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

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Running head:
Selenium and risk of skin lesions

Grants and acknowledgments: This research was supported by U.S. National Institute of Environmental Health Sciences Grants P42ES10349, P30ES09089, and National Cancer Institute Grants R01CA107431, and R01CA102484. The authors would like to thank the staff, field workers and study participants in Bangladesh without whom this work would not have been possible. The authors would also like to thank Dr. Wei-Yann Tsai for his helpful comments.

Abbreviations:

Health Effects of Arsenic Longitudinal Study (HEALS)
Arsenic (As)
Selenium (Se)
Food frequency questionnaire (FFQ)

Key words:

Arsenic
Bangladesh
Case-cohort study
Premalignant skin lesions
Selenium

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 As and Se in the body. We performed a case-cohort analysis to prospectively evaluate the association between As-related premalignant skin lesions and prediagnostic blood Se 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 As Longitudinal Study with available baseline blood and urine samples collected in 2000. Incidence rate ratios for skin lesions in increasing blood Se quintiles were 1.00 (ref), 0.68 (95% confidence interval (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, BMI, smoking status, excessive sunlight exposure (in men), well water As concentration at baseline, and nutritional intakes of folate, iron, protein, Vitamin E, and B Vitamins. At any given As exposure level, the risk of premalignant skin lesions was consistently greater among participants with blood Se lower than the average level. The findings support the hypothesis that dietary Se intake may reduce the incidence of As-related premalignant skin lesions among populations exposed to As exposure from drinking water.

Background

The presence of inorganic arsenic (As) in groundwater has been recognized as a public health hazard in many countries. The International Agency for Research on Cancer has classified arsenic as a group 1 human carcinogen. Epidemiologic studies have documented associations between As 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 As concentrations exceeding the WHO standard (10 μg/L) (4). We have estimated the cancer burden to be doubling in Bangladesh (5). Clearly, As mitigation and cancer preventive programs are urgently needed to reduce As toxicity in the population.

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

It has been hypothesized that susceptibility to As toxicity differs by dietary selenium (Se) intake levels (12, 13). Se is an essential human dietary trace element required for synthesis of a variety of Se-containing proteins, some of which are selenoproteins that incorporate Se in the form of the amino acid selenocysteine (SeCys) during translation (14). Selenoproteins and their metabolites are critical in maintaining antioxidant/anti-inflammatory homeostasis. In experimental studies, As 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 demonstrated an interaction between Se and As, 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 Se intake on risks of As-related diseases such as premalignant skin lesions and blackfoot disease (a unique peripheral vascular disease in lower extremities related to high levels of As exposure) in populations exposed to As exposure have been inconclusive (13, 18-21). Limitations of these studies include small sample sizes, unavailability of prediagnostic Se levels (in observational studies), and methodological shortcomings such as the lack of blindness in randomization (in intervention studies).

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

Methods

The Health Effects of As Longitudinal Study (HEALS)

The parent study HEALS is an ongoing prospective cohort study in Araihazar, Bangladesh. Details of the study methodologies have been presented elsewhere (22, 23). Briefly, prior to 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 Araihazar. Between October, 2000 and May, 2002, 11,746 men and women aged 18 years and above were recruited, with a participation rate of 97.5% (22). The cohort is being followed with in-person visits at two 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 2–8 hours, blood and urine samples were processed and transferred to -20° C freezers in the study office located in Dhaka city. All samples were kept frozen and shipped to Columbia University on dry ice within 1–2 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 previously described (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, shape of skin lesions and extent of skin involvement. Physicians were blind to information on the As 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 Se level and risk of skin lesions. The case-cohort study design has been used to analyze cohort data efficiently when most observations are censored (non-diseased) (24). It provides the advantages of a cohort study in that it allows the direct calculation of a rate ratio 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 previously consumed 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 two-year follow up from the 8,092 participants between November, 2002 and April, 2004; 221 of the cases had only melanosis, while 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 As 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 minutes (25, 26). Total As concentration was determined by graphite furnace atomic-absorption spectrometry (GFAA) with a Hitachi Z-8200 system at the Lamont-Doherty Earth observatory of Columbia University (25). Samples that fell below the detection limit of GFAA (5 μg/L) were subsequently analyzed by inductively coupled plasma mass spectrometry (ICP-MS), with a detection limit of 0.1 μg/L (27). Analyses for time-series samples collected from 20 tube wells in the study area showed that the As concentration in well water is relatively stable over time (27). Therefore, we derived a time-weighted As concentration (TWA) as a function of drinking durations and well As concentrations (28, 29). The TWA represents the average As exposure that accrued for 9 years on average in the cohort members prior to the time of baseline visits.

