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Blood concentrations of methionine, selenium, beta-carotene, and other micronutrients in a case–control study of arsenic-induced skin lesions in West Bengal, India ☆

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Abstract

Previous studies have suggested that susceptibility to arsenic toxicity could be influenced by micronutrients, in particular selenium, methionine, and beta-carotene. A case–control study was conducted in West Bengal, India, in a region known to have groundwater arsenic contamination, to determine whether differences in micronutrient status contribute to susceptibility to arsenic-induced skin lesions. Micronutrient status was assessed by blood levels of specific micronutrients and metabolic indicators. Blood was obtained from 180 cases with skin lesions and 192 controls. Blood assays measured micronutrients and carotenoids (folate, selenium, vitamin B12, vitamin B6, retinol, alpha-tocopherol, lutein/zeaxanthin, beta-carotene, lycopene, beta-cryptoxanthin) and metabolic indicators such as glucose, cholesterol, transthyretin, amino acids, and proteins potentially associated with methylation (cysteine, homocysteine, methionine, glutathione). The distributions of nutrient concentrations were similar in cases and controls. The median selenium concentrations in cases and controls were both 1.15 μmol/L, and there was little evidence of differences in other micronutrients. Odds ratios (ORs) for arsenic-induced skin lesions were estimated for each quartile of nutrient concentrations, using the quartile with the highest nutrient level as the referent group. There were no clear trends associated with deficiencies of any micronutrient or metabolic indicator. For decreasing quartiles of selenium, the OR estimates were 1.00, 0.67, 0.99, 0.80; $P = 0.81$; for methionine, the OR estimates were 1.00, 0.83, 0.78, 0.72; $P = 0.29$. For beta-carotene, the ORs were 1.00, 0.53, 0.51, 0.96, demonstrating no increased risk at the lower quartiles. The measured micronutrients and metabolic indicators investigated do not appear to modify the risk of developing arsenic-induced skin lesions. The lack of any trend of increasing risk with lower selenium, vitamin E, and beta-carotene concentrations has important implications for proposed therapeutic interventions. The emphasis of interventions should be on reducing arsenic exposure.

Keywords: Arsenic; Drinking water; Skin lesions; Micronutrients; Selenium; Methionine; Vitamin E; Beta-Carotene

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1. Introduction

Chronic ingestion of arsenic causes the development of skin lesions (keratoses and hyperpigmentation), skin cancer, lung cancer, and cardiovascular disease (National Research Council, 1999). Studies in Taiwan, India, and Argentina, where populations were exposed to arsenic via drinking water, have suggested that malnutrition and poor dietary patterns increase the risk of arsenic-induced diseases (Guha Mazumder et al., 1998; Yang and Blackwell, 1961; Zaldivar et al., 1978; Hsueh et al., 1995; Chen et al., 1988). Some researchers have postulated that deficiencies in specific nutrients, such as beta-carotene, methionine, and zinc, may increase susceptibility to arsenic (Hsueh et al., 1997, 1998a, b; Engel and Receveur, 1993). However, skin lesions (Smith et al., 2000) and arsenic-related mortality (Lewis et al., 1999) have also been found in populations that are considered to have adequate nutrition. Laboratory experiments have demonstrated that specific micronutrients can modify arsenic metabolism and toxicity (Vahter and Marafante, 1987; Styblo and Thomas, 2001). The present study investigates whether nutritional factors increase susceptibility to arsenic-induced skin lesions.

This study examines a population in West Bengal, India that has been exposed to well water contaminated with inorganic arsenic leached from the earth's crust into the groundwater supply (Guha Mazumder et al., 1998; Das et al., 1996; Chowdhury et al., 2000), and food composites containing arsenic (Roychowdhury et al., 2003). Arsenic water concentrations in this region have been measured up to 3500 μg/L (Guha Mazumder et al., 1998), many times higher than the maximum guideline (10 μg/L) recommended by the World Health Organization (World Health Organization, 1993) and US EPA (Environmental Protection Agency, 2001).

