

# A Prospective Study of Blood Selenium Levels and the Risk of Arsenic-Related Premalignant Skin Lesions

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## Abstract

Arsenic exposure from drinking water is considered to be a risk factor for skin and internal cancers. Animal studies suggest a potential antagonism between arsenic and selenium in the body. We did a case-cohort analysis to prospectively evaluate the association between arsenic-related premalignant skin lesions and prediagnostic blood selenium levels in 303 cases of skin lesions newly diagnosed from November 2002 to April 2004 and 849 subcohort members randomly selected from the 8,092 participants in the Health Effects of Arsenic Longitudinal Study with available baseline blood and urine samples collected in 2000. Incidence rate ratios for skin lesions in increasing blood selenium quintiles were 1.00 (reference), 0.68 [95% confidence interval (95% CI), 0.39-1.18],

0.51 (95% CI, 0.29-0.87), 0.52 (95% CI, 0.30-0.91), and 0.53 (95% CI, 0.31-0.90). Effect estimates remained similar with adjustments for age, sex, body mass index, smoking status, excessive sunlight exposure (in men), well water arsenic concentration at baseline, and nutritional intakes of folate, iron, protein, vitamin E, and B vitamins. At any given arsenic exposure level, the risk of premalignant skin lesions was consistently greater among participants with blood selenium lower than the average level. The findings support the hypothesis that dietary selenium intake may reduce the incidence of arsenic-related premalignant skin lesions among populations exposed to arsenic exposure from drinking water. (Cancer Epidemiol Biomarkers Prev 2007;16(2):207-13)

## Introduction

The presence of inorganic arsenic in groundwater has been recognized as a public health hazard in many countries. The IARC has classified arsenic as a group 1 human carcinogen. Epidemiologic studies have documented associations between arsenic exposure from drinking water and elevated risks of premalignant skin lesions, skin and internal cancers, and cardiovascular diseases (1-3). In Bangladesh, more than 50 million people have been chronically exposed to drinking groundwater with arsenic concentrations exceeding the WHO standard (10 µg/L; ref. 4). We have estimated the cancer burden to be doubling in Bangladesh (5). Clearly, arsenic mitigation and cancer preventive programs are urgently needed to reduce arsenic toxicity in the population.

Cutaneous abnormalities are well-known early signs of chronic inorganic arsenic poisoning. Melanosis is considered as early-stage skin lesions. Keratosis is the most frequent manifestation preceding the appearance of arsenic-related skin cancer (6). Unlike arsenic-related internal cancers that could have long latencies, these premalignant skin lesions may appear with shorter periods of arsenic exposure (7). They cause the majority of arsenic-induced basal and squamous cell skin cancers (6, 8, 9). In 428 cases of skin cancer in an arsenic-exposed population in Taiwan, 90% were associated with hyperpigmentation and 72% were associated with keratosis (6). In other historical case series, 81% to 100% of arsenic-related skin cancer cases were related to keratosis (10, 11).

It has been hypothesized that susceptibility to arsenic toxicity differs by dietary selenium intake levels (12, 13). Selenium is an essential human dietary trace element required for synthesis of a variety of selenium-containing proteins, some of which are selenoproteins that incorporate selenium in the form of the amino acid selenocysteine during translation (14). Selenoproteins and their metabolites are critical in maintaining antioxidant/anti-inflammatory homeostasis. In experimental studies, arsenic exposure has been associated with a greater production of free radicals and increased oxidative stress (15) that may be reduced by selenoproteins. Additionally, animal studies have shown an interaction between selenium and arsenic, such that uptake of one of these elements causes release, redistribution, or elimination of the other element by urinary and/or biliary routes (16, 17). However, findings from epidemiologic studies about the protective effect of selenium intake on risks of arsenic-related diseases, such as premalignant skin lesions and blackfoot disease (a unique peripheral vascular disease in lower extremities related to high levels of arsenic exposure), in populations exposed to arsenic exposure have been inconclusive (13, 18-21). Limitations of these studies include small sample sizes, unavailability of prediagnostic selenium levels (in observational studies), and methodologic shortcomings, such as the lack of blindness in randomization (in intervention studies).

We conducted a case-cohort study nested in the Health Effects of Arsenic Longitudinal Study to prospectively assess the association between prediagnostic levels of selenium in whole blood and the subsequent risk of premalignant skin lesions. We also evaluated whether the relationship between long-term arsenic exposure from drinking water and risk of skin lesions is modifiable by blood selenium levels.

## Materials and Methods

**The Health Effects of Arsenic Longitudinal Study.** The parent study Health Effects of Arsenic Longitudinal Study is an ongoing prospective cohort study in Araihazar, Bangladesh.