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

Measurements of Se and As in whole blood

Whole blood samples collected at baseline were analyzed for blood Se and As concentrations using a Perkin-Elmer Elan DRC II ICP-MS equipped with an AS 93+ autosampler. ICP-MS-DRC 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% HNO_3 + 0.2% Triton-X-100 + 0.5% NH_4OH, and centrifuged for 10 minutes at 3500 rpm with the supernatant reserved for analysis. A multi-element standard solution was used for instrument calibration, with Se and As 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 instrument's Dynamic Reaction Cell (DRC) technology feature, utilizing 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 ICP-MS Comparison Program in Quebec), was 0.99 and 0.90 for blood Se and As, respectively.

Measurements of Dietary Intakes

Dietary intakes were measured at baseline with a validated semi-quantitative 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 (FD) were completed in two separate seasons by trained interviewers for 189 of the 200 participants randomly selected from the overall HEALS 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 (USDA) 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 rate ratios (RRs) for skin lesions were estimated using Cox proportional hazards models with the PROC PHREG procedure in SAS. Standard errors were estimated using the robust variance estimator proposed by Barlow (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 ≤ 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 Se 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

231 factors, along with well As concentration, were considered the primary potential
232 confounders in evaluating the main effect of blood Se level because these factors may
233 also be related to Se intake level. Other risk factors of premalignant skin lesions
234 including indicators of short-term changes in As exposure (well switching status since
235 baseline and total urinary As level at the time of follow-up), excessive sunlight exposure
236 (in men) (28), and nutrient intakes that have been related to As toxicity in the literature
237 (37-39) were also considered. These were evaluated in a separate model (model 2)
238 because values were not available for all the study participants.
239 RRs in relation to joint effects of long-term As exposure and blood Se were also
240 estimated. Since RRs for the main effect of blood Se did not differ by additional
241 adjustments, RRs for joint effect of As exposure and Se were adjusted for primary
242 potential confounders (except for As exposure) only. We further calculated relative
243 excess risk due to interaction (RERI) to assess the additivity of the joint effects (40).
244 The subcohort is a good representation of the underlying source population.
245 We performed linear regression models to evaluate the relationships of blood Se with
246 various socio-demographics, lifestyles, As exposure-related variables, food intakes that
247 have been shown to be related to blood Se, and nutrient intakes that have been associated
248 with modification of As toxicity in the literature. In addition, we evaluated the cross-
249 sectional relationships of blood Se with blood As and total urinary As (all measured at
250 baseline) in the subcohort. Factors such as well As level and water consumption that may
251 be related to As intake were additionally adjusted for in this analysis.
252
253 **Results**
254 Cases were more likely to be male, older, less educated, and ever to have smoked
255 at baseline (Table 1). Total urinary As, well water As level, blood As level, and the time-
256 weighted well As level measured at baseline were all higher in cases than in the
257 subcohort. Cases were more likely to have switched to another well water source since
258 baseline. Nevertheless, total urinary As measured two years later was higher in cases.
259 In the subcohort, the proportion of men was higher among participants with
260 higher levels of blood Se (p-trend <0.01) (Table 1). Average baseline BMI and
261 educational attainment were higher in higher quintiles of blood Se (p-trend <0.05). There
262 were no apparent associations of blood Se with age, cigarettes smoking status, and all of