A case–control study was undertaken in this Indian region of individuals with arsenic-induced skin lesions and controls. We recently published findings on the dose–response relationship between arsenic in drinking water and skin lesions (Haque et al., 2003), and on the relationship between diet and susceptibility to arsenic-caused skin lesions (Mitra et al., 2004). The micronutrients and biochemical indicators that we report on here are: (1) indicators of recent nutritional status such as proteins and cholesterol (transthyretin, cholesterol, glucose), (2) micronutrients and carotenoids (retinol, alpha-tocopherol, beta-carotene, beta-cryptoxanthin, lutein/zeaxanthin, lycopene, vitamin B6, vitamin B12), (3) cofactors of arsenic methylation or one-carbon metabolism (cysteine, folate, glutathione, homocysteine, methionine, vitamin B6, vitamin B12), and (4) potential antagonists to arsenic (selenium). We present the blood concentrations of specific micronutrients and biochemical indicators and evaluate their roles in the susceptibility to developing skin lesions. In future papers we will present findings integrating the nutritional and micronutrient data.

2. Materials and methods

2.1. Study design

Study area and description. This study was conducted in West Bengal, India in the rural areas of South 24-Parganas, where high levels of naturally occurring arsenic have been found in groundwater (Das et al., 1994). Because agricultural irrigation in West Bengal is dependent on groundwater, many foods produced and consumed in this region—such as vegetables, cereals, baked goods, and spices—also have elevated concentrations of arsenic (Roychowdhury et al., 2003). In 1995–1996, a cross-sectional survey of 7683 residents was conducted to determine the prevalence of arsenic-induced skin lesions (Guha Mazumder et al., 1998). The present study is a case–control study, nested in this larger cross-sectional survey, to evaluate the blood concentrations of micronutrients and biochemical indicators in cases with arsenic-induced skin lesions and controls without skin lesions. Cases and controls were interviewed and blood samples obtained between 1998 and 2000. The project was reviewed and approved by the institutional review boards of the University of California, Berkeley and the Institute of Post Graduate Medical Education and Research, Calcutta.

The case–control study base included participants from the cross-sectional survey with measured arsenic well water concentrations below 500 μg/L. Cases were randomly selected from this study base, and the highest known mean tube well concentration among cases was 325 μg/L (standard deviation 183 μg/L). Cases were defined as having a positive skin lesion classification, with either hyperpigmentation (mottled dark brown pigmentation bilaterally distributed on the trunk) or keratoses (diffuse bilateral thickening of palms or soles, with or without nodules) at the time of the survey. Controls were defined as not having skin lesions, were randomly selected from the study base, and were matched to the case by sex and age within 4 years. The highest known mean tube well concentration among controls was 180 μg/L (standard deviation 159 μg/L). A more detailed description of the study population and recruitment is presented elsewhere (Haque et al., 2003). There were no nutritional intervention programs introduced in the study area.

Data and sample collection. Field physicians interviewed participants using a structured questionnaire, conducted a general medical examination, and obtained blood samples the same day participants were located. Informed consent was obtained from all participants. Nonfasting blood samples were obtained on 180 cases and 196 controls. Blood samples were stored in a covered ice chest (0 °C) in the field to prevent degradation of light- and temperature-sensitive compounds. Approximately half (58%) of the blood samples were centrifuged and aliquotted into serum and plasma samples that day, while the rest were refrigerated overnight (4 °C) and aliquotted the next day. Aliquots were kept frozen at −20 °C in India, delivered on dry ice, and stored at −70 °C in the US, until analysis.

