Public Health Nutrition: 16(9), 1644–1655 doi:10.1017/S1368980012004697

Nutritional deficiency and arsenical manifestations: a perspective study in an arsenic-endemic region of West Bengal, India

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Submitted 23 May 2012: Final revision received 6 September 2012: Accepted 13 September 2012: First published online 27 November 2012

Abstract

Objective: To assess whether nutritional deficiency increases susceptibility to arsenic-related health effects.

Design: Assessment of nutrition was based on a 24 h recall method of all dietary constituents.

Setting: Epidemiological cross-sectional study was conducted in an arsenic-endemic area of West Bengal with groundwater arsenic contamination.

Subjects: The study was composed of two groups – Group 1 (cases, *n* 108) exhibiting skin lesions and Group 2 (exposed controls, *n* 100) not exhibiting skin lesions – age- and sex-matched and having similar arsenic exposure through drinking water and arsenic levels in urine and hair.

Results: Both groups belonged to low socio-economic strata (Group 1 significantly poorer, $P < 0{\cdot}01$) and had low BMI (prevalence of BMI $< 18{\cdot}5\,kg/m^2$: in 38 % in Group 1 and 27 % in Group 2). Energy intake was below the Recommended Daily Allowance (set by the Indian Council of Medical Research) in males and females in both groups. Increased risk of arsenical skin lesions was found for those in the lowest quintile of protein intake (*v.* highest quintile: OR = 4·60, 95 % CI 1·36, 15·50 in males; OR = 5·62, 95 % CI 1·19, 34·57 in females). Significantly lower intakes of energy, protein, thiamin, niacin, Mg, Zn and choline were observed in both males and females of Group 1 compared with Group 2. Significantly lower intakes of carbohydrate, riboflavin, niacin and Cu were also observed in female cases with skin lesions compared with non-cases.

Conclusions: Deficiencies of Zn, Mg and Cu, in addition to protein, B vitamins and choline, are found to be associated with arsenical skin lesions in West Bengal.

Keywords
Arsenic manifestations
Energy intake
Micronutrients
Protein

Arsenic exposure through drinking water is a major health problem affecting many countries such as Bangladesh, India, Argentina, Mongolia, China, Chile, Taiwan, Mexico and some parts of the USA[1–4]. Arsenicosis results from prolonged exposure to arsenic at a dose of 5 to 90 μg/kg body weight per d[5]. The clinical features are characterized by hyper- and hypo-pigmentation, keratosis and various systemic manifestations like weakness, anaemia, chronic lung disease, peripheral neuropathy, liver fibrosis, gangrene of the limbs and cancers of the skin, lung and urinary bladder[6–8]. Studies on populations in Taiwan, India and Argentina exposed to arsenic through drinking water have suggested that malnutrition increases the risk of arsenic-induced diseases[9–13]. Several human studies have identified associations between malnutrition and arsenic-induced skin lesions, skin cancer and cardiovascular effects[12,14]. Inhabitants of Taiwan and the Antofagasta region in northern Chile suffering from severe health effects due to ingestion of high-arsenic contaminated drinking water were reported to have poor nutritional status[4,11]. Differential response of the cellular antioxidant mechanism to arsenic exposure in relation to dietary protein deficiency has been shown in experimental animals[15]. Studies done in experimental animals have shown that severe protein deficiency can impair arsenic methylation and excretion[16]. Dietary protein, Fe, Zn and niacin are associated with urinary excretion of monomethyl arsenic (MMA) and dimethyl arsenic (DMA)[17].

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Previous studies suggest that persons with more complete methylation have a lower risk of adverse arsenic-related health outcomes[18]. Dietary deficiency of methionine from protein is likely to decrease the ability to methylate arsenic and increase arsenic toxicity[19]. Folic acid and cyanocobalamin have been suggested to play an important role in the detoxification of ingested arsenic[20].

The objective of the present study was to determine if increased risk of arsenical skin lesions was associated with inadequate nutrition (or low intake of specific nutrients) among individuals who were exposed to arsenic-contaminated drinking water.

Experimental methods

Study design

A cross-sectional study was conducted among two groups with exposure to arsenic. Both groups were drawn from geographical areas in West Bengal known to have high levels of arsenic in groundwater above the permissible limit in India, i.e. >50 μg/l. Group 1 consisted of 108 individuals (cases) exhibiting arsenical skin lesions (diagnosed on the basis of WHO criteria[5]) and Group 2 consisted of 100 individuals (exposed controls) not exhibiting skin lesions.

Selection of participants

Of the seventeen arsenic-affected blocks in the district of Nadia, two blocks were chosen as the sample frame for reasons of convenience as much of the study area is rural and remote. A village-level sampling frame was created from all villages within these two blocks which had at least one tube-well contaminated with arsenic at a level greater than 50 μg/l, as described previously[21].

Of 174 villages in the sampling frame, six were selected, with proportional allocation across the two blocks, giving four villages in block 1 (Chakdah) and two villages in block 2 (Haringhata).

Further selection of the villages from within each block was carried out using a probability proportional to size sampling technique. To adjust for differences in the levels of contamination between villages, the size measure took into account the proportion of arsenic-contaminated tube-wells in the village as well as the total population count. Household selection within each of the six villages was done through systematic sampling with a random start in the list of households. A total of 212 households were eventually covered in the sample and the total number of inhabitants in these selected households turned out to be 900. This implied that about 4% of the households in a selected village were canvassed in the present study. Participants for Group 1 (cases) and Group 2 (exposed controls) were selected from these 212 households.

Participants of the first group (Group 1) consisted of 108 arsenicosis cases affected with typical skin lesions of pigmentation and/or keratosis[5], selected randomly from 187 out of 191 arsenicosis cases (four cases declined to participate) belonging to 900 arsenic-exposed residents of the 212 selected households.

Participants of the second group (Group 2) consisted of 100 individuals who were exposed controls without arsenical skin lesions and with definite evidence of arsenic exposure >50 μg/l, selected randomly from the remaining 709 individuals residing in the 212 households examined in the two blocks.

Field study

For assessment of total individual arsenic exposure, total arsenic level in drinking water and a dietary survey taken over a period of 24 h were determined for each participant in both groups. Biomarkers, namely arsenic level in urine and hair, were also analysed for each participant. All individuals included in the 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 criteria of the Declaration of Helsinki and the recommendation of the Indian Council of Medical Research, Government of India.

Measurement of exposure

Each participant was questioned on his or her current and previous sources of drinking and cooking water, and the duration of water use from each previous source. Responses were used to calculate the cumulative arsenic exposure for each participant. Cumulative arsenic exposure was calculated using the formula: $\Sigma(C_i \times D_i)$, where C_i is the concentration of arsenic in the water of a particular well that the participant had used during the period i and D_i is the duration of use.

Measurement of skin lesions

This was carried out as part of a general medical examination by physicians with extensive clinical experience of arsenical skin lesions in West Bengal. Of the 208 exposed individuals, 108 (Group 1 cases) had arsenical skin lesions and were diagnosed with arsenicosis on the basis of WHO criteria[5], while 100 exposed controls (Group 2) did not have skin lesions.

Collection of water, urine and hair

Water samples were collected from the present source of drinking and cooking water for each family, and also from previous water sources when they were still available, in a polyethylene bottle. Total daily water consumption by each participant was determined from self-report of the number of glasses (250 ml capacity) of water he/she consumed in a 24 h period. A first-morning-void urine sample was also collected from each participant. Both water and urine samples were kept in an ice box before transport from the field and stored at −20°C. For collection of hair, a whole length hair sample was cut from the scalp of each

participant with a stainless steel blade and kept in a plastic packet. All these samples were collected on the same day as the dietary survey and stored according to the WHO standard protocol[5] until further analysis.