263 the As exposure measures. The proportion of participants who switched to a different
264 well since baseline was greater among participants with higher levels of blood Se (p-
265 trend = 0.06). Adjusted average intakes of large fresh water fish, bread, dried beans, and
266 milk were higher in participants with higher levels of blood Se. No significant
267 associations were observed between blood Se level and intakes of meats, small fish, eggs,
268 or any specific vegetables (data not shown). Average intakes of protein, iron, folate, and
269 Vitamin B2 were positively related to blood Se levels (p-trend $\leq$ 0.05); spearman
270 correlations of blood Se with these nutritional parameters were $\leq$ 0.12.
271 Blood Se level was inversely related to risk of premalignant skin lesions (Table
272 2). Comparing the higher four quintiles to the bottom quintile of blood Se, age- and sex-
273 adjusted RRs ranged from 0.56 to 0.81. The inverse association remained apparent with
274 additional adjustments for BMI, cigarettes smoking status, and baseline well As level;
275 RRs were 0.51 (95% confidence interval (CI): 0.29, 0.87), 0.52 (95% CI: 0.30, 0.91), and
276 0.53 (95% CI: 0.30, 0.91) comparing the third, fourth, and fifth quintile to the bottom
277 quintile, respectively (model 1). Additional adjustments for well switching status, total
278 urinary As and urinary creatinine at the time of follow-up, total energy intake, excessive
279 sunlight exposure in men, and intakes of protein, folate, iron, Vitamins E, B2, B6, and
280 B12 did not change the estimates appreciably (model 2).
281 The cross-sectional relationship between baseline blood Se and baseline urinary
282 As in the subcohort is presented in Table 3. Partial spearman correlation controlling for
283 age, well As level, BMI, and urinary creatinine was -0.10 (p = 0.02) between blood Se
284 and urinary As and 0.07 (p = 0.05) between blood Se and blood As. Participants with
285 higher blood Se levels had lower urinary As levels, adjusting for urinary creatinine, age,
286 sex, BMI, smoking status, baseline well As concentration, and daily water consumption.
287 The inverse association was statistically significant in multiple linear regression (p for
288 trend = 0.03). On the other hand, no apparent association was observed between Se and
289 As concentrations in the blood.
290 Low blood Se was associated with a greater risk for skin lesions at each level of As
291 exposure (Table 4). The increased risk associated with low blood Se appeared to be
292 additive to the risk related to higher levels of As exposure. The pattern of effect
293 estimates was consistent with all four As exposure measurements. Additional adjustment
294 for well switching status since baseline did not change the pattern of RRs. An RERI

estimate significantly greater or lower than zero (perfect additivity) indicates that the joint effects are significantly greater or lesser than additivity, respectively. All the RERI estimates were close to zero, ranging from -0.35 to 0.5 (data not shown). For instance, the RERI for joint effects of low blood Se and well As 25.1-117.0 μg/L is -0.26 (2.56-1.70-2.12+1). Therefore, there is no evidence that the joint effect of As exposure and low blood Se departs from additivity.

Discussion

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

Findings from previous studies were mostly inconclusive on the relationship between Se intake and As toxicity. A case-control study in Taiwan found that patients with blackfoot disease had lower blood Se levels than controls, while a similar case-control study found that blood Se was higher in patients with late-stage blackfoot disease compared to that in controls (18, 19). In another case-control study in West Bengal, odds ratios for As-related skin lesions did not differ by blood Se levels (21). It is unclear, however, whether the blood Se levels observed in cases were a consequence or a contributing factor to blackfoot disease or As-related skin lesions in these case-control analyses. A placebo-controlled trial in Inner Mongolia found that Se supplementation significantly improved skin lesions (20). However, the trial was neither randomized nor double-blind, and the drop-out rates in both the placebo and the treatment groups were high. A pilot randomized, placebo-controlled trial conducted by our group found that Se 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) (44). Second, the observed inverse association between blood Se and risk of skin lesions in the present analysis is likely due to both the chemopreventive effect of Se and the interaction between Se and As; the latter is absent in populations not exposed to As exposure. Third, it has been postulated that sub-clinical health effects of Se deficiency may be manifest at the low-end of "adequate" Se intake (45) and that physiological stressors may exert additional demand on Se-dependent systems. Indeed, the negative effects of selenium supplementation for secondary prevention of nonmelanoma skin cancer appear to be greater in those with high baseline plasma selenium (44). We observed that the risk associated with any given level of As exposure was consistently greater among persons with blood Se lower than the average level. Using the equation suggested by Yang et al (46), we estimated the average Se daily intake for participants with blood Se lower than the average level (150.2 μg/L) to be 61 μg/day, close to the low-end of the recommended daily intake (RDI) of Se (55 μg/day), which are established to maintain adequate levels of selenoenzymes. When the level of As exposure was statistically held constant, the reduced RRs associated with the higher three quintiles of blood Se were significant with similar magnitude, indicating that the Se dose-response curve may have a threshold above which no additional benefit occurs. Future As mitigation programs or randomized trials of Se supplementation may consider this finding. It should be noted that Se toxicity, although rare in human populations, has been observed at selenium intakes above 600 μg/day (47).