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Details of the study methodologies have been presented elsewhere (22, 23). Briefly, before subject recruitment, water samples and geographic positional system data were collected for 5,966 contiguous wells in a well-defined geographic area of 25 square km in Araihasar. Between October 2000 and May 2002, 11,746 men and women ages 18 years and above were recruited, with a participation rate of 97.5% (22). The cohort is being followed with in-person visits at 2-year intervals. Verbal consent was obtained from study participants. The study procedures were approved by the Columbia University Institutional Review Board and the Ethical Committee of the Bangladesh Medical Research Council.

At baseline recruitment, venous whole-blood samples were collected in 3 mL Vacutainers containing EDTA as anticoagulant for 91.8% of the overall 11,746 cohort participants. At baseline and the follow-up visits, a spot urine sample was collected in 50-mL acid-washed tubes for 95.6% and 94.5% of the cohort participants, respectively. Both blood and urine samples were kept in portable coolers immediately after collection. Within 2 to 8 h, blood and urine samples were processed and transferred to  $-20^{\circ}\text{C}$  freezers in the study office located in Dhaka city. All samples were kept frozen and shipped to Columbia University (New York, NY) on dry ice within 1 to 2 months.

Trained physicians completed a comprehensive physical examination at baseline and follow-up visits. Details of the clinical examination protocol for premalignant skin lesion diagnosis were described previously (22). We instituted a structured protocol adapting the method for quantitative assessment of the extent of body surface involvement in burn patients. The principle is based on dividing the entire body skin surface into 11 segments and assigning percentages to each of them based on their size relative to the whole body surface. This method requires a physician to record presence/absence, type, size, and shape of skin lesions and extent of skin involvement. Physicians were blind to information on the arsenic level in participants' drinking wells. In the present study, presence of premalignant skin lesions was defined as existence of any melanosis and/or keratosis.

**Selection of Cases and Subcohort.** A case-cohort study design (24) was used to evaluate the relationship between blood selenium level and risk of skin lesions. The case-cohort study design has been used to analyze cohort data efficiently when most observations are censored (nondiseased; 24). It provides the advantages of a cohort study in that it allows the direct calculation of a rate ratio (RR) without the collection and analysis of full information on every member of the cohort. A random sample of the cohort, or "subcohort," is designated as the comparison group for the newly diagnosed cases of skin lesion observed in the overall cohort.

Among the 9,727 participants who gave both urine and blood samples and completed the physical examination at baseline, 712 were prevalent cases of skin lesions. They were excluded from the current analysis. Additionally excluded from the study were 923 randomly selected subjects whose blood samples were consumed previously in a study of genetic susceptibility. The present analysis included a 10.5% random sample of the remaining 8,092 participants ( $n = 849$ ) and 303 cases of newly diagnosed skin lesions. The 303 cases of skin lesions were diagnosed at the first 2-year follow-up from the 8,092 participants between November 2002 and April 2004; 221 of the cases had only melanosis, whereas the remaining 82 had both hyperkeratosis and melanosis. Among the 303 newly diagnosed cases, 31 were also part of the 849 subcohort members.

**Measurements of Arsenic Exposure.** At baseline, water samples from all 5,966 tube wells in the study area were collected in 50-mL acid-washed tubes following well pumping for 5 min (25, 26). Total arsenic concentration was determined

by graphite furnace atomic-absorption spectrometry with a Hitachi (Tokyo, Japan) Z-8200 system at the Lamont-Doherty Earth Observatory of Columbia University (25). Samples that fell below the detection limit of graphite furnace atomic-absorption spectrometry ( $5\ \mu\text{g/L}$ ) were subsequently analyzed by inductively coupled plasma mass spectrometry, with a detection limit of  $0.1\ \mu\text{g/L}$  (27). Analyses for time-series samples collected from 20 tube wells in the study area showed that the arsenic concentration in well water is relatively stable over time (27). Therefore, we derived a time-weighted arsenic (TWA) concentration as a function of drinking durations and well arsenic concentrations (28, 29). The TWA represents the average arsenic exposure that accrued for 9 years on average in the cohort members before the time of baseline visits.

Total urinary arsenic concentration in urine samples collected at both baseline and follow-up visits was measured by graphite furnace atomic-absorption spectrometry, using a Perkin-Elmer (Wellesley, Massachusetts) Analyst 600 graphite furnace system as described previously (30). Urinary creatinine was analyzed using a method based on the Jaffe reaction for adjustment of urinary total arsenic concentration (31).