Measurement of confounders

At the time of the field study, demographic data and socio-economic variables including age, sex, housing and BMI were also measured.

Diet survey

Food (raw and cooked rice, cooked and dry cereals, cooked pulses, cooked vegetables, chapatti, cooked animal protein and fruits) 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 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)[22] were used. All raw food items used to prepare each meal were noted and their weights in grams were recorded in the questionnaire. Raw food materials were weighed whenever the participating woman was unable to state the actual weight of the food used. Total cooked food was weighed to calculate the intake of raw food by the participant. Sugar and oil consumption was assessed using a standard-size spoon. Participants who worked outside often carried food from home. If not, then the participant was questioned about purchased food items.

Individual intake in terms of each raw food item (rice, legumes, potato) was calculated using the following formula: $F = (P/Q) \times R$, where F is the intake of raw food by the participant, P is the amount in grams of each raw food ingredient used for cooked food, Q is volume in millilitres of cooked food and R is the volume in millilitres of the cooked food consumed by the participant[23]. Milk and water consumption in the home, working place and cultivation field were also recorded, along with their sources and amount using a graduated glass beaker (Borosil, India). In the case of cooking water, only the sources were recorded.

Assessment of nutrient intake and nutritional status

The nutrients in each food item (carbohydrate, protein, fat, vitamins, minerals, fibre) and energy consumption were calculated according to the Indian Council of Medical Research reference standard[24] by using a spreadsheet program. For this purpose a detailed database was prepared of the nutrient composition per 100 g of raw food items. The nutritive value for ready-to-eat items like biscuits was obtained from their packaging. The amount and nutritive value were averaged for those food items prepared outside the home. Both cases and exposed controls were stratified by sex for comparison of their nutrient intakes with the respective Recommended Daily Allowance (RDA) set by the Indian Council of Medical Research[23]. The amount of nutrients consumed was compared with the RDA for India to determine the excess or deficient intake of individual nutrients, and the proportions of cases and exposed controls with nutrient intakes below the RDA were then compared. Height and weight were measured, and BMI (weight/height^2, kg/m^2) was calculated.

Statistical methods

Differences between cases (Group 1) and exposed controls (Group 2) with respect to demographic and socio-economic characteristics, arsenic exposure levels and arsenic concentrations in urine and hair were tested using two-population binomial tests. Information acquired from the dietary survey of participants was used to elucidate their daily nutrient intake. The median and interquartile range were computed for intakes of total energy, total protein, protein from animal sources, fat, carbohydrates, fibre, Ca, Fe, choline, Zn, Cu, Mg, carotene, retinol, thiamin, riboflavin, niacin, vitamin B_6, vitamin B_{12}, folate and vitamin C. Since all nutrient values displayed asymmetric behaviour in terms of distribution, non-parametric Mann–Whitney tests were conducted to test the difference in median value of nutrient intake between cases and controls separately for each sex. Intake of each nutrient was next stratified into quintiles of the distribution in both groups and odds ratios with 95 % confidence intervals were estimated for each quintile, taking the highest quintile as the reference group. Tests for trend were based on the χ^2 distribution using the median of each quintile range for both sexes. The number of participants with skin lesions in each quintile of nutrient intake is presented separately for males and females.

A multivariate logistic regression model was conducted to find important socio-economic and dietary predictors of the presence of arsenical skin lesions. We dichotomized the response variable into two categories according to whether or not a participant had skin lesions resulting from arsenic exposure. The predictors of age, sex, housing and BMI were included in the analysis for skin lesions. We did not include in the analysis some nutrients which showed no association with skin lesions but included the following: total energy consumption, total protein, animal protein, carbohydrates, fibre, choline, Zn, Cu, Mg, thiamin, riboflavin and niacin.

The amounts of various food categories consumed by participants in Group 1 and Group 2 were also compared separately for males and females. Further, we tested the

median values in both sexes combined to see if there were differences in nutrient intake among those participants who had been taking arsenic-contaminated water (≥50 μg/l) and safe water (<50 μg/l) for Group 1 and Group 2, separately. The quintile values of energy and nutrient intakes are also presented separately for Group 1 and Group 2 participants of both sexes.

Results

Baseline characteristics of the 108 cases (Group 1) and 100 controls (Group 2) are given in Table 1. There was no difference in regard to age and sex distribution between cases and controls. Distribution of BMI was also found to be similar among both groups, with 35 % of the cases and 27 % of the exposed controls being underweight. However, there were more poor participants among cases than among controls (74 % *v.* 56 % lived in mud houses, $P<0{\cdot}01$). Peak and cumulative arsenic exposure through drinking water were similar for participants in Group 1 (250 (SD 199) μg/l and 4 (SD 4) mg/l-years, respectively) and Group 2 (259 (SD 161) μg/l and 4 (SD 4) mg/l-years, respectively). There was no difference in biomarkers like arsenic concentration in urine and hair among the two groups (Table 1).

Daily intakes of energy and nutrients were calculated in Group 1 and Group 2 participants by sex and are presented in Table 2a (males) and Table 2b (females). Among the male participants, cases were found to have a lower energy intake than exposed controls (median: 9372 and 10 527 kJ/d, respectively; $P<0{\cdot}05$) and both groups had an intake below the RDA for Indians (11 422 kJ/d). The same was found for female participants, with median energy intake of 7887 kJ/d among cases and 9088 kJ/d among exposed controls ($P<0{\cdot}01$), and these were also below the RDA (9330 kJ/d). For males, the median protein intake of cases was 51 g/d and that of exposed controls was 63 g/d; this difference was found to be statistically significant ($P<0{\cdot}01$). For females, median protein intake was 40 g/d among cases and 49 g/d among exposed controls, and this difference was also found to be statistically significant ($P<0{\cdot}01$). For animal protein, median intake for male cases was 8 g/d, which was significantly lower in comparison to that of exposed controls (i.e. 13 g/d; $P<0{\cdot}01$); however, there was no significant difference in animal protein intake for females. Significantly lower intakes of thiamin (both $P<0{\cdot}05$), niacin (both $P<0{\cdot}05$), Mg (both $P<0{\cdot}05$), Zn ($P<0{\cdot}01$ males, $P<0{\cdot}05$ females) and choline ($P<0{\cdot}01$ males, $P<0{\cdot}05$ females) were observed in both male and female cases compared with their respective controls. Significantly lower fibre intake ($P<0{\cdot}05$) was also observed in male cases with skin lesions as compared with controls. Female cases with skin lesions also had significantly lower intakes of carbohydrate ($P<0{\cdot}01$), riboflavin ($P<0{\cdot}05$) and Cu ($P<0{\cdot}05$) compared with exposed controls.

