	−0.075	0.64	0.028	0.73

after installation of the filters (p <0.0001), which suggests a positive effect of producing and using decontaminated water. However, no significant difference in the urine arsenic concentration could be observed between groups IA and IIA (p=0.25, Fig. 5-left). This may be attributed to the fact that there was no significant change over time (p=0.23) for intake through diet (Fig. 5-right). This suggests that even with arsenic-poor drinking and cooking water, people still take up significant arsenic amounts through their diet, which is reflected in urinary arsenic release.

Discussion

To our knowledge, this is the first study in which daily doses of arsenic intake through drinking and cooking water as well as diet, and their possible correlation with urinary arsenic release was reported in an endemic population for two consecutive years. The main difference between the two study years was the use of water containing arsenic concentrations above the Indian permissible limit of 50 μg L^{-1} for drinking and cooking in the first year (group I) and use of arsenic-poor water in the second year after installation of a filter removing arsenic (group II). Gender of the participants was taken into account in the data analysis. There were no variations in intake of major dietary items between the male participants of groups I and II, except for dry cereals (p=0.0085), cooked egg (p=0.0099), and in case of cooked chicken/meat (p=0.017). Intake of raw rice for the females of group II was slightly lower (p=0.044) compared to group I. Intake of chapatti and cooked egg are bit higher for the females of group I compared to group II, but this could perhaps be attributed to the fact that only very few samples could be collected for these items. For group I, the average water intake amounts reported by Guha Mazumder et al. (2013) were 2.62 L day^{-1} for females and 3.86 L day^{-1} for males. In group II, the average water intake amount was 3.5 L day^{-1} (range 1–6.5 L day^{-1}) for males and 2 L day^{-1} (range 1–4 L day^{-1}) for females. This water-intake level matched values reported previously by others, e.g., 3.1 and 2.9 L day^{-1} for men and women, respectively, reported by Kile et al. (2007a) and 3 L day^{-1} for men and women reported by Watanabe et al. (2004). Cooked rice and vegetables were taken up by all participants of both groups, and cooked rice constituted the major bulk of the diet. This was previously also observed in studies on dietary arsenic

Table 6 Linear regression analysis of urine arsenic concentration with arsenic exposure (dose) through diet and water as exposure routes. Estimate refers to estimated effect of the corresponding predictor

Parameters and cases	Group I		Group II	
	Estimate	*p* Value	Estimate	*p* Value
Male participants with skin lesion				
Arsenic dose from diet (μg kg body wt.$^{-1}$ day^{-1})	31.09	0.16	15.43	0.065
Arsenic dose from water (μg kg body wt.$^{-1}$ day^{-1})	3.13	0.8	88.99	0.0017
Diet and water interaction (μg kg body wt.$^{-1}$ day^{-1})	–	–	−36.89	0.0031
Male participants without skin lesion				
Arsenic dose from diet (μg kg body wt.$^{-1}$ day^{-1})	−39.27	<0.0001	−6.68	0.11
Arsenic dose from water (μg kg body wt.$^{-1}$ day^{-1})	−8.02	0.15	4.96	0.67
Diet and water interaction (μg kg body wt.$^{-1}$ day^{-1})	3.51	0.0005	–	–
Female participants with skin lesion				
Arsenic dose from diet (μg kg body wt.$^{-1}$ day^{-1})	−24.28	0.46	1.51	0.79
Arsenic dose from water (μg kg body wt.$^{-1}$ day^{-1})	23.74	0.06	35.78	0.0017
Female participants without skin lesion				
Arsenic dose from diet (μg kg body wt.$^{-1}$ day^{-1})	−8.09	0.42	−0.56	0.92
Arsenic dose from water (μg kg body wt.$^{-1}$ day^{-1})	34.62	<0.0001	21.42	0.010

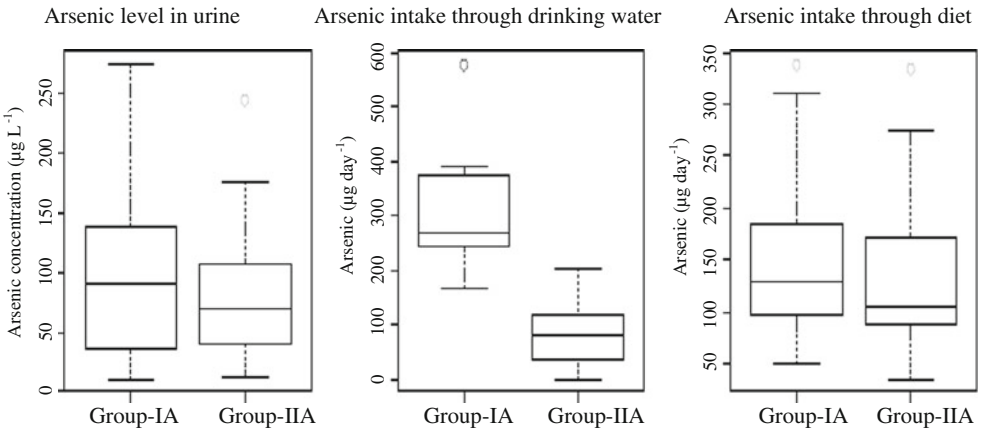

Fig. 5 Boxplots of arsenic concentration in urine (*left*), arsenic intake through drinking water (*middle*) and arsenic intake through diet (*right*) of 27 common participants who occur both in groups I and II (i.e., groups IA and IIA)