2.2. Laboratory methods

Pacific Biometrics, Inc. (Seattle, WA) conducted serum and plasma analyses for the micronutrients and biochemical indicators unless otherwise noted. Assays were conducted blind to the case or control status of each sample. Methods used for each nutrient are briefly presented. Plasma indicators measured include homocysteine, glutathione, cysteine, methionine, and vitamin B6. Plasma levels of retinol (vitamin A), alpha-tocopherol (vitamin E), alpha-carotene, beta-carotene, lycopene, lutein/zeaxanthin, and beta-cryptoxanthin were also measured. Plasma thiols (homocysteine, glutathione, and cysteine) were measured by high-performance liquid chromatography (HPLC) using an internal standard and monobromobimane derivatization (Fiskerstrand et al., 1993). Plasma methionine was measured by amino acid analyzer (Beckman 6300 Amino Acid Analyzer) using a cation–ion exchange column at the Scientific Research Consortium, Inc. (St. Paul, MN). Vitamin B6 (pyridoxal phosphate) was determined by HPLC and fluorescence detection involving precolumn derivatization of plasma vitamers with sodium bisulfite (Kimura et al., 1996). Plasma retinol, alpha-tocopherol, beta-carotene,

lycopene, lutein/zeaxanthin, and beta-cryptoxanthin were analyzed by isocratic reverse-phase HPLC after addition of internal standards and total lipid extraction.

Serum measurements include glucose, cholesterol, vitamin B12, folate, transthyretin, and selenium. Glucose was assayed using an automated version of the Barthelmai and Czok glucose assay (i.e., the hexokinase/glucose-6-phosphate dehydrogenase). Total serum cholesterol was quantified with the CDC-standardized Trinder end-point reaction in an automated chemistry analyzer. Levels of vitamin B12 and folate were analyzed by CEDIA assays (Henderson et al., 1986) on the Hitachi 911 using Roche reagents. Transthyretin levels were determined by immunoprecipitin analysis. Sample transthyretin concentrations were calculated from a standard curve established for each assay batch. Selenium was measured by graphite furnace atomic absorption spectrophotometry at the Nutrition Research Laboratory in the Department of Laboratory Medicine at the University of Washington or by inductively coupled plasma mass spectometry at the Associated Regional and University Pathologists trace mineral laboratory at the University of Utah Health Science Center (Salt Lake City, UT).

Table 1
Characteristics of study participants

	Cases	Controls
n	180	196
Male (%)	112 (62%)	127 (65%)
Female (%)	68 (38%)	69 (35%)
Age distribution (%)		
<24 years	27 (15%)	37 (19%)
25–34 years	28 (16%)	37 (19%)
35–44 years	47 (26%)	41 (21%)
45–54 years	29 (16%)	32 (16%)
55–64 years	25 (14%)	30 (15%)
⩾65 years	24 (13%)	19 (10%)
BMI (kg/m^2)[a]	18.8 (3.5)	18.9 (3.1)
Weight (kg)[a]	46.3 (11.1)	46.4 (10.1)

[a]Mean (standard deviation).

In the blood measurement data, there were five samples that had nondetectable values of a particular nutrient (three for lycopene and two for folate). These samples were considered missing and not included in calculations.

2.3. Data analysis

Case and control demographic information was compared and Student *t* tests were conducted for body mass index (BMI) and weight between the groups. The median concentrations and 10th, 90th percentile values for the blood nutrient measurements were determined for cases and controls, and Wilcoxon rank sum tests were used to evaluate differences in the blood nutrients distribution.

The participants were divided into quartiles based on distribution of the blood nutrients in the control group. The odds ratios (ORs) and 95% confidence intervals (CI) for arsenic-induced skin lesions were estimated for quartiles of each blood nutrient using unconditional logistic regression. Using the highest quartile of the blood nutrient as a reference group, we calculated ORs for each descending quartile category. Unconditional logistic regression was used to include the unpaired observations in analysis, since blood samples were not obtained from all case–control pairs. An indicator variable for sex and a variable indicating the age group (<24 years, 25–34, 35–44, 45–54, 55–64, ⩾65) were also added to the regression model. A score test for trend was used to evaluate whether there was variation across the quartiles for each blood nutrient.

