Arsenic Exposure from Drinking Water and Risk of Premalignant Skin Lesions in Bangladesh: Baseline Results from the Health Effects of Arsenic Longitudinal Study (HEALS)*

Habibul Ahsan[1,3,7]
Yu Chen[1]
Faruque Parvez[2]
Lydia Zablotska[1]
Maria Argos[1]
AZM Iftikhar Hussain[4]
Hassina Momotaj[4]
Diane Levy[6]
Zhongqi Cheng[5]
Vesna Slavkovich[2]
Alexander van Geen[5]
Geoffrey R. Howe[1]
Joseph H. Graziano[2]

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Authors' affiliations:

[1]Department of Epidemiology, Mailman School of Public Health, Columbia University
[2]Department of Environmental Health Sciences, Mailman School of Public Health, Columbia University
[3]Herbert Irving Comprehensive Cancer Center, Columbia University
[4]National Institute of Preventive and Social Medicine, Bangladesh
[5]Lamont-Doherty Earth Observatory, Columbia University
[6]Department of Biostatistics, Mailman School of Public Health, Columbia University

[7]To whom reprint requests and correspondence should be addressed:
Department of Epidemiology, Mailman School of Public Health, Columbia University Medical Center, 722 West 168th Street, Room 720-G, New York, N.Y. 10032. Phone: (212) 305 7636; Fax: (212) 342 2129; E-mail: habibul.ahsan@columbia.edu.

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Running head:

Dose-response effects of arsenic on skin lesions.

Abbreviations:

Arsenic (As)
Health Effects of Arsenic Longitudinal Study (HEALS)
Graphite furnace atomic absorption (GFAA)
Body mass index (BMI)
Cumulative As index (CAI)
Relative excess risk due to interaction (RERI)

Medical subject headings:

Arsenic exposure; Environmental epidemiology; Skin lesion; Bangladesh; Cross-sectional study; Additive Interaction; Relative excess risk due to interaction (RERI)

Abstract

Millions of individuals around the world are exposed to low doses of arsenic (As) through drinking water. Estimates for health effects associated with low-dose As exposure, however, were based on extrapolations from the high-dose studies. In Bangladesh, a large number of persons have been exposed to a wide range of doses of As from drinking water over a significant period of time. We evaluated dose-response relationships between As exposure from drinking water and pre-malignant skin lesions using baseline data among 11,746 participants in the Health Effects of Arsenic Longitudinal Study (HEALS) in Araihazar, Bangladesh. Several measures of As exposure were estimated for each cohort participant based on well water As concentration and usage pattern of the wells and urinary As concentration. Consistent dose-response effects were observed for all As exposure measures in different regression models. Compared to people drinking water containing <8.1 μg/L of As, adjusted prevalence odds ratios of skin lesions for those drinking water with 8.1-40.0, 40.1-91.0, 91.1-175.0, and 175.1-864.0 μg/L of As were 1.91 (95% CI: 1.26, 2.89), 3.03 (95% CI: 2.05, 4.50), 3.71 (95% CI: 2.53, 5.44), and 5.39 (95% CI: 3.69, 7.86), respectively. The effect of As on skin lesions seemed to be influenced by gender, age, and body mass index. The findings provide information that should be taken into consideration in future research and policy decisions.

Introduction

Millions of persons in the world – including more than three million in the United States and more than 70 million in Bangladesh and adjoining West Bengal, India – are chronically exposed to arsenic (As) through drinking water (1-4). Chronic exposure to As has been associated with a variety of health outcomes including neoplastic (5-9), cardiovascular (10-12), endocrine (13, 14) and neuro-developmental (15, 16) disorders. Several studies have shown that elevated risks of cancers persist even decades after exposure has ceased (17-19).

The International Agency for Research on Cancer classified As as a Group 1 human carcinogen (20). Although health effects of As have been studied extensively, many research questions remain unanswered. First, scientific evidence is sparse for health effects of low-level As exposure. Our knowledge about the health effects of As exposure at doses <100 μg/L is primarily based on extrapolations from high-dose studies (5). Second, most of the studies conducted to date, including cohort studies, have employed retrospective ecological exposure measurements in their dose-response analyses. This is either due to the fact that the exposure had ceased many years prior to the conduct of the study or that the population drank water from multiple sources, making individual-level exposure assessment extremely difficult. In Bangladesh, where the majority of the population uses a single well as their primary source of drinking water, a unique opportunity exists for epidemiological study with chronic As exposure measured directly at the individual level.