#### **Measurements of Selenium and Arsenic in Whole Blood.**

Whole-blood samples collected at baseline were analyzed for blood selenium and arsenic concentrations using a Perkin-Elmer Elan DRC II ICP-MS equipped with an AS 93+ autosampler. Inductively coupled plasma mass spectrometry-dynamic reaction cell methods for metals in whole blood were developed (with modifications) based on published methods (32). Whole-blood samples were thawed, thoroughly mixed, diluted 50 times with diluent containing 1%  $\text{HNO}_3$  + 0.2% Triton X-100 + 0.5%  $\text{NH}_4\text{OH}$ , and centrifuged for 10 min at 3500 rpm with the supernatant reserved for analysis. A multielement standard solution was used for instrument calibration, with selenium and arsenic concentrations chosen to cover the expected ranges of analyte in the blood samples. We used iridium to correct matrix-induced interferences. A stock internal standard spiking solution was added to all calibrators and samples in the same concentration, 10 ng iridium per tube. Polyatomic interferences were suppressed with the dynamic reaction cell technology feature of the instrument, using oxygen as a second gas. Interclass correlation coefficient between the expected and observed concentrations in quality control samples (blood samples with known analyte concentrations obtained from the laboratory for Inductively Coupled Plasma Mass Spectrometry Comparison Program in Quebec), was 0.99 and 0.90 for blood selenium and arsenic, respectively.

**Measurements of Dietary Intakes.** Dietary intakes were measured at baseline with a validated semiquantitative food frequency questionnaire (FFQ) designed for the study population. Detailed information on the design and the validation of the FFQ has been published elsewhere (33). Briefly, to assess the validity of the FFQ, two 7-day food diaries were completed in two separate seasons by trained interviewers for 189 of the 200 participants randomly selected from the overall Health Effects of Arsenic Longitudinal Study population. Correlations for macronutrients and common micronutrients, including total fat, monounsaturated fat, polyunsaturated fat, saturated fat, protein, carbohydrate, dietary fiber, sodium, potassium, vitamin B6, vitamin B12, riboflavin, manganese, thiamin, and iron, ranged from 0.30 to 0.76 (33). We used both the United States Department of Agriculture Nutrient Database for standard reference (abbreviated version; 34) and an Indian food nutrient database (35) to convert food intakes to nutrient intake values (33).

**Statistical Analysis.** Incidence RRs for skin lesions were estimated using Cox proportional hazards models with the PROC PHREG procedure in SAS. SEs were estimated using the

**Table 1. Characteristics of the 849 subcohort members and 303 newly diagnosed skin lesion cases in the Health Effects of Arsenic Longitudinal Study cohort**

Characteristic*	Skin lesion cases	Subcohort	Quintile of blood Se levels in the subcohort					<i>P</i> <sub>trend</sub>
			Q1	Q2	Q3	Q4	Q5	
No. participants	303	849	170	173	167	171	168	
Range of blood Se levels, µg/L	88.5-258.8	69.8-262.6	69.8-132.4	132.3-145.0	145.1-156.6	156.7-169.8	169.9-262.6	
Mean blood Se, µg/L	150.1	152.3	120.9	139.3	150.5	163.4	188.1	
Baseline characteristic								
Males, %	70.3	37.0	26.5	35.8	37.7	39.8	45.2	<0.01
Mean age, y	45.0	36.6	36.4	35.7	37.2	35.7	37.8	0.27
Mean BMI	19.4	19.9	19.2	19.6	20.1	20.2	20.5	<0.01
Cigarette smoking status								
Ever smokers in men, %	81.7	70.7	62.2	72.3	65.1	73.4	76.3	0.12
Ever smokers in women, %	11.1	5.6	5.6	9.0	4.8	2.9	5.4	0.36
Excessive sunlight exposure in men, % <sup>†</sup>	8.5	5.1	8.9	3.2	7.9	1.5	5.3	0.32
Mean educational level, y	2.9	3.7	3.0	3.7	3.6	3.6	4.6	<0.01
Mean baseline well As, µg/L	157.4	103.1	96.7	103.1	93.0	117.4	104.9	0.27
Mean well water TWA, µg/L	147.4	101.8	93.8	106.6	95.9	109.2	103.2	0.44
Mean baseline total urinary As, µg/L	172.0	137.3	137.0	134.1	132.9	142.2	140.0	0.71
Mean urinary creatinine, g/L	60.6	58.1	54.7	55.9	54.6	60.2	65.1	0.02
Mean blood As, µg/L	14.3	10.8	10.2	10.7	10.7	11.1	11.1	0.20
Follow-up characteristic								
Mean total urinary As, µg/L	139.1	119.9	115.5	122.7	126.2	111.3	123.9	0.89
Mean urinary creatinine, g/L	67.8	63.8	58.1	62.0	68.1	61.6	69.1	0.05
Switched to other well since baseline, %	52.2	40.5	34.7	39.8	40.1	43.3	44.5	0.06
Mean daily food or nutrient intake <sup>‡</sup>								
No. participants	292	824	162	167	161	164	164	
Protein, g/d	91.6	86.9	84.9	85.7	86.4	89.6	88.7	0.02
Iron, mg/d	26.7	25.1	24.3	24.2	25.0	26.8	25.2	<0.01
Folate, µg/d	137.2	131.7	126.2	129.7	129.7	139.7	134.5	0.05
Vitamin B2, mg/d	1.1	1.1	1.02	1.05	1.05	1.10	1.07	0.01
Vitamin B12, mg/d	1.9	1.9	1.92	1.89	1.77	1.98	1.92	0.82
Vitamin B6, mg/d	3.7	3.5	3.56	3.54	3.55	3.48	3.49	0.12
Vitamin E, mg/d	5.7	5.5	5.2	5.5	5.5	5.6	5.5	0.36
Big fish, g/d	23.5	22.5	21.5	21.6	19.8	23.0	27.9	0.02
Small fish, g/d	34.5	32.2	34.7	36.0	27.7	29.6	30.5	0.18
Bread, g/d	25.5	14.5	7.0	10.5	17.2	15.0	22.8	<0.01
Dried Beans, g/d	86.7	81.0	69.8	74.8	80.0	89.4	89.8	<0.01
Beans, g/d	42.8	37.1	33.8	40.4	36.1	39.6	35.1	0.80
Beef lamb, g/d	13.6	15.4	14.2	13.5	16.9	19.5	13.5	0.51
Poultry, g/d	3.4	4.0	3.2	5.0	4.7	3.3	4.3	0.89
Milk, g/d	32.3	25.7	21.6	22.6	22.2	31.1	30.1	0.01
Eggs, g/d	6.7	8.5	7.6	8.9	8.4	8.4	8.7	0.72