The analyses were repeated with an indicator variable to account for differences that may have resulted from instability and degradation in the blood samples stored overnight. The inclusion of this variable did not affect the results; therefore, the unadjusted results are presented. The analyses were also conducted using decile levels of nutrients. Since there were no striking differences, only the quartile level information is presented.

Data analyses were performed using the statistical package STATA (StataCorp, 1995). All reported *P* values are two-tailed, and confidence intervals were calculated at the 95% level.

3. Results

Information on the study participants ($n = 376$) collected from the interview is presented in Table 1. Sex, age,

Table 2
Median concentrations (10th, 90th percentile values) of blood micronutrients and biochemical indicators for cases and controls

Micronutrients and biochemical indicators	Cases ($n = 180$) Median (10th, 90th)	Controls ($n = 196$) Median (10th, 90th)	*P*-value[a]	Reference ranges
Alpha-tocopherol (μg/dl) (plasma)	597 (434, 873)	596 (406, 911)	0.86	500–2650[b] (Gunter et al., 1996)
Beta-carotene (μg/dl) (plasma)	56 (18, 229)	51 (21, 132)	0.39	2–80[b] (Gunter et al., 1996)
Beta-cryptoxanthin (μg/dl) (plasma)	3.9 (1.8, 14.0)	4.0 (1.6, 11.1)	0.71	2–40[b] (Gunter et al., 1996)
Cholesterol (mg/dl) (serum)	154 (111, 198)	150 (114, 208)	0.92	140–300[b] (Gunter et al., 1996)
Cysteine (μmol/L) (plasma)	214 (174, 260)	212 (166, 261)	0.61	202–338 (El-Khairy et al., 1999)
Folate (ng/mL) (serum)	2.9 (1.3, 5.6)	2.6 (1.5, 5.8)	0.19	2.6–12.2[b] (Gunter et al., 1996)
Glucose (mg/dl)(serum)	82 (53, 130)	81 (55, 113)	0.18	64–112[b] (Gunter et al., 1996)
Glutathione (total) (μmol/L) (plasma)	8.3 (3.8, 15.8)	6.9 (3.5, 15.3)	0.23	0.3–9.4[b] (Flagg et al., 1993)
Homocysteine (μmol/L) (plasma)	12.7 (8.1, 21.4)	13.0 (8.6, 23.8)	0.45	5.5–15.0[b] (Gunter et al., 1996)
Lutein/Zeaxanthin (μg/dl) (plasma)	61 (34, 102)	62 (32, 104)	0.75	5–65[b] (Gunter et al., 1996)
Lycopene (μg/dl) (plasma)	2.00 (0.54, 6.92)	1.80 (0.45, 7.90)	0.87	3–55[b] (Gunter et al., 1996)
Methionine (μmol/L) (plasma)	19.2 (12.9, 26.4)	18.8 (12.4, 27.0)	0.58	6–40 (Tietz, 1995)
Retinol (μg/dl) (plasma)	32.8 (18.7, 48.5)	34.0 (22.2, 48.5)	0.24	25–115[b] (Gunter et al., 1996)
Selenium (μmol/L) (serum)	1.15 (0.69, 1.74)	1.15 (0.66, 1.75)	0.53	1.2–1.9[b] (Gunter et al., 1996)
Transthyretin (mg/L) (plasma)	235 (162, 307)	240 (173, 306)	0.34	100–400 (Tietz, 1995)
Vitamin B12 (pg/mL) (serum)	376 (218, 712)	385 (217, 676)	0.93	165–1600[b] (Gunter et al., 1996)
Vitamin B6 (nmol/L) (plasma)	34.3 (20.0, 51.5)	35.2 (21.8, 58.3)	0.09	20–121 (Tietz, 1995)

[a]*P*-values of Wilcoxon rank sum test.
[b]Ranges found in studies conducted in US populations.

and smoking status distributions, in addition to mean BMI and weight, were similar in cases and controls.