We recently established the Health Effects of Arsenic Longitudinal Study (HEALS), a prospective cohort study of nearly 12,000 men and women in Araihazar, Bangladesh, to investigate the health effects of As exposure for doses ranging from very high to very low, utilizing individual-level exposure assessment. In this paper, we report the results of the dose-response effects of As on risk of skin lesions and the influence of key host factors on this association.

Materials and Methods

The overall goal of HEALS is to study short-, intermediate- and long-term health consequences of As exposure from epidemiologic, molecular, and clinical perspectives. Detailed descriptions of the background, purpose, design and methods of HEALS are described elsewhere (21, 22) and are briefly presented here.

Study Area and Study Population

We identified a population exposed to the full dose range of As exposure (0.1 μg/L and 864 μg/L) (23) in a 25 square-kilometer area southeast of the capital city which had not been subject to prior As testing or other As-related research/mitigation activities. In 2000, following identification, enumeration, and As testing of all 5,966 tube wells in the

study area we interviewed the well owners (or their close relatives) to create a roster of all users of the 5,966 tube wells. This source population consisted of 65,876 individuals and was used to sample and recruit cohort participants (23). We identified a total of 14,828 potential study participants who met the following study eligibility criteria: 1) married and between 18 and 75 years of age, 2) resident of the study area for at least five years prior to recruitment, and 3) primary users of one of the 5,966 tube wells, designated as the "index" well, for at least three years. We targeted married individuals mainly to reduce the potential loss to follow-up due to migration because they are less likely than unmarried individuals to move out of the study area during the follow-up period.

Trained study teams consisting of interviewers and physicians visited potential study participants in their homes to recruit them and to perform in-person interviews, including a full dietary instrument (24). In addition, participants were clinically assessed for skin lesions and other health conditions. Biological samples (blood and urine) were also collected. The physicians were blind to the As concentrations in the tube wells. Detailed As exposure information was disseminated to the subjects along with the pertinent health education information (21).

Between October 22, 2000 and May 19, 2002, a total of 11,746 participants (5,042 men and 6,704 women) were recruited into the HEALS from the total 14,828 eligible participants. Nineteen percent of the eligible (n=2,778) were not at home during study visits. Of the 12,050 who were available and approached, 11,746 (97.5% response rate) participated. Eighty-six percent of study participants (n=10,494) shared tube wells with 0-5 other study participants, while the remaining fourteen percent shared their wells with 6-13 individuals.

The study protocol and field procedures were approved by the Columbia University Institutional Review Board and by the Ethical Committee of the Bangladesh Medical Research Council.

Arsenic Exposure Assessment

Water samples from all 5,966 tube wells in the study area were collected in 50 ml acid-washed tubes after pumping the well for 5 minutes. Water As concentrations were analyzed by graphite furnace atomic absorption (GFAA). Details of the methods of sample analysis and quality control procedures have been published elsewhere (25). Since the standard GFAA method has a detection limit of 5 μg/L water samples found to have As concentration at or below the detection limit were reanalyzed by inductively-coupled plasma-mass spectrometry (ICP-MS), which has a detection limit of 0.1 μg/L (26).

In addition to information on the index well, we also collected usage information on any other wells and at least one previous well. The average durations of well use for wells with known As concentration were 10.0 and 8.3 years for males and females, respectively, accounting for an average of 25% of lifetime for both genders. We derived a time-weighted As measure (TWA) as a function of drinking durations and well As

concentrations [TWA in μg/L =$\sum C_i T_i / \sum T_i$, where C_i and T_i denote the well As concentration and drinking duration for the *i*th well]. Eighty-six percent of study participants used the index well as their exclusive source of drinking water. For participants who reported drinking water from a second well, the average concentration of the two wells was considered for the same drinking duration in TWA calculation. In addition, a "cumulative As exposure index" (CAI), was calculated to also incorporate amount of water drank [CAI in mg = C (well water As concentration, mg/L) x Q (daily consumption of well water, L/day) x D (duration of well use, days; 365.25 x duration of well use in years)]. For participants who reported drinking water from a second well, we collected information on the proportion of drinking from each of the wells and included the information into CAI calculation [CAI in mg = $\sum C_i Q_i D$; where C_i and Q_i denote the well As concentration and daily water consumption for the *i*th well]. Similarly, for participants who reported use of a different well as a prior drinking source that was one of our tested wells, we were able to take past exposure into consideration.