Table 1 Demographic and socio-economic characteristics of participants in Group 1 (cases) and Group 2 (exposed controls): males and females from an arsenic-endemic area of West Bengal, India

	Group 1 (*n* 108)		Group 2 (*n* 100)		
	n	%	*n*	%	*P* value
Age (years)					
15–29	15	14	20	20	>0·05
30–44	45	41	47	47	>0·05
45–59	43	40	29	29	>0·05
≥60	5	5	4	4	>0·05
Sex					
Male	66	61	60	60	>0·05
Female	42	39	40	40	>0·05
Type of dwelling					
Mud house	80	74	56	56	<0·01
Mixed house	17	16	20	20	>0·05
Brick house	11	10	24	24	<0·01
BMI classification					
Underweight (<18·50 kg/m^2)	38	35	27	27	>0·05
Normal weight (18·50–24·99 kg/m^2)	61	57	61	61	>0·05
Pre-obese (25·00–29·99 kg/m^2)	7	6	11	11	>0·05
Obese (≥30·00 kg/m^2)	2	2	1	1	>0·05
	Arsenic concentration in drinking water and biological samples				
	Group 1		Group 2		
	Mean	SD	Mean	SD	*P* value
Highest known tube-well concentration (peak, μg/l)	250	199	259	161	>0·05
Cumulative exposure through water (mg/l-years)	4	4	4	4	>0·05
Urine (μg/l)	123	10	112	88	>0·05
Hair (mg/kg)	1·11	1·22	1·03	0·64	>0·05

Table 2a Comparison of nutrient intakes between participants in Group 1 (cases) and Group 2 (exposed controls): males from an arsenic-endemic area of West Bengal, India

		Group 1 (*n* 66)		Group 2 (*n* 60)		
Daily nutrient intake	RDA	Median	IQR	Median	IQR	*P* value
Energy (kJ)	11 422	9372	4067	10 527	711	<0·05
Carbohydrate (g)	NA	450	206	510	163	>0·05
Protein (g)	60	51	21	63	29	<0·01
Animal protein (g)	NA	8	10	13	17	<0·01
Fat (g)	30	22	14	25	13	>0·05
Carotene (μg)	4800	347	2975	380	1449	>0·05
Retinol (μg)	600	210	119	168	84	>0·05
Thiamin (mg)	1·4	1·39	0·66	1·57	0·42	<0·05
Riboflavin (mg)	1·6	0·53	0·39	0·61	0·26	>0·05
Niacin (mg)	18	22	9	26	8	<0·05
Vitamin C (mg)	40	59	67	55	56	>0·05
Fe (mg)	17	10	5	11	4	>0·05
Ca (mg)	600	369	394	317	492	>0·05
Dietary folate (μg)	200	78	49	81	51	>0·05
Fibre (g)	25–40	28	14	36	11	<0·05
Total vitamin B_6 (mg)	2	0·88	0·49	1·07	0·50	>0·05
Mg (mg)	350	425	200	494	177	<0·05
Cu (mg)	2	1·05	0·66	1·17	0·64	>0·05
Zn (mg)	16	8	3	10	3	<0·01
Choline (mg)	550	198	162	377	318	<0·01
Vitamin B_{12} (μg)	1	0·90	0·75	0·90	0·51	>0·05

RDA, Recommended Daily Allowance set by the Indian Council of Medical Research[23]; IQR, interquartile range; NA, not applicable.

Table 2b Comparison of nutrient intakes between participants in Group 1 (cases) and Group 2 (exposed controls): females from an arsenic-endemic area of West Bengal, India

		Group 1 (*n* 42)		Group 2 (*n* 40)		
Daily nutrient intake	RDA	Median	IQR	Median	IQR	*P* value
Energy (kJ)	9330	7887	2573	9088	2607	<0·01
Carbohydrate (g)	NA	371	117	430	174	<0·01
Protein (g)	55	40	21	49	17	<0·01
Animal protein (g)	NA	8	9	10	10	>0·05
Fat (g)	25	20	10	23	9	>0·05
Carotene (μg)	4800	250	1468	327	1845	>0·05
Retinol (μg)	600	119	87	95	53	>0·05
Thiamin (mg)	1·1	1·10	0·41	1·31	0·38	<0·05
Riboflavin (mg)	1·3	0·39	0·22	0·49	0·28	<0·05
Niacin (mg)	14	18	5	21	7	<0·05
Vitamin C (mg)	40	49	28	65	43	>0·05
Fe (mg)	21	9	4	10	4	>0·05
Ca (mg)	600	252	366	266	318	>0·05
Dietary folate (μg)	200	62	41	69	43	>0·05
Fibre (g)	25–40	23	9	26	11	>0·05
Total vitamin B_6 (mg)	2	0·80	0·39	0·78	0·35	>0·05
Mg (mg)	350	349	149	412	189	<0·05
Cu (mg)	2	0·78	0·57	1·01	0·63	<0·05
Zn (mg)	16	6	2	8	3	<0·05
Choline (mg)	425	208	155	291	232	<0·05
Vitamin B_{12} (μg)	1	0·41	0·35	0·38	0·12	>0·05

RDA, Recommended Daily Allowance set by the Indian Council of Medical Research[23]; IQR, interquartile range; NA, not applicable.

Intake of each nutrient was stratified into quintiles of the distribution in both groups. OR and 95 % CI for skin lesions were computed for each quintile, using the highest quintile as the reference group, for both male (Table 3a) and female (Table 3b) participants. The strongest trends in OR for males were for protein ($P<0·05$), animal protein ($P<0·01$), fibre ($P<0·01$), Zn ($P<0·01$), Mg ($P<0·05$) and choline ($P<0·01$); and for females the strongest trends were for protein ($P<0·05$), thiamin ($P=0·05$), riboflavin ($P<0·05$), choline ($P<0·01$), Zn ($P<0·05$) and Cu ($P<0·05$).

The number of cases with skin lesions according to intake quintile of each nutrient is presented separately for male and female participants (Table 4a and 4b, respectively). It can be seen that the highest number of participants with skin lesions is found in the lowest intake quintile of each nutrient with a significant deficiency.

Table 3a Odds ratios and 95 % confidence intervals for presence of arsenical skin lesions by quintile of nutrient intake (OR comparing highest *v.* lowest quintile, with quintile 1 as the reference group): males from an arsenic-endemic area of West Bengal, India