The median (and 10th, 90th percentile) concentrations for each blood nutrient are presented separately for cases and controls in Table 2. The selenium medians for cases and controls were both 1.15 μmol/L. The medians for methionine cases and controls were similar, at 19.2 and 18.8 μmol/L, respectively. There were also no notable

Table 3
Adjusted[a] odds ratios and 95% confidence intervals (CI) for arsenic-induced skin lesions according to quartile of blood micronutrients and median values for each quartile

Micronutrients and biochemical indicators	Quartile	Medians	Odds ratio (95% CI)	*P* value test for trend
Alpha-tocopherol (mg/dl)	1(high)	876	1.00 (reference)	
	2	657	1.35 (0.75, 2.42)	
	3	544	1.35 (0.75, 2.42)	
	4 (low)	433	1.04 (0.56, 1.93)	
				0.82
Beta-carotene (μg/dl)	1(high)	133	1.00 (reference)	
	2	67	0.53 (0.29, 0.97)	
	3	41	0.51 (0.28, 0.93)	
	4 (low)	24	0.96 (0.54, 1.71)	
				0.89
Beta-cryptoxanthin (μg/dl)	1(high)	10.9	1.00 (reference)	
	2	5.8	1.08 (0.57, 2.04)	
	3	3.4	0.92 (0.52, 1.62)	
	4 (low)	2.0	1.23 (0.69, 2.20)	
				0.56
Cholesterol (mg/dl)	1(high)	198	1.00 (reference)	
	2	164	1.15 (0.65, 2.05)	
	3	141	0.83 (0.44, 1.54)	
	4 (low)	116	1.18 (0.65, 2.14)	
				0.76
Cysteine (μmol/L)	1(high)	257	1.00 (reference)	
	2	224	1.10 (0.62, 1.95)	
	3	202	0.94 (0.52, 1.70)	
	4 (low)	172	1.04 (0.56, 1.93)	
				0.96
Folate (ng/mL)	1(high)	4.9	1.00 (reference)	
	2	3.1	0.94 (0.53, 1.65)	
	3	2.4	0.71 (0.39, 1.29)	
	4 (low)	1.5	0.70 (0.39, 1.26)	
				0.14
Glucose (mg/dl)	1(high)	113	1.00 (reference)	
	2	86	0.60 (0.33, 1.10)	
	3	74	0.73 (0.42, 1.26)	
	4 (low)	56	0.75 (0.43, 1.34)	
				0.40
Glutathione (total) (μmol/L)	1(high)	14.6	1.00 (reference)	
	2	8.9	2.35 (1.33, 4.15)	
	3	5.8	0.88 (0.46, 1.65)	
	4 (low)	3.7	0.90 (0.48, 1.69)	
				0.13
Homocysteine (μmol/L)	1(high)	20.8	1.00 (reference)	
	2	14.7	1.09 (0.60, 1.98)	
	3	11.9	0.96 (0.52, 1.76)	
	4 (low)	9.1	1.30 (0.71, 2.38)	
				0.45
Lutein/zeaxanthin (μg/dl)	1(high)	99	1.00 (reference)	
	2	71	1.48 (0.81, 2.69)	
	3	55	1.51 (0.84, 2.72)	
	4 (low)	36	1.27 (0.69, 2.32)	
				0.39
Lycopene (μg/dl)	1(high)	7.5	1.00 (reference)	
	2	2.6	1.70 (0.95, 3.06)	
	3	1.4	1.17 (0.63, 2.17)	
	4 (low)	0.6	1.20 (0.65, 2.21)	
				0.97

Table 3 (*continued*)