A total of 11,224 HEALS participants provided urine samples. Urine samples were stored in coolers until their transfer to -20°C freezers at the end of the day and were batch-shipped on dry ice to Columbia University for further testing. Total urinary As concentration was measured by GFAA, using the Analyst 600 graphite furnace system, as previously described (27). This newer version of the GFAA system has a detection limit of 1 μg/L and therefore no additional ICP-MS analyses were required for urine samples. Urinary creatinine levels were also assayed by a colorimetric Sigma Diagnostics Kit (Sigma, St. Louis, MO) for adjustment of urinary total As concentration.

Assessment and Diagnosis of Premalignant Skin Lesions at Baseline Recruitment:

Non-malignant skin lesions have a short latency period and may appear within a few years of exposure. The typical natural progression of the disease starts with hyperpigmentation of the skin, known as 'melanosis', followed by (or in parallel with) a characteristic bilateral thickening of the palms and soles known as 'hyperkeratosis', which often includes nodular protrusions. The majority of the basal and squamous cell skin cancers among As-exposed individuals are thought to develop from these lesions which are considered precursors of skin cancer (28, 29).

To ensure uniformity of the clinical examination of skin lesions across the entire body, we instituted a structured protocol following the plan for the quantitative assessment of the extent of body surface involvement in burn patients (30). The principle is based on dividing the entire body skin surface into 11 segments (e.g., front of arm, back of arm, face, etc.) and assigning percentages to each of them based on their size relative to the whole body surface. This method requires a physician to record not only the presence/absence of skin lesions in each segment but also to estimate the size, shape and extent of skin involvement. Both male and female physicians performed the examinations to ensure the best possible cooperation from study subjects.

A total of 810 pre-malignant skin lesions were identified at baseline examination of the cohort. Upon further clinical review, 96 of them were determined as cases of either solar

or occupational keratosis and were excluded. This analysis included 714 confirmed cases of pre-malignant skin lesions of which 421 (337 men and 84 women) had only melanosis while the remaining 293 (247 men and 46 women) had both hyperkeratosis and melanosis.

Statistical Analysis

Our primary analysis was to estimate prevalence odds ratio (POR) for skin lesions using unconditional logistic regression modeling. We also estimated prevalence ratio (PR) using log-binomial (31) and Poisson regression models to compare our results under different assumptions to evaluate the robustness of the study findings. Since multiple cohort members shared the same well, we used generalized estimating equations (GEE) for estimating effects while accounting for the correlated errors (32).

We also examined variations in the risk estimates for different types of pre-malignant skin lesions (i.e., melanosis vs. hyperkeratosis) by employing polytomous logistic regression models comparing each of the different types of skin lesions using the non-diseased cohort members as a common referent group.

In addition, to assess the linear relationship between As exposure and risk of skin lesions we estimated excess relative risk (ERR) and excess absolute risk (EAR) measures (33). The general model for the linear ERR takes the form: $R_D = R_0 [1.0 + \beta_1 D]$, where R_D is the risk of skin lesions at exposure D, R_0 is the background risk (parametrically adjusted for potential confounders), β_1 is the ERR, and D is the estimate of As exposure. Adjusted parameter estimates from this model can be directly (i.e., without exponentiation) interpreted as the increase in risk of skin lesions per unit dose of exposure in this population. Thus, any risk associated with dose multiplies the background risk, and the relationship between risk and dose is linear. The general model for the linear EAR takes the form: $R_D = R_0 + \beta_1 D$, where β_1 is the estimate of EAR which can be interpreted as an excess of cases for a given size of the population per unit dose of exposure above the background. In this paper, we estimated EAR per 10,000 persons.