	Quintile (1 = highest, 5 = lowest)									
	1	2		3		4		5		Test for trend*
Daily nutrient intake		OR	95 % CI	OR	95 % CI	OR	95 % CI	OR	95 % CI	
Energy (kJ)	1·00	1·55	0·58, 4·14	1·40	0·49, 3·98	2·38	0·60, 9·37	2·21	0·84, 6·85	>0·05
Carbohydrate (g)	1·00	1·09	0·39, 3·02	1·59	0·56, 4·45	2·71	0·84, 8·72	1·86	0·62, 5·69	>0·05
Protein (g)	1·00	1·54	0·58, 4·06	2·37	0·81, 6·97	2·26	0·73, 6·97	4·60	1·36, 15·50	<0·05
Animal protein (g)	1·00	3·64	1·25, 10·59	6·37	1·99, 20·34	6·00	1·57, 22·88	4·63	1·54, 13·96	<0·01
Fat (g)	1·00	0·67	0·23, 1·94	1·14	0·39, 3·35	1·86	0·59, 5·78	2·77	0·89, 8·64	>0·05
Carotene (μg)	1·00	0·49	0·16, 1·51	0·58	0·19, 1·71	0·76	0·23, 2·49	0·73	0·24, 2·19	>0·05
Retinol (μg)	1·00	2·25	0·41, 12·44	2·37	0·41, 12·96	1·56	0·24, 10·03	1·00	0·16, 6·42	>0·05
Thiamin (mg)	1·00	1·14	0·41, 3·17	1·17	0·44, 3·11	2·67	0·75, 9·45	4·00	1·19, 13·46	>0·05
Riboflavin (mg)	1·00	0·62	0·22, 1·72	0·71	0·25, 1·97	1·06	0·36, 3·15	3·82	0·91, 16·05	>0·05
Niacin (mg)	1·00	0·83	0·29, 2·35	1·35	0·49, 3·67	2·32	0·75, 7·18	3·25	1·02, 11·04	>0·05
Vitamin B_6 (mg)	1·00	1·17	0·44, 3·11	2·13	0·76, 6·01	2·48	0·79, 7·72	1·33	0·44, 4·02	>0·05
Vitamin B_{12} (μg)	1·00	0·74	0·15, 3·50	1·55	0·24, 9·91	1·00	0·16, 5·98	0·44	0·07, 2·66	>0·05
Vitamin C (mg)	1·00	0·61	0·19, 1·87	0·46	0·16, 1·32	0·81	0·26, 2·56	0·89	0·32, 2·53	>0·05
Fe (mg)	1·00	0·43	0·15, 1·22	0·60	0·18, 1·94	1·22	0·39, 3·80	1·36	0·41, 4·54	>0·05
Ca (mg)	1·00	1·90	0·66, 5·46	1·89	0·62, 5·76	0·92	0·33, 2·59	2·62	0·78, 8·84	>0·05
Dietary folate (μg)	1·00	1·69	0·59, 4·80	1·29	0·47, 3·54	0·64	0·21, 1·93	2·47	0·71, 8·67	>0·05
Fibre (g)	1·00	0·54	0·19, 1·53	1·85	0·67, 5·15	2·16	0·59, 7·85	3·20	1·01, 10·23	<0·05
Choline (mg)	1·00	3·16	0·92, 10·87	5·98	1·68, 21·31	11·50	3·23, 40·86	16·07	5·46, 39·34	<0·01
Zn (mg)	1·00	1·77	0·63, 4·96	4·20	1·43, 12·36	4·73	1·41, 15·81	11·82	2·84, 41·19	<0·01
Cu (mg)	1·00	0·76	0·27, 2·13	1·09	0·38, 3·15	0·65	0·21, 1·98	2·80	0·81, 9·74	>0·05
Mg (mg)	1·00	1·12	0·42, 3·00	0·87	0·29, 2·58	3·39	1·05, 10·95	4·75	1·28, 17·57	<0·05

*P value based on χ^2 test.

Table 3b Odds ratios and 95 % confidence intervals for presence of arsenical skin lesions by quintile of nutrient intake (OR comparing highest *v.* lowest quintile, with quintile 1 as the reference group): females from an arsenic-endemic area of West Bengal, India

	Quintile (1 = highest, 5 = lowest)									
	1	2		3		4		5		Test for trend*
Daily nutrient intake		OR	95 % CI	OR	95 % CI	OR	95 % CI	OR	95 % CI	
Energy (kJ)	1·00	0·91	0·34, 2.42	1·41	0·49, 3·98	2·37	0·73, 7·76	2·20	0·71, 6·85	>0·05
Carbohydrate (g)	1·00	0·74	0·12, 4.73	0·72	0·12, 4·39	2·86	0·89, 16·36	2·33	0·41, 13·17	>0·05
Protein (g)	1·00	2·50	0·34, 18.30	1·75	0·27, 11·15	4·33	1·13, 26·53	5·62	1·19, 34·57	<0·05
Animal protein (g)	1·00	7·50	1·19, 47.05	10·80	1·69, 69·90	2·70	0·33, 21·90	4·80	1·03, 25·90	>0·05
Fat (g)	1·00	1·22	0·26, 5.66	1·14	0·27, 4·86	1·24	0·28, 5·53	3·64	1·02, 17·00	>0·05
Carotene (μg)	1·00	1·64	0·35, 6.05	0·63	0·14, 2·82	0·93	0·24, 3·58	4·95	0·98, 24·87	>0·05
Retinol (μg)	1·00	5·00	0·47, 52.96	6·00	0·35, 101·5	3·00	0·25, 35·33	0·40	0·03, 6·17	>0·05
Thiamin (mg)	1·00	0·50	0·06, 4.09	0·43	0·09, 2·01	2·00	0·43, 9·29	2·14	0·46, 9·89	0·05
Riboflavin (mg)	1·00	3·33	0·56, 19.94	2·00	0·39, 10·31	1·57	0·33, 7·48	2·71	0·60, 12·32	<0·05
Niacin (mg)	1·00	2·25	0·31, 16.41	1·50	0·22, 10·17	1·87	0·37, 11·52	1·87	0·37, 11·52	>0·05
Vitamin B_6 (mg)	1·00	0·89	0·12, 6.31	2·07	0·37, 11·52	1·45	0·26, 8·01	1·78	0·31, 10·01	>0·05
Vitamin B_{12} (μg)	1·00	1·33	0·17, 10.25	2·00	0·22, 17·89	5·00	0·38, 64·39	0·20	0·01, 2·57	>0·05
Vitamin C (mg)	1·00	0·85	0·21, 3.51	1·46	0·34, 6·11	1·75	0·43, 7·17	1·63	0·33, 7·95	>0·05
Fe (mg)	1·00	0·85	0·14, 5.23	0·79	0·21, 2·97	1·14	0·29, 4·51	3·43	0·82, 14·36	>0·05
Ca (mg)	1·00	2·80	0·58, 13.47	0·82	0·18, 3·58	1·96	0·38, 9·93	1·93	0·48, 7·99	>0·05
Dietary folate (μg)	1·00	1·29	0·23, 7.05	1·80	0·31, 10·52	1·25	0·23, 6·63	2·71	0·53, 13·91	>0·05
Fibre (g)	1·00	2·33	0·41, 13.61	1·00	0·17, 5·77	2·17	0·44, 10·65	6·00	1·08, 33·37	>0·05
Choline (mg)	1·00	1·00	0·23, 4.69	1·80	0·44, 7·31	2·88	1·07, 13·75	4·50	1·11, 19·11	<0·05
Zn (mg)	1·00	1·47	0·18, 11.72	1·33	0·16, 10·74	2·17	0·30, 15·71	4·50	1·13, 33·71	<0·05
Cu (mg)	1·00	1·80	0·45, 8.68	1·60	0·37, 6·82	1·31	0·31, 5·43	7·20	1·53, 33·85	<0·05
Mg (mg)	1·00	0·75	0·12, 4.89	1·17	0·22, 6·19	3·71	0·71, 19·59	4·00	1·04, 20·31	>0·05

*P value based on χ^2 test.

The results of the multivariate regression analysis are given in Table 5. Age was found to be a significant predictor, whereas BMI, housing (an indicator of socio-economic status) and sex were not significant predictors of skin lesions among socio-economic and biological factors. Among included nutrients, carbohydrate ($P = 0·05$), protein ($P < 0·01$), animal protein ($P < 0·01$), Zn ($P < 0·01$), Mg ($P < 0·01$), choline ($P < 0·05$), thiamin ($P < 0·05$) and riboflavin ($P < 0·01$) significantly influenced the occurrence of skin lesions in a participant. The goodness-of-fit test

Table 4a Number of participants (cases) with skin lesions according to quintile of nutrient intake: males from an arsenic-endemic area of West Bengal, India

	Quintile (1 = highest, 5 = lowest)				
Daily nutrient intake	1	2	3	4	5
Energy (kJ)	13	9	11	17	16
Carbohydrate (g)	16	11	14	13	12
Protein (g)	8	11	13	16	18
Animal protein (g)	8	10	10	21	17
Fat (g)	13	10	10	16	17
Carotene (μg)	16	11	14	11	14
Retinol (μg)	4	3	4	5	1
Thiamin (mg)	15	12	14	10	15
Riboflavin (mg)	17	12	12	12	13
Niacin (mg)	13	6	15	15	17
Vitamin B_6 (mg)	11	12	12	18	13
Vitamin C (mg)	18	10	11	11	16
Iron (mg)	14	8	13	14	17
Ca (mg)	13	16	13	12	12
Dietary folate (μg)	16	15	15	9	11
Fibre (g)	11	8	13	16	18
Choline (mg)	5	11	13	20	17
Zn (mg)	11	5	13	17	20
Cu (mg)	13	14	11	12	16
Mg (mg)	13	11	10	14	18
Vitamin B_{12} (μg)	6	10	7	6	4