Abbreviations: As, arsenic, Se, selenium.

\*Data on BMI were missing for four cases of skin lesions and seven subcohort members. Data were also missing on TWA for 18 and 36 subjects, respectively; on follow-up total urinary arsenic for 0 and 27 subjects; and on switching status for 0 and 26 subjects.

<sup>†</sup>Men who worked outside with a bare upper body were categorized as having excessive sun exposure (28). As women in Bangladesh universally wear traditional dresses that almost completely cover the skin of their trunk, sunlight exposure of female respondents was considered minimal and therefore was not assessed in the study.

<sup>‡</sup>Dietary intakes were measured with a validated FFQ at baseline. A total of 824 subcohort members and 292 cases completed the FFQ. Mean values shown by quintile of blood selenium in the subcohort were adjusted for age, sex, BMI, and total energy intake.

robust variance estimator proposed by Barlow et al. (36). The random cohort was weighted by the inverse of the sampling fraction from the source population. Follow-up time, defined for each person as the time of baseline visit to the time of the first follow-up visit, was 1.9 years on average with a range of 0.9 to 3.5 years. Risk sets were created with age at the time of follow-up visit as a matching variable. For each case, members of the random subcohort whose age at the time of follow-up were older than that of the case by  $\leq 3$  years were included as the comparison for the case (i.e., those who had not been diagnosed with skin lesions at the age the case was diagnosed). Blood selenium categories were determined according to quintile values in the subcohort. Previous studies from our group have suggested that age, sex, body mass index (BMI), and tobacco smoking, may modify the risk of premalignant skin lesions (28, 29). These factors, along with well arsenic concentration, were considered the primary potential confounders in evaluating the main effect of blood selenium level because these factors may also be related to selenium intake level. Other risk factors of premalignant skin lesions, including indicators of short-term changes in arsenic exposure (well

switching status since baseline and total urinary arsenic level at the time of follow-up), excessive sunlight exposure (in men; ref. 28), and nutrient intakes that have been related to arsenic toxicity in the literature (37-39), were also considered. These were evaluated in a separate model (model 2) because values were not available for all the study participants.

RRs in relation to joint effects of long-term arsenic exposure and blood selenium were also estimated. Because RRs for the main effect of blood selenium did not differ by additional adjustments, RRs for joint effect of arsenic exposure and selenium were adjusted for primary potential confounders (except for arsenic exposure) only. We further calculated relative excess risk due to interaction to assess the additivity of the joint effects (40).