Finally, we explored the joint effects of As and key host characteristics (gender, age, and BMI) on risk of skin lesions. The statistical significance of the joint effect of As exposure and host characteristics was assessed by estimating relative excess risk due to interaction (RERI) and its 95% confidence intervals as suggested by Hosmer and Lemeshaw (34). RERI is estimated as follows:

$$RERI \approx POR_{1k} - POR_{10} - POR_{0k} + 1$$

Where POR_{1k} indicates POR for skin lesion comparing participants with As exposure at k level and a hypothesized more susceptible attribute (i.e. male gender) to the reference group, i.e., participants with lowest As exposure level and a less susceptible attribute (i.e. female gender); POR_{0k} indicates POR for skin lesion comparing participants with As exposure at k level alone to the reference group and POR_{10} denotes POR for skin lesion comparing participants with a more susceptible attribute (i.e. male gender) alone to the reference group.

In all analyses, we adjusted for the following *a priori* defined confounding variables: age, gender, cigarette smoking, socioeconomic status indicators, sun exposure, and body mass index (BMI). Variables measured on a continuous scale, including As exposure, were categorized based on their distribution among the total cohort members. In descriptive analysis, we included all 11,438 participants who underwent a physical examination and had a defined skin lesion diagnosis. In subsequent regression analysis, we included 10,951 participants with complete data on duration of well water use and all other covariates in the model. Distributions of As exposure and skin lesion status were similar between the 487 participants with missing data on any of the covariates and the overall study population (data not shown). We used the GMBO module of the Epicure software (33) to conduct linear analysis of the data. Estimation of PORs and PRs was performed using Statistical Analysis Software (SAS) version 8.0.

Results

As shown in Table 1, males were more than four times likely to have skin lesions than females (POR = 4.15, 95% CI: 3.27, 5.26). Older age was positively associated with the risk of skin lesions in the study population. Compared to the participants in the lowest age group (less than 30 years), the risk of skin lesions increased nearly five-fold for participants in the highest age group (60+ years). There was a general inverse trend of the association between BMI and skin lesion risk. Cigarette smoking, hukka smoking, and markers of socioeconomic status in the rural Bangladeshi population including education and land ownership were also associated with the risk of skin lesions in this cohort when As exposure was held constant in the analysis.

The POR estimates increased monotonically with levels of As exposure and the dose-dependent increases were evident for all three measures of As exposure (Table 2, Figure 1). Of particular note is the observation that the risk was significantly higher for the exposure group with 8.1-40 μg/L of TWA, compared to the lowest exposure group (<8.1 μg/L of TWA). While the creatinine-adjusted urinary As and CAI categories do not directly correspond to TWA categories, the elevated risks were statistically significant also for the second lowest category of these two measures. PORs are considered a closer estimate than PRs for incidence rate ratios (35). PR estimates based on log-binomial and Poisson regression models, although slightly towards the null (as expected), were very similar to POR estimates (differences were <10%) and therefore results are not shown. When we evaluated the dose-dependent effect of As separately for early-staged (melanosis) and late-staged (hyperkeratosis) skin lesions, the results were similar for all three measures of exposure (results not shown).

In linear dose-response analyses, we estimated that a 10 μg/L increase of As concentration in the tube well water was associated with an ERR of 0.122 (95% CI: 0.087, 0.171), i.e., those exposed to As doses of 10 μg/L had a 1.22 times higher risk of developing skin lesions compared to those with zero dose (Table 3). We estimated ERRs of 0.416 and 0.008 per 10 μg/g increase in urinary As adjusted for creatinine and per 10 mg increase in CAI, respectively. Based on estimates from linear EAR models in a cohort

of 10,000 people, in one year, exposure to 10 μg/L of As from well water may lead to 14 excess cases of non-malignant skin lesions above the background occurrence typical for this population. The corresponding excess numbers for 10 μg As/g creatinine and CAI were 10 and 2 cases of skin lesions, respectively, per year per 10,000 people.

Joint effects of As and host factors (gender, age, and BMI) on the risk of pre-malignant skin lesions are presented in Tables 4-6. Patterns of PORs and RERIs were similar when we used urinary arsenic and therefore results were not shown. Because RERI is a measure of the differences in risk ratios, if the 95% confidence interval around its point estimate excludes zero, there will be evidence of synergy between two risk factors at the $p<0.05$ level. Males appeared to be disproportionally more susceptible to skin lesions than females at higher levels of TWA /CAI (Table 4). RERIs of higher levels of TWA/CAI and male gender were statistically significant and were greater at higher levels of TWA/CAI, indicating that the synergism between As exposure and male gender status was stronger for higher levels of As exposure categories.