Table 4b Number of participants (cases) with skin lesions according to quintile of nutrient intake: females from an arsenic-endemic area of West Bengal, India

	Quintile (1 = highest, 5 = lowest)				
Daily nutrient intake	1	2	3	4	5
Energy (kJ)	5	4	10	9	14
Carbohydrate (g)	3	4	6	15	14
Protein (g)	1	5	7	13	16
Animal protein (g)	1	10	12	3	16
Fat (g)	4	7	9	9	13
Carotene (μg)	8	7	5	9	13
Retinol (μg)	2	5	3	3	1
Thiamin (mg)	4	2	7	14	15
Riboflavin (mg)	4	3	5	11	19
Niacin (mg)	3	3	6	15	15
Vitamin B_6 (mg)	3	3	13	11	12
Vitamin C (mg)	6	7	10	12	7
Fe (mg)	7	2	9	9	15
Ca (mg)	4	10	7	7	14
Dietary folate (μg)	4	6	5	8	19
Fibre (g)	3	6	5	13	15
Choline (mg)	4	5	10	8	15
Zn (mg)	2	4	5	13	18
Cu (mg)	5	5	8	8	16
Mg (mg)	3	4	6	13	16
Vitamin B_{12} (μg)	4	9	9	5	4

suggested there is insufficient evidence to support that the model does not fit the data adequately.

The amounts of various food categories consumed by male and female participants of the two groups are presented in Table 6a and 6b, respectively. Male cases with skin lesions consumed significantly smaller amounts of animal protein ($P < 0{\cdot}05$), fish ($P = 0{\cdot}05$), roots and tubers ($P < 0{\cdot}01$) and other vegetables ($P < 0{\cdot}05$) compared with

Table 5 Results of multivariate analysis evaluating the association of arsenical skin lesions with dietary intakes of energy and selected nutrients, and age, sex, housing and BMI: males and females from an arsenic-endemic area of West Bengal, India

				95% CI	
Predictor	Coefficient	*P* value	OR	Lower	Upper
Constant	3·19057	<0·05			
Age	0·0794717	<0·01	1·08	1·04	1·13
BMI	−0·116711	>0·05	0·89	0·79	1·01
Housing	−0·444084	>0·05	0·64	0·35	1·18
Sex	0·565008	>0·05	1·76	0·78	3·99
Energy	0·003051	>0·05	1·00	1·00	1·01
Carbohydrate	−0·0275767	0·05	0·97	0·95	1·00
Protein	0·151037	<0·01	1·16	1·05	1·29
Animal protein	−0·147769	<0·01	1·12	1·04	1·19
Thiamin	4·39219	<0·05	0·82	0·63	0·96
Riboflavin	−5·27884	<0·01	0·01	0·00	0·11
Niacin	0·264182	>0·05	1·30	0·77	2·19
Fibre	−0·0179027	>0·05	0·98	0·87	1·11
Mg	0·0122758	<0·01	1·01	1·00	1·02
Cu	−1·26071	>0·05	0·28	0·06	1·40
Zn	−2·01013	<0·01	2·13	1·16	3·26
Choline	−0·00224	<0·05	1·00	1·00	1·00

Goodness-of-fit test: $\chi^2 = 184{\cdot}35$, df = 191, *P* value = 0·622.

exposed male controls; while significantly smaller amounts of cereals ($P < 0{\cdot}05$) and milk ($P < 0{\cdot}05$) were consumed by female cases with skin lesions compared with exposed female controls.

Intakes of each nutrient among Group 1 and Group 2 participants according to arsenic exposure level, i.e. low (<50 μg/l) or high (≥50 μg/l) arsenic concentration in current drinking water source, are given in Table 7a and 7b, respectively. In cases with skin lesions (Group 1), intake of vitamin C was significantly lower ($P < 0{\cdot}05$) and intake of choline was significantly higher ($P < 0{\cdot}05$) in those with low compared with high arsenic concentration in drinking water. In exposed controls (Group 2), intakes of animal protein ($P = 0{\cdot}05$) and Ca ($P < 0{\cdot}05$) were significantly lower in participants currently drinking water with a low concentration of arsenic compared with a high concentration of arsenic. The quintile distribution of each nutrient intake among male and female participants of Group 1 and Group 2 is presented in Table 8a and 8b, respectively.

Discussion

The present study shows that among poor underweight people, several nutrient deficiencies were associated with arsenical skin lesions in an arsenic-endemic region of West Bengal. This was also reflected in the quantitative differences in intakes of quality foods like protein including fish, milk, cereals, roots and tubers, and vegetables in the group with skin lesions compared with the group with no skin lesions. Interestingly, male cases had lower intakes of animal protein including fish, roots and

Table 6a Comparison of food group intakes between Group 1 (cases) and Group 2 (exposed controls): males from an arsenic-endemic area of West Bengal, India

	Group 1			Group 2			
Daily food group intake (g/ml)	*n*	Median	IQR	*n*	Median	IQR	*P* value
Cereals*	66	512	238	60	564	238	>0·05
Pulses and legumes†	36	24	31	21	33	24	>0·05
Roots and tubers‡	63	118	103	59	157	129	<0·01
Green leafy vegetables§	31	100	109	27	90	160	>0·05
Other vegetables‖	66	129	93	60	134	111	<0·05
Total animal protein¶	49	60	71	49	100	120	<0·05
Only fish	43	23	37	30	34	109	0·05
Only milk	8	100	200	9	125	109	>0·05
Fruits**	3	75	142	2	281	–	>0·05
Others††	66	20	26	56	20	11	>0·05

IQR, interquartile range.
*Cereals: rice, wheat flour, puffed rice, flaked rice, *semai*, etc.
†Pulses and legumes: lentils, mung beans, green peas, Bengal gram, dhal, etc.
‡Roots and tubers: potato, carrot, onion, colocasia, radish, etc.
§Green leafy vegetables: amaranth, spinach, drumstick leaves, colocasia leaves, cabbage, cauliflower, etc.
‖Other vegetables: pumpkin, bitter gourd, bottle gourd, brinjal, papaya, tomato, beans, ladies finger, etc.
¶Animal protein: fish, egg, meat, chicken, milk and milk products.
**Fruits: banana, mango, palmyrah, etc.
††Other: mustard oil, sugar, etc.