The subcohort is a good representation of the underlying source population. We did linear regression models to evaluate the relationships of blood selenium with various sociodemographics, lifestyles, arsenic exposure-related variables, food intakes that are related to blood selenium, and nutrient intakes that have been associated with modification of arsenic toxicity in the literature. In addition, we evaluated the

**Table 2. Adjusted RRs for skin lesions by quintile of blood selenium level**

Blood Se quintile (µg/L)	Mean blood Se level (µg/L)	Subcohort, n (%)	Cases, n (%)	Age- and sex-adjusted RRs (95% CI)*	Model 1		Model 2	
					Subcohort/case, n	Multivariate adjusted RRs (95% CI)†	Subcohort/cases, n	Multivariate adjusted RRs (95% CI) ‡
68.8-132.4	121.0	170 (20.0)	72 (23.8)	1.00	168/72	1.00	158/68	1.00
132.5-145.0	139.0	173 (20.4)	62 (20.5)	0.81 (0.49-1.34)	172/60	0.68 (0.39-1.18)	160/58	0.60 (0.33-1.10)
145.1-156.6	150.7	167 (19.7)	52 (17.2)	0.58 (0.35-0.96)	166/51	0.51 (0.29-0.87)	156/50	0.52 (0.30-0.89)
156.7-169.8	163.4	171 (20.1)	59 (19.5)	0.62 (0.37-1.04)	169/58	0.52 (0.30-0.91)	157/57	0.53 (0.30-0.96)
169.9-262.6	187.3	168 (19.8)	58 (19.0)	0.56 (0.33-0.93)	167/58	0.53 (0.31-0.90)	160/55	0.51 (0.29-0.89)

\*RRs were adjusted for age and sex.

†RRs were adjusted for age, sex, BMI, smoking status, and baseline well arsenic. A total of 11 subjects with unknown BMI were excluded from the analysis.

‡RRs were adjusted for age, sex, BMI, smoking status, baseline well arsenic, well switching status at follow-up, urinary arsenic at follow-up, excessive sunlight exposure in men, total energy intake, and dietary intakes of folate, iron, protein, vitamin E, B2, B12, and B6. A total of 83 subjects with unknown information on BMI, well switching status since baseline, urinary arsenic level at the time of follow-up, or dietary intakes of arsenic-related nutrients were excluded from the analysis.

cross-sectional relationships of blood selenium with blood arsenic and total urinary arsenic (all measured at baseline) in the subcohort. Factors, such as well arsenic level and water consumption that may be related to arsenic intake, were additionally adjusted for in this analysis.

## Results

Cases were more likely to be male, older, less educated, and ever to have smoked at baseline (Table 1). Total urinary arsenic, well water arsenic level, blood arsenic level, and the well water TWA level measured at baseline were all higher in cases than in the subcohort. Cases were more likely to have switched to another well water source since baseline. Nevertheless, total urinary arsenic measured 2 years later was higher in cases.

In the subcohort, the proportion of men was higher among participants with higher levels of blood selenium ( $P_{\text{trend}} < 0.01$ ; Table 1). Average baseline BMI and educational attainment were higher in higher quintiles of blood selenium ( $P_{\text{trend}} < 0.05$ ). There were no apparent associations of blood selenium with age, cigarette smoking status, and all of the arsenic exposure measures. The proportion of participants who switched to a different well since baseline was greater among participants with higher levels of blood selenium ( $P_{\text{trend}} = 0.06$ ). Adjusted average intakes of large fresh water fish, bread, dried beans, and milk were higher in participants with higher levels of blood selenium. No significant associations were observed between blood selenium level and intakes of meats, small fish, eggs, or any specific vegetables (data not shown). Average intakes of protein, iron, folate, and vitamin B2 were positively related to blood selenium levels ( $P_{\text{trend}} \leq 0.05$ ); Spearman correlations of blood selenium with these nutritional variables were  $\leq 0.12$ .

Blood selenium level was inversely related to risk of premalignant skin lesions (Table 2). Comparing the higher four quintiles to the bottom quintile of blood selenium, age- and sex-adjusted RRs ranged from 0.56 to 0.81. The inverse association remained apparent with additional adjustments for BMI, cigarette smoking status, and baseline well arsenic level; RRs were 0.51 [95% confidence interval (95% CI), 0.29-0.87], 0.52 (95% CI, 0.30-0.91), and 0.53 (95% CI, 0.30-0.91) comparing the third, fourth, and fifth quintile to the bottom quintile, respectively (model 1). Additional adjustments for well switching status, total urinary arsenic and urinary creatinine at the time of follow-up, total energy intake, excessive sunlight exposure in men, and intakes of protein, folate, iron, vitamins E, B2, B6, and B12 did not change the estimates appreciably (model 2).