The primary interaction between Se and As is thought to be via a Se-As-glutathione conjugate formed in the liver and excreted into bile. In recent studies in rabbits, Gailer et al identified the compound excreted into bile as a seleno-bis (S-glutathionyl) arsinium ion, $[(GS)_2AsSe]^-$ (17, 48). Our observation of an inverse association between blood Se level and urinary As is consistent with the hypothesis that Se-induced biliary excretion may occur in human. The association of blood As and blood

Se, on the other hand, was not apparent. These findings require further investigation. Other direct Se/As interactions exist. Berry et al reported that Se decreased As toxicity via the formation of a selenide precipitate (As_2Se) that is deposited into tissues (49). Oxidative stress reducing effects of selenoenzymes including glutathione peroxidases (GPx), iodothyronine deiodinases (ID) and thioredoxine reductases (TR) (50) may also reduce As toxicity. In the mouse model, a significant reduction in the formation of 8-oxo-2'-deoxyguanosine, an oxidative DNA damage biomarker, was observed in ultraviolet radiation (UVR) and As treated mice that were supplemented with Se, compared with those treated with UVR or As alone (51). The initiation of UVR-induced skin tumors has been shown to vary with the activity of GPx and TR (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 As exposure. Donation of blood and urine samples and consent to physical examination were weakly associated with a higher educational attainment (22). While these differences do not affect the internal validity of our findings, compared to the study population, the overall cohort may have a somewhat higher As level and a lower blood Se level given the positive association between blood Se level and educational attainment. The risk difference associated with Se intake thus may be more significant in the overall cohort. Consistent with findings from another study (53), we found that the average blood Se in Bangladeshi population (150 μg/L) was not particularly lower than those reported from populations in developed countries (54), ranging from 87-107 μg/L in Germany, 134-138 μg/L in England, and 166 to 200 μg/L in non-seleniferous areas in the US.

Se levels measured in whole blood are considered as a useful measure for ranking subjects for long-term Se intake (55). The calculation of TWA was based on self-reported use of wells. However, validity of self-reported well use history was good since 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 As exposure and low blood Se were similar using multiple biologic measures of As 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 As, and baseline urinary As, and we demonstrated that blood As is a good

biomarker of As exposure in this population (56). The three measures were highly correlated with one another (pairwise spearman correlation = 0.8) (56). Dietary intakes of other nutrients relevant to As toxicity were measured by FFQ, and therefore measurement errors are expected. The fact that RRs for skin lesions in relation to blood Se 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 1121 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 As exposure among subjects. After the completion of baseline interviews, participants with well As > 50 μg/L were advised to change their drinking well, leading to the changes in As exposure during the 1.9 years period of time from baseline to the follow-up visit. However, the short-term changes in As exposure are less relevant to the risk of skin lesions, compared to the TWA, which is based on an average of 9 years of well use history. In addition, adjustments for switching status and urinary As at the time of follow-up did not change RR estimates for skin lesions in relation to blood Se.