Table 6b Comparison of food group intakes between Group 1 (cases) and Group 2 (exposed controls): females from an arsenic-endemic area of West Bengal, India

	Group 1			Group 2			
Daily food group intake (g/ml)	*n*	Median	IQR	*n*	Median	IQR	*P* value
Cereals*	42	412	142	40	470	234	<0·05
Pulses and legumes†	17	23	33	19	28	20	>0·05
Roots and tubers‡	39	131	116	39	152	123	>0·05
Green leafy vegetables§	18	80	110	16	57	47	>0·05
Other vegetables‖	42	81	86	40	101·5	87	>0·05
Total animal protein¶	27	60	56	24	87·5	121	>0·05
Only fish	30	24	39	34	32	66	>0·05
Only milk	5	100	62	5	250	77	<0·05
Fruits**	2	55	–	3	323	248	>0·05
Others††	37	18	17	36	17	16	>0·05

IQR, interquartile range.
*Cereals: rice, wheat flour, puffed rice, flaked rice, *semai*, etc.
†Pulses and legumes: lentils, mung beans, green peas, Bengal gram, dhal, etc.
‡Roots and tubers: potato, carrot, onion, colocasia, radish, etc.
§Green leafy vegetables: amaranth, spinach, drumstick leaves, colocasia leaves, cabbage, cauliflower, etc.
‖Other vegetables: pumpkin, bitter gourd, bottle gourd, brinjal, papaya, tomato, beans, ladies finger, etc.
¶Animal protein: fish, egg, meat, chicken, milk and milk products.
**Fruits: banana, mango, palmyrah, etc.
††Other: mustard oil, sugar, etc.

tubers, and other vegetables; while female cases had lower intakes of cereals and milk (Table 6a and 6b).

In the present study, the energy intakes of males and females in both groups (cases and controls) were found to be below the RDA. Similar findings were obtained in nutrition surveys conducted in eight states of India by the National Nutrition Bureau of India[25]. Undernourishment has been found to increase the risk of skin lesions and skin cancer in arsenic-exposed populations[9,11]. In Western countries such as the USA (Alaska), studies revealed that populations consuming high concentrations of arsenic from their drinking water often did not show arsenical skin lesions; their good nutritional status was cited as a potential explanation[26]. The present study found that there was widespread deficiency of nutrients in Group 1 participants with skin lesions. Multivariate logistic regression analysis showed that deficiencies of nutrients like carbohydrate, protein, thiamin, riboflavin, Mg, Zn and choline were associated with arsenical skin lesions (Table 5). Significantly lower intakes of protein, thiamin, niacin, Mg, Zn and choline were observed in both male and female cases compared with respective controls. Significantly lower intakes of carbohydrate, riboflavin and Cu were also observed in female cases with skin lesions compared with controls (Table 6). It could be seen that the highest numbers of participants with skin lesions for both males and females were present in the lowest quintiles of nutrient intakes (Table 4a and 4b).

Table 7a Comparison of nutrient intakes in relation to current arsenic exposure level among participants in Group 1 (cases): males and females from an arsenic-endemic area of West Bengal, India

	Current arsenic exposure level ≥50 μg/l (*n* 25)		Current arsenic exposure level <50 μg/l (*n* 83)		
Daily nutrient intake	Median	IQR	Median	IQR	*P* value
Energy (kJ)	8477	2979	8602	3643	>0·05
Carbohydrate (g)	406	161	396	189	>0·05
Protein (g)	45	22	47	24	>0·05
Animal protein (g)	8	7	8	10	>0·05
Fat (g)	20	16	22	13	>0·05
Carotene (μg)	368	557	246	3043	>0·05
Retinol (μg)	155	88	130	93	>0·05
Thiamin (mg)	1·19	0·62	1·23	0·64	>0·05
Riboflavin (mg)	0·49	0·26	0·44	0·40	>0·05
Niacin (mg)	20	8	20	10	>0·05
Vitamin C (mg)	56	38	37	61	<0·05
Fe (mg)	10	6	10	5	>0·05
Ca (mg)	289	402	413	400	>0·05
Dietary folate (μg)	74	39	69	51	>0·05
Fibre (g)	26	18	28	13	>0·05
Total vitamin B_6 (mg)	0·83	0·37	0·81	0·47	>0·05
Mg (mg)	396	264	385	177	>0·05
Cu (mg)	0·94	0·65	0·99	0·71	>0·05
Zn (mg)	7	3	7	3	>0·05
Choline (mg)	181	176	242	161	<0·05
Vitamin B_{12} (μg)	0·90	0·39	0·68	0·42	>0·05

	Arsenic concentration in drinking water (μg/l)		
	Median	IQR	*P* value
Current arsenic exposure level			
<50 μg/l (*n* 83)	BDL	6	<0·01
≥50 μg/l (*n* 25)	96	42	

IQR, interquartile range; BDL, below detection limit.

An earlier dietary survey by the 24 h recall method in an arsenic-exposed population in south Parganas, West Bengal reported that deficiencies in some nutrients (i.e. animal protein, Ca, fibre, folic acid and vitamin C) may increase the risk of arsenic-induced skin lesions[23]. Inadequate intakes of folic acid, methionine, cysteine, vitamins B_6 and B_{12}, energy and protein are associated with arsenic-related health effects in human populations[12,16,23,27,28]. In the present study, we did not find deficiency of folic acid, vitamin B_6, vitamin B_{12} and Ca to be associated with arsenical skin lesions, but we observed deficiency of choline, Cu, Mg and Zn in cases of arsenic-induced skin disease. Experimental studies in animals have shown that low dietary protein and amino acids intake increases the risks of arsenic-related health effects[12,16,27,29]. The present study found statistically significant differences in intake of protein between cases and exposed controls in both male and female participants. Animal protein intake was also significantly lower in cases than exposed controls for males in the present study. Low dietary intake of methionine, choline or protein decreased arsenic excretion (especially urinary excretion of DMA) and increased the tissue retention of arsenic in rabbits[16]. Diets deficient in methionine and choline decreased *S*-adenosylmethionine levels, therefore inhibiting methyltransferase reactions[30], i.e. arsenic detoxification reactions, in rats. In a study in a human population, those in the lower quartile of protein intake excreted a higher proportion of ingested inorganic arsenic (InAs) as MMA and a lower proportion as DMA than did those in the upper quartile of protein intake. Participants in the lower quartiles of Fe, Zn and niacin intakes also had higher urinary percentage MMA and lower urinary percentage DMA levels than did those with higher intakes of these nutrients[17]. In a study from Bangladesh higher intakes of cysteine, methionine, Ca, protein and vitamin B_{12} were found to be associated with lower percentage of InAs and higher MMA:InAs in urine[28]. In an experimental study Zn has been found to induce arsenic tolerance in mice[31].

In a hospital-based study in West Bengal, intake of a nutritious diet was shown to be associated with improvement of arsenical symptoms[32]. There are studies indicating that consumption of a diet rich in riboflavin, pyridoxine and vitamins A, C and E can significantly reduce the harmful effects of developments of skin lesions[20]. Other nutrients like niacin, Fe, Ca, protein and thiamin were also reported to be protective against arsenic toxicity[17].

Nutrient intakes among Group 1 and Group 2 participants by current exposure to low (<50 μg/l) and high (≥50 μg/l)

Table 7b Comparison of nutrient intakes in relation to current arsenic exposure level among participants in Group 2 (exposed controls): males and females from an arsenic-endemic area of West Bengal, India

	Current arsenic exposure level ≥50 μg/l (*n* 61)		Current arsenic exposure level <50 μg/l (*n* 39)		
Daily nutrient intake	Median	IQR	Median	IQR	*P* value
Energy (kJ)	9945	3337	9799	3703	>0·05
Carbohydrate (g)	456	194	480	212	>0·05
Protein (g)	53	23	59	38	>0·05
Animal protein (g)	12	20	12	13	0·05
Fat (g)	24	12	24	16	>0·05
Carotene (μg)	368	1453	373	3174	>0·05
Retinol (μg)	105	94	160	50	>0·05
Thiamin (mg)	1·38	0·48	1·47	0·54	>0·05
Riboflavin (mg)	0·55	0·25	0·55	0·39	>0·05
Niacin (mg)	23	9	24	10·	>0·05
Vitamin C (mg)	63	56	58	67	>0·05
Fe (mg)	9	4	11	5	>0·05
Ca (mg)	258	549	351	342	<0·05
Dietary folate (μg)	79	37	77	56	>0·05
Fibre (g)	32	13	32	17	>0·05
Total vitamin B_6 (mg)	0·96	0·60	0·97	0·51	>0·05
Mg (mg)	435	146	475	250	>0·05
Cu (mg)	1·03	0·63	1·01	0·73	>0·05
Zn (mg)	9	4	9	4	>0·05
Choline (mg)	334	295	373	344	>0·05
Vitamin B_{12} (μg)	1·06	0·34	0·68	0·36	>0·05

	Arsenic concentration in drinking water (μg/l)		
	Median	IQR	*P* value
Current arsenic exposure level			
<50 μg/l (*n* 39)	BDL	26	<0·01
≥50 μg/l (*n* 61)	97	71	

IQR, interquartile range; BDL, below detection limit.