The cross-sectional relationship between baseline blood selenium and baseline urinary arsenic in the subcohort is

presented in Table 3. Partial Spearman correlation controlling for age, well arsenic level, BMI, and urinary creatinine was  $-0.10$  ( $P = 0.02$ ) between blood selenium and urinary arsenic and  $0.07$  ( $P = 0.05$ ) between blood selenium and blood arsenic. Participants with higher blood selenium levels had lower urinary arsenic levels, adjusting for urinary creatinine, age, sex, BMI, smoking status, baseline well arsenic concentration, and daily water consumption. The inverse association was statistically significant in multiple linear regression ( $P_{\text{trend}} = 0.03$ ). On the other hand, no apparent association was observed between selenium and arsenic concentrations in the blood.

Low blood selenium was associated with a greater risk for skin lesions at each level of arsenic exposure (Table 4). The increased risk associated with low blood selenium seemed to be additive to the risk related to higher levels of arsenic exposure. The pattern of effect estimates was consistent with all four arsenic exposure measurements. Additional adjustment for well switching status since baseline did not change the pattern of RRs. A relative excess risk due to interaction estimate significantly greater or lower than 0 (perfect additivity) indicates that the joint effects are significantly greater or lesser than additivity, respectively. All the relative excess risks due to interaction estimates were close to 0, ranging from  $-0.35$  to  $0.5$  (data not shown). For instance, the relative excess risk due to interaction for joint effects of low blood selenium and well arsenic 25.1 to 117.0 µg/L is  $-0.26$  ( $2.56-1.70 - 2.12+1$ ). Therefore, there is no evidence that the joint effect of arsenic exposure and low blood selenium departs from additivity.

## Discussion

To our knowledge, this is the first prospective study that evaluates the association between selenium levels and risk of arsenic-related disease in a population exposed to arsenic from drinking water. Higher prediagnostic blood selenium level was related to as much as a 50% reduction in risk of arsenic-related premalignant skin lesions. This estimate did not change appreciably with adjustments for age, sex, BMI, smoking status, arsenic exposure level, and dietary intakes related to arsenic toxicity, including dietary folate, iron, protein, vitamin E, and B vitamins (37-39). The pattern of RRs suggests that the effects of arsenic exposure and selenium deprivation on risk of skin lesions are additive. These findings are in line with the hypothesis that dietary selenium intakes may reduce the incidence of skin lesions among populations with arsenic exposure from drinking water.

Findings from previous studies were mostly inconclusive on the relationship between selenium intake and arsenic toxicity. A case-control study in Taiwan found that patients with blackfoot disease had lower blood selenium levels than

**Table 3. Relationships of blood selenium with urinary and blood arsenic in the subcohort at baseline**

Blood Se quintile ( $\mu\text{g/L}$ )	<i>n</i>	Adjusted means of baseline urinary As ( $\mu\text{g/L}$ )*		Adjusted means of baseline blood As ( $\mu\text{g/L}$ ) <sup>†</sup>	
		Means (SD)	<i>P</i> <sub>trend</sub>	Means (SD)	<i>P</i> <sub>trend</sub>
68.8-132.4	170	142.94 (9.03)	0.03	10.81 (0.48)	0.66
132.5-145.0	173	135.58 (8.88)		10.68 (0.48)	
145.1-156.6	167	142.37 (8.96)		11.22 (0.48)	
156.7-169.8	171	126.08 (8.88)		10.57 (0.48)	
169.9-262.6	168	125.41 (8.95)		11.06 (0.48)	

\*Adjustments were made for baseline age, sex, smoking status, BMI, well arsenic concentration, daily water consumption, and urinary creatinine.

<sup>†</sup>Adjustments were made for baseline age, sex, smoking status, BMI, well arsenic concentration, daily water consumption.

controls, whereas a similar case-control study found that blood selenium was higher in patients with late-stage blackfoot disease compared with that in controls (18, 19). In another case-control study in West Bengal, odds ratios for arsenic-related skin lesions did not differ by blood selenium levels (21). It is unclear, however, whether the blood selenium levels observed in cases were a consequence or a contributing factor to blackfoot disease or arsenic-related skin lesions in these case-control analyses. A placebo-controlled trial in Inner Mongolia found that selenium supplementation significantly improved skin lesions (20). However, the trial was neither randomized nor double blind, and the dropout rates in both the placebo and the treatment groups were high. A pilot randomized, placebo-controlled trial conducted by our group found that selenium supplementation slightly improved skin lesion status; however, the sample size of the study was small and the improvement was not significant (13).