Micronutrients and biochemical indicators	Quartile	Medians	Odds ratio (95% CI)	*P* value test for trend
Methionine (μmol/L)	1(high)	25.8	1.00 (reference)	
	2	20.4	0.83 (0.46, 1.48)	
	3	17.4	0.78 (0.44, 1.41)	
	4 (low)	13.4	0.72 (0.40, 1.28)	
				0.29
Retinol (μg/dl)	1(high)	47.1	1.00 (reference)	
	2	37.8	1.20 (0.66, 2.18)	
	3	31.5	1.10 (0.60, 2.00)	
	4 (low)	23.2	1.54 (0.87, 2.74)	
				0.12
Selenium (μmol/L)	1(high)	1.65	1.00 (reference)	
	2	1.28	0.67 (0.37, 1.20)	
	3	1.00	0.99 (0.57, 1.72)	
	4 (low)	0.69	0.80 (0.45, 1.41)	
				0.81
Transthyretin (mg/L)	1(high)	300	1.00 (reference)	
	2	254	0.88 (0.49, 1.59)	
	3	225	0.80 (0.44, 1.45)	
	4 (low)	178	1.20 (0.68, 2.10)	
				0.42
Vitamin B12 (pg/mL)	1(high)	632	1.00 (reference)	
	2	441	0.87 (0.48, 1.57)	
	3	328	1.03 (0.58, 1.84)	
	4 (low)	231	0.99 (0.56, 1.76)	
				0.89
Vitamin B6 (nmol/L)	1(high)	57.5	1.00 (reference)	
	2	40.6	2.20 (1.17, 4.13)	
	3	31.4	1.62 (0.85, 3.10)	
	4 (low)	22.3	1.79 (0.95, 3.36)	
				0.18

[a]Multivariate odds ratios adjusted for sex and age.

differences in other blood nutrient medians. Ranges found in population surveys and clinical/laboratory reference values are presented with corresponding source information. In both cases and controls, the median values were near or below the lower end of the reference ranges for folate, lycopene, and selenium. For lutein/zeaxanthin, nearly 50% of the participants had values above the upper end of the reference range.

The odds ratios for arsenic-induced skin lesions according to descending quartiles for each micronutrient and biochemical indicator are presented in Table 3. There were no clear trends across quartiles for any of the blood nutrients. For decreasing quartiles of selenium, the OR estimates were 1.00, 0.67, 0.99, 0.80; $P = 0.81$, and for methionine, the OR estimates were 1.00, 0.83, 0.78, 0.72; $P = 0.29$. The lower quartiles had higher ORs for skin lesions than the highest quartile for vitamin B6 (OR = 1.00, 2.20, 1.62, 1.79, from highest to lowest quartile, respectively), lycopene (OR = 1.00, 1.70, 1.17, 1.20), lutein/zeaxanthin (OR = 1.00, 1.48, 1.51, 1.27), and retinol (OR = 1.00, 1.20, 1.10, 1.54), but the trends were not significant. In contrast to the other carotenoids, the risks of developing skin lesions in the 2nd and 3rd quartiles of beta-carotene were lower (OR = 0.53, 95%CI 0.29, 0.97; OR = 0.51, 95%CI 0.28, 0.93; respectively) than the reference group. There was no evidence of increased risks with low levels of selenium or methionine.

4. Discussion

It has been postulated that poor nutrition causes susceptibility to health effects from chronic arsenic ingestion via drinking water (Yang and Blackwell, 1961; Zaldivar et al., 1978; Chen et al., 1988). We present the results of a case–control study that did not find evidence of blood micronutrients being related to arsenic-induced skin lesions.

Undernourishment was previously found to increase the risk of skin lesions (Guha Mazumder et al., 1998) and skin cancer (Hsueh et al., 1995) in arsenic-exposed populations. We found no relationship between skin lesions and blood levels of transthyretin, a sensitive indicator of protein-depletion status (Ingenbleek and Young, 1994). A Taiwanese study on arsenic-related ischemic heart disease (ISHD) did not demonstrate an association with cholesterol (Hsueh et al., 1998a). This present study also did not find cholesterol levels to be related to skin lesions. It is noteworthy that almost 90% (331 of 376) of the participants had blood cholesterol levels less than 200 mg/dL. We also evaluated glucose levels

in both the cases and the controls and found no association with skin lesions. Glucosuria and diabetes have been found to be associated with arsenic exposure in some studies but were unrelated to skin lesions (Rahman et al., 1998, 1999).