Table 8a Distribution of nutrient intakes associated with each quintile among participants of Group 1 (cases) by sex: males (M) and females (F) from an arsenic-endemic area of West Bengal, India

	Quintile (1 = highest, 5 = lowest)									
	1		2		3		4		5	
Daily nutrient intake	F	M	F	M	F	M	F	M	F	M
Energy (kJ)	13 948	19 700	8983	12 405	8113	10 117	7150	8770	5364	7217
Carbohydrate (g)	725	1920	451	594	386	490	345	412	255	325
Protein (g)	76	191	51	68	42	58	38	47	27	37
Animal protein (g)	28	42	10	12	7	7	3	4	0	0
Fat (g)	45	112	26	33	22	24	19	19	14	14
Carotene (μg)	12 577	16 261	2306	3566	469	535	186	252	120	131
Retinol (μg)	210	506	176	270	132	210	103	159	87	71
Thiamin (mg)	2·11	5·92	1·36	1·75	1·15	1.51	1·01	1·21	0·86	0·86
Riboflavin (mg)	0·94	1·65	0·55	0·80	0.43	0·60	0.32	0.47	0·26	0·35
Niacin (mg)	34	88	22	29	19	23	17	20	12	16
Vitamin B_6 (mg)	1·53	3·04	0·92	1·24	0·82	1·05	0·71	0·83	0·45	0·65
Vitamin C (mg)	216	524	86	124	54	71	42	45	34	28
Fe (mg)	19	54	13	14	10	11	7	8	6	7
Ca (mg)	1044	1258	553	602	359	463	168	275	106	151
Dietary folate (μg)	176	238	88	118	66	89	50	71	40	56
Fibre (g)	48	170	33	40	24	30	21	26	17	19
Choline (mg)	820	890	334	328	242	231	172	178	122	143
Zn (mg)	11	30	8	10	6	8	6	7	5	6
Cu (mg)	2·61	4·80	1·00	1·00	0·91	1·23	0·69	0.94	0·56	0·64
Mg (mg)	628	3314	447	605	380	479	323	394	274	313
Vitamin B_{12} (μg)	1·76	2·08	0·90	1·68	0·45	0·90	0·36	0·90	0·35	0·42

Table 8b Distribution of nutrient intakes associated with each quintile among participants of Group 2 (exposed controls) by sex: males (M) and females (F) from an arsenic-endemic area of West Bengal, India

	Quintile (1 = highest, 5 = lowest)									
	1		2		3		4		5	
Daily nutrient intake	F	M	F	M	F	M	F	M	F	M
Energy (kJ)	13244	21516	10924	12891	9569	11506	8682	10000	7414	8376
Carbohydrate (g)	642	1044	525	619	440	550	407	478	326	385
Protein (g)	125	157	61	81	52	67	46	57	39	46
Animal protein (g)	85	61	15	25	6	14	2	9	0	1
Fat (g)	91	45	32	33	24	26	22	24	18	19
Carotene (μg)	26550	24075	3221	1995	489	487	250	302	206	160
Retinol (μg)	504	281	193	281	113	210	78	105	49	52
Thiamin (mg)	2·60	5·04	1·52	1·87	1·39	1·63	1·24	1·47	1·07	1·22
Riboflavin (mg)	1·17	1·79	0·68	0·79	0·54	0·65	0·46	0·55	0·36	0·44
Niacin (mg)	33	58	25	30	22	27	20	23	16	20
Vitamin B_6 (mg)	1·37	1·89	1·06	1·38	0·91	1·16	0·69	0·99	0·55	0.65
Vitamin C (mg)	475	512	95	98	68	63	54	46	37	31
Fe (mg)	34	46	13	13	10	12	9	10	7	8
Ca (mg)	1519	2055	499	719	327	467	236	260	120	156
Dietary folate (μg)	164	247	102	124	76	90	64	76	55	63
Fibre (g)	73	146	34	41	30	37	25	33	21	26
Choline (mg)	1749	1549	486	713	342	459	259	341	172	225
Zn (mg)	16	28	9	12	8	11	7	9	6	8
Cu (mg)	2·89	5·69	1·54	1·54	1·15	1·32	0·89	1·03	0·77	0·78
Mg (mg)	858	1722	539	609	440	534	406	470	317	407
Vitamin B_{12} (μg)	2·16	2·80	0·90	1·18	0·69	1·05	0·32	0·71	0·12	0·28

arsenic levels in drinking water showed few variations (Table 7a and 7b). However, limited inferences could be drawn from observation of any difference in intake of any nutrient among cases and controls on the basis of arsenic exposure through current drinking water source, as the clinical effects like skin lesions develop following prolonged intake of arsenic-contaminated water. We therefore compared cumulative arsenic exposure among cases and controls, and found these two groups to be similarly exposed to arsenic.

In the present study, although there was lack of an observed statistically significant difference in distribution in regard to age, sex and BMI in the two participant groups, this does not necessarily mean that there was no significant difference between the populations that they were sampled from. There is possibly some other factor, e.g. a genetic factor, that may be important in determining susceptibility to developing skin lesions other than nutrition. People in northern Chile exposed to arsenic had a good nutritious dietary intake. However, the prevalence of skin lesions among men and children in the population studied was similar to that reported at corresponding concentrations of arsenic in drinking water in both Taiwan and West Bengal, India – populations in which malnutrition has been thought to increase susceptibility[33].

Conclusions

A cross-sectional study was conducted in two arsenic-endemic blocks of Nadia district of West Bengal to assess whether nutritional deficiency increases the susceptibility to arsenical skin lesions, an important clinical diagnostic criterion of chronic arsenic toxicity. Poor underweight people with several nutrient deficiencies were found to be more susceptible to arsenical skin lesions in this arsenic-endemic region of West Bengal. Significantly lower intakes of protein, thiamin, niacin, Mg, Zn and choline were observed in both male and female cases compared with respective controls. Significantly lower intakes of carbohydrate, riboflavin and Cu were also observed in female cases with skin lesions compared with control females without skin lesions. Moreover, significantly lower amounts of animal protein, roots and tubers, and other vegetables were consumed by males with skin lesions, while significantly lower amounts of cereals and milk were consumed by females with skin lesions, compared with male and female participants without skin lesions. Dietary advice to increase the consumption of animal protein, roots and tubers, and other vegetables by males and to increase the consumption of cereals and milk by females may help reduce the occurrence of arsenical skin lesions in this arsenic-endemic region in West Bengal.