Our findings are consistent with several observational studies that found a protective association between plasma selenium level and the risk of nonmelanoma skin cancer (41-43). A large randomized clinical trial in patients who previously had nonmelanoma skin cancer, on the other hand, found that selenium supplementation increased the risk of skin cancer (44). There are several possible explanations. First, selenium supplementation may not offer benefits for secondary prevention of skin cancer in an older population (median age, 65 years; ref. 44). Second, the observed inverse association

between blood selenium and risk of skin lesions in the present analysis is likely due to both the chemopreventive effect of selenium and the interaction between selenium and arsenic; the latter is absent in populations not exposed to arsenic exposure. Third, it has been postulated that subclinical health effects of selenium deficiency may be manifest at the low-end of "adequate" selenium intake (45) and that physiologic stressors may exert additional demand on selenium-dependent systems. Indeed, the negative effects of selenium supplementation for secondary prevention of nonmelanoma skin cancer seem to be greater in those with high baseline plasma selenium (44). We observed that the risk associated with any given level of arsenic exposure was consistently greater among persons with blood selenium lower than the average level. Using the equation suggested by Yang et al. (46), we estimated the average selenium daily intake for participants with blood selenium lower than the average level (150.2  $\mu\text{g/L}$ ) to be 61  $\mu\text{g/d}$ , close to the low-end of the recommended daily intake of selenium (55  $\mu\text{g/d}$ ), which is established to maintain adequate levels of selenoenzymes. When the level of arsenic exposure was statistically held constant, the reduced RRs associated with the higher three quintiles of blood selenium were significant with similar magnitude, indicating that the selenium dose-response curve may have a threshold above which no additional benefit occurs. Future arsenic mitigation programs or randomized trials of selenium supplementation may consider this finding. It should be noted that

**Table 4. Joint effect of arsenic exposure and low blood selenium on risk of skin lesion**

As exposure measures* (tertiles)	Blood Se, >150.2 $\mu\text{g/L}$ <sup>†</sup>			Blood Se, $\leq$ 150.2 $\mu\text{g/L}$ <sup>†</sup>		
	Cases/ subcohort, <i>n</i>	Median As level <sup>‡</sup>	RRs (95% CI) <sup>§</sup>	Cases/ subcohort, <i>n</i>	Median As level <sup>‡</sup>	RRs (95% CI) <sup>§</sup>
Baseline well As levels ( $\mu\text{g/L}$ )						
0.1-25.0	25/129	7.2	1.00	37/153	7.2	2.12 (1.09-4.10)
25.1-117.0	36/140	67.7	1.70 (0.86-3.36)	45/140	62.1	2.56 (1.33-4.94)
117.1-564.0	87/157	231.7	3.38 (1.86-6.17)	69/130	237.8	4.15 (2.24-7.67)
Well water TWA level ( $\mu\text{g/L}$ )						
0.1-29.0	24/123	8.4	1.00	34/146	9.0	2.11 (1.01-4.34)
29.1-116.0	35/138	68.2	1.85 (0.92-3.74)	45/131	63.9	2.62 (1.30-5.28)
116.1-564.0	79/148	223.8	3.40 (1.75-6.63)	64/127	232.2	4.58 (2.33-8.99)
Baseline blood As ( $\mu\text{g/L}$ )						
1.6-6.8	25/118	5.0	1.00	36/171	4.9	1.55 (0.82-2.92)
6.9-11.3	39/146	8.9	1.36 (0.71-2.59)	45/127	8.9	2.18 (1.16-4.09)
11.4-63.9	84/162	17.8	2.50 (1.40-4.46)	70/125	19.2	3.55 (1.94-6.50)
Baseline total urinary As ( $\mu\text{g/L}$ ) <sup>  </sup>						
3-54	27/142	30.6	1.00	38/141	30.1	1.68 (0.89-3.16)
55-138	44/125	88.0	1.46 (0.78-2.71)	49/154	88.7	2.15 (1.16-3.96)
139-1220	77/159	281.2	2.67 (1.42-5.03)	64/128	301.3	3.12 (1.66-5.84)

\*Cut points were determined based on tertile values in the subcohort.

<sup>†</sup>Cut point was determined based on median value in the subcohort.

<sup>‡</sup>Category-specific median values in the subcohort for each of the four arsenic exposure measures in the left column.

<sup>§</sup>RRs were adjusted for age, BMI, sex, and smoking status. RRs in relation to urinary arsenic were additionally adjusted for urinary creatinine. A total of 11 subjects with unknown information on BMI were excluded from the analysis. A total of 51 subjects with unknown information on the TWA were also excluded from the calculation of RRs in relation to TWA.

<sup>||</sup>RRs associated with total urinary arsenic were additionally adjusted for urinary creatinine level.

selenium toxicity, although rare in human populations, has been observed at selenium intakes  $>600 \mu\text{g}/\text{d}$  (47).