Laboratory studies have shown that methionine and selenium affect the metabolism and toxicity of arsenic (Vahter and Marafante, 1987; Styblo and Thomas, 2001; Maiti and Chatterjee, 2000), and it has been suggested that selenium deficiencies should be taken into account when determining risks from arsenic toxicity (National Research Council, 1999; Kenyon et al., 1997, 2001). In laboratory studies, selenium and arsenic at high doses clearly influence the toxicology of the other, but there is evidence that this occurs in both directions (Levander, 1977; Kraus and Ganther, 1989): while some experiments with arsenic and selenium demonstrated antagonism, with reduced cytotoxic effects (Biswas et al., 1999) and increased excretion of arsenic (Hilmy et al., 1991), others have found synergistic effects such as increased arsenic retention (Styblo and Thomas, 2001), impaired methylation (Kenyon et al., 1997), and higher morbidity and mortality in rats (Kraus and Ganther, 1989). In human populations also, it is unclear how arsenic and selenium interact. One study found blood selenium lower in Blackfoot disease patients (a peripheral vascular disease found in Taiwan, induced specifically by arsenic) than in controls (Lin and Yang, 1988), whereas another study found higher amounts of selenium in the hair of patients than in controls (Pan et al., 1993). A recent study found greater reversal of arsenic-induced physiological effects in patients taking selenium supplements while simultaneously drinking arsenic-free water than those only drinking arsenic-free water (Wang et al., 2001). Through the same supplementation trial, the authors found significantly more improvements in skin lesions of those given selenium supplementation. The subjects were given supplements or placebos according to their resident village; the authors note that theirs was "not a randomized, controlled, double blind trial" and that it involved a relatively small number of people (Yang et al., 2002). Our findings indicate that the blood selenium and methionine levels were similar in cases and controls. There was no increased risk of skin lesions with lower selenium or methionine concentrations. It should also be noted that selenium may actually increase the risk of nonmelanoma skin cancers (Duffield-Lillico et al., 2003) rather than prevent such cancers which are known to be caused by arsenic exposure. This is added reason for caution in considering selenium as a therapeutic agent for persons with arsenic skin lesions.

Several nutrients with antioxidant function and roles in disease prevention were evaluated. Recent investigations in Taiwan have found that arsenic can increase reactive oxidant species (Pi et al., 2002; Wang and Huang, 1994) and decrease antioxidant capacity in blood (Wu et al., 2001), lending support to oxidative stress as a potential mechanism of arsenic toxicity (Nordenson and Beckman, 1991). We found that the ORs in the lowest quartiles of lycopene, lutein/zeaxanthin, alpha-tocopherol, and retinol were greater than 1, but the confidence intervals for all risk estimates included unity. Our results show that the ORs of the 2nd and 3rd quartiles of beta-carotene were 0.53 and 0.51, respectively. This is in contrast to a Taiwanese study that determined that low serum beta-carotene levels were associated with increased risk of arsenic-induced skin cancer (Hsueh et al., 1997) and ISHD (Hsueh et al., 1998a); however, those researchers did not show an association between lycopene and ISHD (Hsueh et al., 1998a). In the present study, no associations were found between skin lesions and other antioxidants, carotenoids, or vitamin E.

Glutathione is critical to arsenic metabolism, specifically the initial reduction of As^{+5} to As^{+3} required for methylation (Thompson, 1993; Scott et al., 1993; Buchet and Lauwerys, 1987, 1994; Zakharyan et al., 1999). Although the median value for glutathione was higher in cases than in controls (8.3 vs. 6.9 μmol/L), there was no statistical evidence that the distributions differed ($P = 0.23$) between the two groups. It should be noted that the glutathione concentrations in this population were high relative to the reference range. Glutathione levels in India have not been previously reported and whether dietary or genetic differences contribute to the high levels found in this study population is unknown. These results suggest that further studies should examine the role of glutathione in arsenic-exposed populations.