Acknowledgements

Sources of funding: This work was supported by a research grant funded by the World Bank under the National Agricultural Innovation Project 'Arsenic in Food Chain: Cause, Effect and Mitigation' from the Indian

Council of Agricultural Research (ICAR), Government of India (ref. no. NAIP/C4/C1005, dated 12/06/2007). *Conflicts of interest:* None declared. *Authors' contributions:* D.D. conducted the diet survey and nutrient intake analysis, and prepared the manuscript. A.B. carried out the laboratory analysis and quantification of arsenic in urine and hair. A.G. performed the epidemiological study, clinical examination and case diagnosis of arsenical skin lesions. A.D. conducted the statistical analysis. K.K.M. performed data evaluation and prepared the manuscript. D.N.G.M. planned and conducted the research, evaluated the data, and corrected and finalized the manuscript.

References

1. World Health Organization (2001) *Arsenic and Arsenic Compounds. Environmental Health Criteria* no. 224. Geneva: WHO.
2. Thornton I & Farago M (1997) The geochemistry of arsenic. In *Arsenic Exposure and Health Effects*, pp. 1–16 [CO Abernathy, RL Calderon and WR Chappell, editors]. London: Chapman & Hall.
3. Alam MGM, Snow ET & Tanaka A (2003) Arsenic and heavy metal contamination of rice, pulses and vegetables grown in Samta village, Bangladesh. In *Arsenic Exposure and Health Effects V*, pp. 103–114 [WR Chappell, CO Abernathy, RL Calderon *et al.*, editors]. London: Elsevier BV.
4. National Research Council (1999) *Arsenic in Drinking Water*. Washington, DC: National Academies Press.
5. World Health Organization (2005) Section 6: Case management. In *A Field Guide for Detection, Management and Surveillance of Arsenicosis Cases*, vol. 30, pp. 19–22 [D Caussy, editor]. New Delhi: WHO Regional Office for South East Asia.
6. Guha Mazumder DN (2003) Criteria for case definition of arsenicosis. In *Arsenic Exposure and Health Effects V*, vol. 9, pp. 117–133 [WR Chappell, CO Abernathy, RL Calderon *et al*., editors]. London: Elsevier BV.
7. Guha Mazumder DN (2001) Clinical aspects of chronic arsenic toxicity. *J Assoc Physicians India* **49**, 650–655.
8. Saha KC (2003) Saha's grading of arsenicosis progression and treatment. In *Arsenic Exposure and Health Effects V*, vol. 30, pp. 391–414 [WR Chappell, CO Abernathy, RL Calderon *et al.*, editors]. London: Elsevier BV.
9. Guha Mazumder DN, Chakraborty AK, Ghose A *et al.* (1988) Chronic arsenic toxicity from drinking tube well water in rural West Bengal. *Bull World Health Organ* **66**, 499–506.
10. Zaldivar R (1978) Arsenic contamination of drinking water and foodstuffs causing endemic chronic poisoning. *Beitr Pathol* **151**, 384–400.
11. Hsueh YM, Cheng GS, Wu MM *et al.* (1995) Multiple risk factors associated with arsenic induced skin cancer: effects of chronic liver disease and malnutritional status. *Br J Cancer* **71**, 109–114.
12. Chen CJ, Kuo TL & Wu MM (1988) Arsenic and cancers. *Lancet* **ii**, 414–415.
13. Chung JS, Haque R, Guha Mazumder DN *et al.* (2006) Blood concentrations of methionine, selenium, β-carotene and other micronutrients in a case control study of arsenic induced skin lesions in West Bengal, India. *Environ Res* **101**, 230–237.
14. Pal A, Chowdhury U, Mandal D *et al.* (2009) Arsenic burden from cooked rice in the population of arsenic affected and non-affected areas and Kolkata city in West Bengal, India. *Environ Sci Technol* **43**, 3349–3355.
15. Maity S & Chatterjee AK (2000) Differential response of cellular antioxidant mechanism of liver and kidney to arsenic exposure and its relation to dietary protein deficiency. *Environ Pharmacol Toxicol* **8**, 227–235.
16. Vahter M & Marafante E (1987) Effects of low dietary intake of methionine, choline or proteins on the biotransformation of arsenite in the rabbit. *Toxicol Lett* **37**, 41–46.
17. Steinmaus C, Carrigan K, Kalman D *et al.* (2005) Dietary intake and arsenic methylation in a US population. *Environ Health Perspect* **113**, 1153–1159.
18. Vahter M (1999) Methylation of inorganic arsenic in different mammalian species and population groups. *Sci Prog* **82**, 69–88.
19. Heck JE, Nieves JW, Chen Y *et al.* (2009) Dietary intake of methionine, cysteine and protein and urinary arsenic excretion in Bangladesh. *Environ Health Perspect* **117**, 99–104.
20. Zablotska LB, Chen Y, Graziano JH *et al.* (2008) Protective effects of B vitamins and antioxidants on the risk of arsenic-related skin lesions in Bangladesh. *Environ Health Perspect* **116**, 1056–1062.
21. Guha Mazumder DN, Ghose A, Majumdar KK *et al.* (2010) Arsenic contamination of ground water and its health impact on population of district of Nadia, West Bengal, India. *Indian J Community Med* **35**, 331–338.
22. Gopaldas T & Seshadri S (editors) (1987) *Nutrition Monitoring and Assessment*. New Delhi: Oxford University Press.
23. Mitra SR, GuhaMazumder DN, Basu A *et al.* (2004) Nutritional factors and susceptibility to arsenic-caused skin lesions in West Bengal, India. *Environ Health Perspect* **112**, 1104–1109.
24. Gopalan C, Rama Sastri BV & Balasubramanian SC (2010) *Nutritive Value of Indian Foods*, pp. 47–98. Hyderabad: National Institute of Nutrition, Indian Council of Medical Research.
25. Bamji MS (1983) Vitamin deficiencies in rice-eating populations: effects of B-vitamin supplements. *Experientia Suppl* **44**, 245–263.
26. Harrington JM, Middaugh JP, Morse DL *et al.* (1978) A survey of a population exposed to high concentrations of arsenic in well water, in Fairbanks, Alaska. *Am J Epidemiol* **108**, 337–385.
27. Yang YH & Blackwell RQ (1961) Nutritional and environmental conditions in the endemic black foot area. *Formos Sci* **15**, 101–129.
28. Heck JE, Gamble MV & Chen Y (2007) Consumption of folate-related nutrients and metabolism of arsenic in Bangladesh. *Am J Clin Nutr* **85**, 1367–1374.
29. Maity S & Chatterjee AK (2001) Effects on levels on glutathione and some related enzymes in tissues after an acute arsenic exposure in rats and their relationship to dietary protein deficiency. *Arch Toxicol* **75**, 531–537.
30. Shivapurkar N & Poirier LA (1983) Tissue levels of S-adenosylmethionine and S-adenosylhomocysteine in rats fed methyl-deficient, amino acid-defined diets for one to five weeks. *Carcinogenesis* **4**, 1051–1057.
31. Kreppel H, Liu J, Liu Y *et al.* (1994) Zinc-induced arsenic tolerance in mice. *Fundam Appl Toxicol* **23**, 32–37.
32. Guha Mazumder DN, Ghoshal UC, Saha J *et al.* (1998) Randomized placebo-controlled trial of 2,3-dimercaptosuccinic acid in therapy of chronic arsenicosis due to drinking arsenic-contaminated subsoil water. *J Toxicol Clin Toxicol* **36**, 683–690.
33. Smith AH, Arroyo AP, Guha Mazumder DN *et al.* (2000) Arsenic-induced skin lesions among Atacameno people in Northern Chile despite good nutrition and centuries of exposure. *Environ Health Perspect* **108**, 617–620.