In conclusion, our results are consistent with the notions that 1) higher dietary Se intake may reduce the risk of As-related skin lesions, and 2) Se RDI may not be adequate in the presence of physiological stressors such as chronic As exposure from drinking water. Future studies should continue to evaluate the effect of Se in treating As-related skin lesions and skin cancers, as well as the influence of Se on relationships between As exposure and other As-related disorders.

References

1. Tseng WP. Blackfoot disease in Taiwan: a 30-year follow-up study. Angiology 1989;40:547-558.

2. Chen CJ, Kuo TL, Wu MM. Arsenic and cancers. Lancet 1988;1:414-415.

3. Chiou HY, Huang WI, Su CL, et al. Dose-response relationship between prevalence of cerebrovascular disease and ingested inorganic arsenic. Stroke 1997;28:1717-1723.

4. The British Geological Survey. Groundwater studies for arsenic contamination in Bangladesh-Phase 1 findings. Available: http://www.bgs.ac.uk/arsenic/ [accessed March 3rd, 2006].

5. Chen Y, Ahsan H. Cancer burden from arsenic in drinking water in Bangladesh. Am J Public Health 2004;94:741-744.

6. Tseng WP, Chu HM, How SW, et al. Prevalence of skin cancer in an endemic area of chronic arsenicism in Taiwan. J Natl Cancer Inst 1968;40:453-463.

7. Saha KC. Diagnosis of arsenicosis. J Environ Sci Health Part A Tox Hazard Subst Environ Eng 2003;38:255-272.

8. Alain G, Tousignant J, Rozenfarb E. Chronic arsenic toxicity. Int J Dermatol 1993;32:899-901.

9. Centeno JA, Mullick FG, Martinez L, et al. Pathology related to chronic arsenic exposure. Environ Health Perspect 2002;110 Suppl 5:883-886.

10. Neubauer O. Arsenical cancer; a review. Br J Cancer 1947;1:192-251.

11. Arguello RA, Conget DD, Tello EE. Cancer and. endemic arsenism in the Cordoba Region. RevArgent Dermatol 1939;22:461-487.

12. Spallholz JE, Mallory BL, Rhaman MM. Environmental hypothesis: is poor dietary selenium intake an underlying factor for arsenicosis and cancer in Bangladesh and West Bengal, India? Sci Total Environ 2004;323:21-32.

13. Verret WJ, Chen Y, Ahmed A, et al. A randomized, double-blind placebo-controlled trial evaluating the effects of vitamin E and selenium on arsenic-induced skin lesions in Bangladesh. J Occup Environ Med 2005;47:1026-1035.

14. Behne D, Kyriakopoulos A. Mammalian selenium-containing proteins. Annu Rev Nutr 2001;21:453-473.

15. Shi H, Shi X, Liu KJ. Oxidative mechanism of arsenic toxicity and carcinogenesis. Mol Cell Biochem 2004;255:67-78.

16. Levander OA, Baumann CA. Selenium metabolism. VI. Effect of arsenic on the excretion of selenium in the bile. Toxicol Appl Pharmacol 1966;9:106-115.

17. Gailer J, George GN, Pickering IJ, et al. Biliary excretion of [(GS)(2)AsSe](-) after intravenous injection of rabbits with arsenite and selenate. Chem Res Toxicol 2002;15:1466-1471.

18. Wang CT. Concentration of arsenic, selenium, zinc, iron and copper in the urine of blackfoot disease patients at different clinical stages. Eur J Clin Chem Clin Biochem 1996;34:493-497.

19. Lin SM, Yang MH. Arsenic, selenium, and zinc in patients with Blackfoot disease. Biol Trace Elem Res 1988;15:213-221.

20. Yang L, Wang W, Hou S, Peterson PJ, Williams WP. Effects of Selenium Supplementation on Arsenism: An Intervention Trial in Inner Mongolia. Environmental Geochemistry and Health 2002;24:359-374.

21. Chung JS, Haque R, Guha Mazumder DN, et al. Blood concentrations of methionine, selenium, beta-carotene, and other micronutrients in a case-control study of arsenic-induced skin lesions in West Bengal, India. Environ Res 2005.