exposure in Indo-Bangla subcontinent (Smith et al. 2006; Roychowdhury et al. 2002; Ohno et al. 2007; Kile et al. 2007a; Williams et al. 2006). Variations in daily consumption of raw rice between males and females were also reported in Bangladesh (Watanabe et al. 2004). Compared to the median values we reported (0.5 and 0.441 kg for males of groups I and II; 0.407 and 0.362 kg for females of groups I and II), a higher intake value of 0.750-kg raw rice day^{-1} was previously reported for participants from Murshidabad, West Bengal (Roychowdhury et al. 2002). Median arsenic concentration in raw rice consumed by groups I and II was found to be 311 and 369 μg kg^{-1} (wet weight basis), and in cooked rice 108 and 88 μg kg^{-1} (wet weight basis), respectively (Table 3). This means that cooking reduced arsenic from raw rice by 65 and 76 % for groups I and II, respectively. In another study in Bangladesh, a reduction of 61 % (calculated on wet weight basis) was observed (Ohno et al. 2007) upon using of arsenic-poor water in cooking. The relatively low concentrations of arsenic in cooked rice in our study may be attributed to the use of traditional rice cooking methods in the Bengal delta. In these procedures, cooking water is thrown away after boiling. Therefore, actual exposure to arsenic from rice would be much lower than raw rice arsenic contents, as was also previously demonstrated (Rahman et al. 2006; Bae et al. 2002; Signes et al. 2008).

Arsenic content of individual dietary items did not change very much when arsenic filters were installed, which can be considered as a major finding of our present study. Several previous reports also demonstrated the presence of higher amounts of arsenic in raw dietary materials (Roychowdhury et al. 2002; Biswas et al. 2012). Kile et al. (2007a) carried out a duplicate diet survey to quantify daily arsenic intake in 47 women residing in Bangladesh. Combined median daily arsenic intake from food and drinking water was 68 μg day^{-1}. They found significant relations between concentrations of arsenic in household's drinking water and total arsenic concentration in food. When drinking water concentrations exceeded the Bangladesh drinking water standard of 50 μg arsenic L^{-1}, ingested water was the dominant source of exposure (Kile et al. 2007b). In our study involving 24 women participants in West Bengal, there is higher median total arsenic intake through the water and the diet, being 456 μg day^{-1} in group I who uses also water with arsenic contents above 50 μg L^{-1} for drinking and cooking purposes (Table 4).

Our study showed that when arsenic levels in water were reduced to below 50 μg L^{-1} for both drinking and cooking purposes, water played a major role in contributing to the arsenic level in urine as also in the case of participants using arsenic-contaminated (>50 μg L^{-1}) drinking and water. Urinary arsenic release was higher in group I participants compared to group II participants. This was the case for both males and females. However, urinary arsenic release of the 27 participants who were included in both groups I and II did not significantly decrease when the less-contaminated water was used. This confirms that not only the drinking water, but also the diet, plays an important role in influencing urinary arsenic release. Although water-borne arsenic is directly available for intestinal uptake, food-borne arsenic should first be released from its matrix (Alava et al. 2013; Sun et al. 2012). This may explain why dietary arsenic release had a much lower influence on urinary arsenic excretion compared to drinking water. The fact that urine samples were not taken throughout the day may have also contributed to this. Although total arsenic intake through water only contributed more significantly to urine arsenic levels than dietary intake does, gender and having clinical symptoms of arsenical skin lesion also played a major modifying factor for both groups I and II participants. Male participants experienced a lower decrease of urinary arsenic concentration upon using arsenic-poor water, compared to females. Differences between male and female participants may indicate not only differences in arsenic uptake, but also many physiological factors affect arsenic behavior in the body and its excretion. In a study conducted in Bangladesh, it was illustrated that individuals possessing GSTT1-null genotypes had significantly more arsenic in their toenails in contrast to GSTT1 wild-type individuals (Kile et al. 2005).

It should be noted that participants taking water from sources other than those which could be collected were excluded from the study. However, this cannot be completely controlled. Therefore, some participants may also have collected water from other sources, resulting in an overestimation or underestimation of arsenic uptake.

Conclusion

We assessed the association between total arsenic intake through diet and water, and arsenic levels in urine under variable conditions of water contamination. Therefore, we assessed the relative contribution of arsenic intake through water and diet to arsenic levels in urine in people from an endemic population of West Bengal being exposed to different levels of water contamination.

When arsenic levels in the water were reduced to below 50 μg L^{-1} (the Indian permissible limit), arsenic intake through the water significantly decreased, but arsenic uptake through the diet was found to be not significantly affected. Moreover, it was found that drinking water mainly contributed to variations in urine arsenic concentrations. However, differences in response between male and female participants also indicate that not only arsenic uptake, but also many physiological factors affect arsenic behavior in the body and its excretion.

As total median arsenic exposure still often exceeded 3.0 μg kg body $wt.^{-1}$ day^{-1} (the permissible lower limit established by the Joint Expert Committee on Food Additives) after installation of the drinking water filters, it can be concluded that only supplying the filtered water may not be sufficient to minimize arsenic availability for an already endemic population.

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