The primary interaction between selenium and arsenic is thought to be via a selenium-arsenic-glutathione conjugate formed in the liver and excreted into bile. In recent studies in rabbits, Gailer et al. (17, 48) identified the compound excreted into bile as a seleno-bis(S-glutathionyl) arsinium ion,  $[(\text{GS})_2\text{AsSe}]^+$ . Our observation of an inverse association between blood selenium level and urinary arsenic is consistent with the hypothesis that selenium-induced biliary excretion may occur in human. The association of blood arsenic and blood selenium, on the other hand, was not apparent. These findings require further investigation. Other direct selenium/arsenic interactions exist. Berry et al. (49) reported that selenium decreased arsenic toxicity via the formation of a selenide precipitate ( $\text{As}_2\text{Se}$ ) that is deposited into tissues. Oxidative stress-reducing effects of selenoenzymes, including glutathione peroxidases, iodothyronine deiodinases, and thioredoxine reductases (50), may also reduce arsenic toxicity. In the mouse model, a significant reduction in the formation of 8-oxo-2'-deoxyguanosine, an oxidative DNA damage biomarker, was observed in UV radiation- and arsenic-treated mice that were supplemented with selenium compared with those treated with UV radiation or arsenic alone (51). The initiation of UV radiation-induced skin tumors has been shown to vary with the activity of glutathione peroxidases and thioredoxine reductases (52).

The underlying source population represents those who gave both blood and urine samples, who underwent the baseline clinical examination, and who did not have skin lesions at baseline and thus had a lower level of arsenic exposure. Donation of blood and urine samples and consent to physical examination were weakly associated with a higher educational attainment (22). Although these differences do not affect the internal validity of our findings, compared with the study population, the overall cohort may have a somewhat higher arsenic level and a lower blood selenium level, given the positive association between blood selenium level and educational attainment. The risk difference associated with selenium intake thus may be more significant in the overall cohort. Consistent with findings from another study (53), we found that the average blood selenium in Bangladeshi population ( $150 \mu\text{g}/\text{L}$ ) was not particularly lower than those reported from populations in developed countries (54), ranging from  $87$  to  $107 \mu\text{g}/\text{L}$  in Germany,  $134$  to  $138 \mu\text{g}/\text{L}$  in England, and  $166$  to  $200 \mu\text{g}/\text{L}$  in nonseleniferous areas in the United States.

Selenium levels measured in whole blood are considered as a useful measure for ranking subjects for long-term selenium intake (55). The calculation of TWA was based on self-reported use of wells. However, validity of self-reported well use history was good because the correlation between arsenic concentration in the baseline well and baseline urinary arsenic was  $0.70$  (22). In addition, the patterns of RRs for the joint effects of arsenic exposure and low blood selenium were similar using multiple biological measures of arsenic exposure, which further strengthen the findings. In a separate analysis, we have also shown consistent dose-response relationships of the risk of skin lesions with TWA, baseline blood arsenic, and baseline urinary arsenic, and we showed that blood arsenic is a good biomarker of arsenic exposure in this population (56). The three measures were highly correlated with one another (pairwise Spearman correlation,  $0.8$ ; ref. 56). Dietary intakes of other nutrients relevant to arsenic toxicity were measured by FFQ; therefore, measurement errors are expected. The fact that RRs for skin lesions in relation to blood selenium levels remained the same after controlling for dietary folate, iron, protein, vitamin E, and B vitamins excludes the possibility of strong confounding effect due to these dietary factors. Sharing of the wells in the study population was minimal; the 1,121

subjects included in the present analysis were users of 908 wells at baseline. Therefore, the findings are not likely to have been affected by correlated arsenic exposure among subjects. After the completion of baseline interviews, participants with well arsenic  $>50 \mu\text{g}/\text{L}$  were advised to change their drinking well, leading to the changes in arsenic exposure during the 1.9 years period from baseline to the follow-up visit. However, the short-term changes in arsenic exposure are less relevant to the risk of skin lesions, compared with the TWA, which is based on an average of 9 years of well use history. In addition, adjustments for switching status and urinary arsenic at the time of follow-up did not change RR estimates for skin lesions in relation to blood selenium.

In conclusion, our results are consistent with the notions that (a) higher dietary selenium intake may reduce the risk of arsenic-related skin lesions and (b) selenium recommended daily intake may not be adequate in the presence of physiologic stressors, such as chronic arsenic exposure from drinking water. Future studies should continue to evaluate the effect of selenium in treating arsenic-related skin lesions and skin cancers as well as the influence of selenium on relationships between arsenic exposure and other arsenic-related disorders.

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