22. Ahsan H, Chen Y, Parvez F, et al. Health Effects of Arsenic Longitudinal Study (HEALS): description of a multidisciplinary epidemiologic investigation. J Expo Sci Environ Epidemiol 2006;16:191-205.

23. Parvez F, Chen Y, Argos M, et al. Prevalence of arsenic exposure from drinking water and awareness of its health risks in a Bangladeshi population: results from a large population-based study. Environ Health Perspect 2006;114:355-359.

24. Prentice RL. A Case-Cohort Design for Epidemiologic Cohort Studies and Disease Prevention Trials. Biometrika 1986;73:1-11.

25. van Geen A, Ahsan H, Horneman AH, et al. Promotion of well-switching to mitigate the current arsenic crisis in Bangladesh. Bull World Health Organ 2002;80:732-737.

26. van Geen A, Zheng Y, Versteeg R, et al. Spatial variability of arsenic in 6000 tube wells in a 25 km 2 area of Bangladesh. Water Resour Res 2003;39:1140.

27. Cheng Z, van Geen A, Seddique AA, Ahmed KM. Limited temporal variability of arsenic concentrations in 20 wells monitored for 3 years in Araihazar, Bangladesh. Environ Sci Technol 2005;39:4759-4766.

28. Chen Y, Graziano JH, Parvez F, et al. Modification of Risk of Arsenic-Induced Skin Lesions by Sunlight Exposure, Smoking, and Occupational Exposures in Bangladesh. Epidemiology 2006;17:459-467.

29. Ahsan H, Chen Y, Parvez F, et al. Arsenic exposure from drinking water and risk of premalignant skin lesions in Bangladesh: baseline results from the health effects of arsenic longitudinal study. Am J Epidemiol 2006;163:1138-1148.

30. Nixon DE, Mussmann GV, Eckdahl SJ, Moyer TP. Total arsenic in urine: palladium-persulfate vs nickel as a matrix modifier for graphite furnace atomic absorption spectrophotometry. Clin Chem 1991;37:1575-1579.

31. Slot C. Plasma creatinine determination. A new and specific Jaffe reaction method. Scand J Clin Lab Invest 1965;17:381-387.

32. Stroh A. Determination of Pb and Cd in Whole Blood Using Isotope Dilution ICP-MS. Atomic Spectroscopy 1993;37:1575-1579.

33. Chen Y, Ahsan H, Parvez F, Howe GR. Validity of a food-frequency questionnaire for a large prospective cohort study in Bangladesh. Br J Nutr 2004;92:851-859.

34. U.S.Department of Agriculture ARS, Nutrient Data Laboratory Home Page. USDA Nutrient Database for Standard Reference, Release 15. Available: http://www.nal.usda.gov/fnic/foodcomp/Data/SR14/dnload/sr14dnld.html [accessed June 3rd, 2006].

35. Gopalan C, Rama Sastri BV, Balasubramanian SC: Nutritive value of indian foods. Hyderabad, India, Indian Council of Medical Research, National Institute of Nutrition, 1989.

36. Barlow WE, Ichikawa L, Rosner D, Izumi S. Analysis of case-cohort designs. J Clin Epidemiol 1999;52:1165-1172.

37. Gamble MV, Liu X, Ahsan H, et al. Folate, Homocysteine, and Arsenic Metabolism in Arsenic-Exposed Individuals in Bangladesh. E 2005;113:1683-1688.

38. Steinmaus C, Carrigan K, Kalman D, et al. Dietary intake and arsenic methylation in a U.S. population. Environ Health Perspect 2005;113:1153-1159.

39. Mitra SR, Mazumder DN, Basu A, et al. Nutritional factors and susceptibility to arsenic-caused skin lesions in West Bengal, India. Environ Health Perspect 2004;112:1104-1109.

40. Rothman KJ: Modern Epidemiology. Boston/Toronto, Little Brown, 1986.

41. Clark LC, Graham GF, Crounse RG, et al. Plasma selenium and skin neoplasms: a case-control study. Nutr Cancer 1984;6:13-21.