Few studies have examined the role of vitamin B6 in arsenic effects. We found no evidence of a linear dose–response relationship between vitamin B6 and skin lesion status; however, ORs were elevated in the lower concentrations of vitamin B6 (OR = 2.20, 1.62, 1.79 for decreasing quartiles). A study in rats demonstrated an interaction between dietary vitamin B6 and arsenic, possibly through global one-carbon metabolism (Uthus and Poellot, 1991). Lack of vitamin B6 may be associated with dermatitis and development of lesions in animals (Berdanier, 1998).

Clearly this population suffers from both the effects of drinking water and food contaminated with high arsenic levels and the prevalence of undernourishment. The mean BMI (19 kg/m^2) for the entire study population indicated overall undernourishment. Also, the median blood levels of several nutrients fell near the low ends of the reference ranges. The folate concentrations in this population were low: 18 cases and 15 controls had folate levels below 1.4 ng/mL, indicative of inadequate folate intake.

Dietary selenium is found in significant amounts only in animal protein and then only when the animals themselves obtain enough selenium in their diets. The regional diet was expected to be low in selenium, not only because animal protein is infrequently eaten but also because the soil in the study area is very low in selenium, leading to low selenium in crops and therefore low selenium in animals (Spallholz et al., 2004). Although the median selenium concentration (1.15 μmol/L) in this study fell below ranges in the United States, the worldwide selenium concentrations in healthy

adults range from 0.52 to 2.50 μmol/L (Alfthan and Neve, 1996), and more than 90% of the study population fell within this range.

Since the latency from first arsenic exposure to the development of skin lesions is usually longer than 10 years (Haque et al., 2003; Cebrian et al., 1983), we realize the limitations of using a single cross-sectional blood measurement to assess past nutritional status during the period of skin lesion development. In this particular population, we can assume relative constancy in the rank order of the individuals over time: South 24-Parganas is a predominantly rural area, and the diet and portion sizes remain unchanged over time for much of the population. Therefore, those with high blood levels will tend to maintain their high rank over time, and those with low concentrations will maintain a low rank order.

One potential source of bias was the possibility that nutritional status was diminished in those with skin lesions because of debilitation or loss of livelihood. If that was the case, those with skin lesions would have had lower concentrations of micronutrients. These data do not support this hypothesis, since the group-level concentrations were similar in cases and controls. Furthermore, if this bias was present, its direction would have resulted in spurious data suggesting increased risk of skin lesions with lower levels of nutrients rather than resulting in null associations and masking true effects. In our data, we found no strong associations with lower levels of micronutrients, suggesting the lack of this bias.

With regard to seasonal variations in diet, the participants' interviews and blood draws were conducted randomly during the study period, independent of case and control status, and should not bias these findings. In the present study, 45% of the cases and controls were sampled during the autumn and winter months.

Socioeconomic and educational confounding factors were considered, but we have previously reported that the cases and controls in this study do not differ in housing status or education level (Mitra et al., 2004).

In conclusion, we found little difference in the distributions of micronutrients and biochemical indicators measured in cases with arsenic-induced skin lesions and controls without skin lesions. There were suggestions that further research could entail examining the roles of glutathione, vitamin B6, and beta-carotene. However, there were no clear findings that the blood concentrations of micronutrients and biochemical indicators commonly thought to be associated with arsenic toxicity—such as methionine and selenium—affect susceptibility to developing arsenic-induced skin lesions, nor was there any evidence of increasing risk with low concentrations of vitamin E and beta-carotene, both of which have been proposed for therapy. Based on existing knowledge, it does not seem that these factors modify the risks of developing arsenic-caused skin lesions. The emphasis of interventions in arsenic-exposed populations should be on reductions in arsenic exposure.

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