42. Breslow RA, Alberg AJ, Helzlsouer KJ, et al. Serological precursors of cancer: malignant melanoma, basal and squamous cell skin cancer, and prediagnostic levels of retinol. Cancer Epidemiol Biomarkers Prev 1995;4:837-842.

43. Karagas MR, Greenberg ER, Nierenberg D, et al. Risk of squamous cell carcinoma of the skin in relation to plasma selenium, alpha-tocopherol, beta-carotene, and

retinol: a nested case-control study. Cancer Epidemiol Biomarkers Prev 1997;6:25-29.

44. Duffield-Lillico AJ, Slate EH, Reid ME, et al. Selenium supplementation and secondary prevention of nonmelanoma skin cancer in a randomized trial. J Natl Cancer Inst 2003;95:1477-1481.

45. Rayman MP. The importance of selenium to human health. Lancet 2000;356:233-241.

46. Yang G, Zhou R, Yin S, et al. Studies of safe maximal daily dietary selenium intake in a seleniferous area in China. I. Selenium intake and tissue selenium levels of the inhabitants. J Trace Elem Electrolytes Health Dis 1989;3:77-87.

47. Yang GQ, Xia YM. Studies on human dietary requirements and safe range of dietary intakes of selenium in China and their application in the prevention of related endemic diseases. Biomed Environ Sci 1995;8:187-201.

48. Gailer J, George GN, Pickering IJ, et al. Structural basis of the antagonism between inorganic mercury and selenium in mammals. Chem Res Toxicol 2000;13:1135-1142.

49. Berry JP, Galle P. Selenium-arsenic interaction in renal cells: role of lysosomes. Electron microprobe study. J Submicrosc Cytol Pathol 1994;26:203-210.

50. Morton WE, Dunnette DA: Health Effects of Environmental Arsenic; in: Nriagu J O (ed): Arsenic in the Environment, Part II: Human Health and Ecosystem Effects. New York, John Wiley & Sons, Inc., 1994.

51. Uddin AN, Burns FJ, Rossman TG. Vitamin E and organoselenium prevent the cocarcinogenic activity of arsenite with solar UVR in mouse skin. Carcinogenesis 2005;26:2179-2186.

52. Burke KE, Combs GF, Jr., Gross EG, Bhuyan KC, Abu-Libdeh H. The effects of topical and oral L-selenomethionine on pigmentation and skin cancer induced by ultraviolet irradiation. Nutr Cancer 1992;17:123-137.

53. Iyengar GV, Kawamura H, Parr RM, et al. Dietary intake of essential minor and trace elements from Asian diets. Food Nutr Bull 2002;23:124-128.

54. Combs GF, Jr. Selenium in global food systems. Br J Nutr 2001;85:517-547.

55. Longnecker MP, Stram DO, Taylor PR, et al. Use of selenium concentration in whole blood, serum, toenails, or urine as a surrogate measure of selenium intake. Epidemiology 1996;7:384-390.

56. Hall M, Chen Y, Ahsan H, et al. Blood arsenic as a biomarker of arsenic exposure: Results from a prospective study. Toxicology 2006;225:225-233.

Table 1. Characteristics of the 849 Subcohort Members and 303 Newly Diagnosed Skin Lesion Cases in the HEALS Cohort

Characteristic*	Skin lesion cases	Subcohort	Quintile of blood selenium levels in the Subcohort					p-value for trend
			Q1	Q2	Q3	Q4	Q5	
No. of 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	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
Cigarettes 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, % †	8.5	5.1	8.9	3.2	7.9	1.5	5.3	0.32
Mean educational level, years	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 time-weighted well As, 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 ‡								
No. of participants	292	824	162	167	161	164	164	
Protein, g/day	91.6	86.9	84.9	85.7	86.4	89.6	88.7	0.02

Iron, mg/day	26.7	25.1	24.3	24.2	25.0	26.8	25.2	<0.01
Folate, µg/day	137.2	131.7	126.2	129.7	129.7	139.7	134.5	0.05
Vitamin B2, mg/day	1.1	1.1	1.02	1.05	1.05	1.10	1.07	0.01
Vitamin B12, mg/day	1.9	1.9	1.92	1.89	1.77	1.98	1.92	0.82
Vitamin B6, mg/day	3.7	3.5	3.56	3.54	3.55	3.48	3.49	0.12
Vitamin E, mg/day	5.7	5.5	5.2	5.5	5.5	5.6	5.5	0.36
Big fish, g/day	23.5	22.5	21.5	21.6	19.8	23.0	27.9	0.02
Small fish, g/day	34.5	32.2	34.7	36.0	27.7	29.6	30.5	0.18
Bread, g/day	25.5	14.5	7.0	10.5	17.2	15.0	22.8	<0.01
Dried Beans, g/day	86.7	81.0	69.8	74.8	80.0	89.4	89.8	<0.01
Beans, g/day	42.8	37.1	33.8	40.4	36.1	39.6	35.1	0.80
Beef lamb, g/day	13.6	15.4	14.2	13.5	16.9	19.5	13.5	0.51
Poultry, g/day	3.4	4.0	3.2	5.0	4.7	3.3	4.3	0.89
Milk, g/day	32.3	25.7	21.6	22.6	22.2	31.1	30.1	0.01
Eggs, g/day	6.7	8.5	7.6	8.9	8.4	8.4	8.7	0.72

***** Data on body mass index were missing for 4 cases skin lesions and 7 subcohort members. Data were also missing on time-weighted As for, respectively, 18 and 36 subjects; on follow-up total urinary As for 0 and 27 subjects; and on switching status for 0 and 26 subjects.

† 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.

‡ 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 Se in the subcohort were adjusted for age, sex, BMI, and total energy intake.

Table 2. Adjusted Rate Ratios for Skin Lesions by Quintile of Blood Selenium Level

Blood Se Quintile (μg/L)	Mean Blood Se Level (μg/L)	No. of subcohort (%)	No. of cases (%)	Age- & Sex-adjusted Rate Ratios (95% CI) *	Model 1		Model 2	
					No. of subcohort/ cases	Multivariate Adjusted Rate Ratios (95% CI) †	No. of subcohort/ cases	Multivariate Adjusted Rate Ratios (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)

* Rate Ratios were adjusted for age and sex

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

‡ Rate Ratios were adjusted for age, sex, BMI, smoking status, baseline well As, well switching status at follow-up, urinary As 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 As level at the time of follow-up, or dietary intakes of As-related nutrients were excluded from the analysis

Table 3. Relationships of blood Se with Urinary and Blood As in the Subcohort at Baseline

Blood Se Quintile (μg/L)	n	Adjusted means of baseline urinary As (μg/L) *		Adjusted means of baseline blood As (μg/L) †	
		Means (*SD*)	p-value for trend	Means (*SD*)	p-value for trend
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 As concentration, daily water consumption, and urinary creatinine.

† Adjustments were made for baseline age, sex, smoking status, BMI, well As concentration, daily water consumption.

Table 4. Joint Effect of As Exposure and Low Blood Se on Risk of Skin Lesion

As exposure measures [§] (Tertiles)	Blood Se > 150.2 μg/L†			Blood Se ≤ 150.2 μg/L†		
	N (Cases/ Subcohort)	Median As level[‡]	Rate Ratios (95% CI)*	N (Cases/ Subcohort)	Median As level[‡]	Rate Ratios (95% CI)*
Baseline well As levels (μg/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)
Time-weighted water As levels (TWA) (μg/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 (μg/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 (μg/L) [‡‡]						
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)

*RRs were adjusted for age, BMI, sex, and smoking status. RRs in relation to urinary As 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.

[‡] Category-specific median values in the subcohort for each of the four As exposure measures in the left column.

† Cut point was determined based on median value in the subcohort.

[§] Cut points were determined based on tertile values in the subcohort.

[‡‡] RRs associated with total urinary arsenic were additionally adjusted for urinary creatinine level.