At each level of TWA/CAI, older participants were more susceptible to skin lesions than their younger counterparts (Table 5). The synergistic effects between higher levels of TWA and older age were statistically significant. Analysis results based on CAI show a similar pattern of PORs and RERIs. The calculation of CAI incorporated exposure time. Therefore, when we used CAI as the measure of As exposure to evaluate the influence of older age, exposure time was accounted for. When compared to participants in the highest tertile of BMI and lowest quintile of TWA/CAI, we observed a trend for the adjusted PORs to be higher for participants with the highest levels of TWA/CAI and lower levels of BMI than participants with the highest levels of TWA/CAI and the highest level of BMI (Table 6). The synergistic effects of a very low level of BMI (< 18.1) with the highest two quintiles of TWA and the highest quintile of CAI were statistically significant. We have also assessed joint effects of As exposure and age and BMI in men and women separately. Patterns of PORs and RERIs were similar in men and women and therefore only the results for the overall study population were shown.

Discussion

In this paper, we report findings from cross-sectional analysis of the baseline data from HEALS, a prospective cohort study with individual-level exposure and outcome data. Because of the wide range of As exposure in the HEALS study population and the relatively large sample size, we were able to estimate and report dose-response relationships even at the very low end of the As exposure range.

We observed a dose-response effect of As on the risk of skin lesions based on all statistical models. In particular, As exposure seems to increase the risk of skin lesions even at the low end of exposure in this population. Of the three measures of As exposure used in this study, well water As concentration gives the most direct measure for assessing disease risk that can be directly incorporated into public policy decisions.

This study clearly provides evidence that a population exposed to well water As concentrations <50 μg/L is at risk for skin lesions. Previous studies in other countries, including those in Bangladesh and West Bengal, have failed to show any increased risk at the lower As dose range. This was partly because those studies lacked sufficient sample size at the low-level As exposure (36-38). An obvious difference between the rural population of Bangladesh and other studied populations is that the Bangladesh rural population consumes a large amount of water compared to other populations (2.5 to 3 liters per day on average versus <1 liter in the US). Moreover, almost 100% of drinking water for this population comes from 1-2 wells with relatively stable As concentration, while in the US people usually drink water from multiple sources.

We found that male, older, and/or thinner participants were more likely to be affected by As exposure. The more pronounced effect of As exposure in men is consistent with other studies conducted in Bangladesh and elsewhere (36, 38, 39). It is possible that hormonal and other biological differences between men and women could be responsible for part of the gender differences in the skin lesion risks. Although this line of evidence has yet to be examined in humans, animal data have shown that As interacts with steroid hormones (37). Women in rural Bangladesh tend to cover their body more extensively than men. Although our study employed female physicians for examining female participants, it is plausible that there was some underascertainment of skin lesions for females in our study population if some of the women did not allow a full body examination under sufficient light. However, when we restricted the analyses by locations of skin lesions in body; the increased risk of skin lesions in the trunk for male participants was not statistically greater than that for female participants (data not shown). On the other hand, if sun exposure acts as a causal partner in As-induced skin disease, then women, due to their reduced exposure to sun, would have a lower risk of skin lesions. While the evidence of interactions between sun exposure and As exposure has been mainly suggested by animal and in-vitro experiments (40) we have also observed such evidence in our cohort (41).

The stronger effect of As exposure on skin lesion risk among older participants has also been reported in other studies in Bangladesh and other countries (36, 38, 39). The calculation of CAI incorporated exposure time, and the median values of CAI within CAI quintiles in the two age groups are comparable. Since the excess risk for older participants persists when As was measured by CAI, it is possible that biological factors associated with aging, rather than longer exposure time per se, are related to the susceptibility to As-induced skin lesions. For example, it is possible that the enzyme systems responsible for detoxification of As are less active in older individuals. Other potential mechanisms responsible for age-related susceptibility to As toxicity include decreased immune function and decreased DNA repair (42, 43). The biologic reactions to As toxicity, once initiated, may vary in different age groups depending on immune system status and alterations in other regulatory factors such as angiogenesis.

Our study also found some evidence that participants with higher BMI were at a lower risk of skin lesions than participants with lower BMI (Table 6). Previous studies in West Bengal also found that prevalence of skin lesions was higher among people with lower body weight. However, BMI was not considered and joint effect of As exposure and

body weight on risk of skin lesion was not formally evaluated (44). Lower BMI reflects poorer nutritional status in rural Bangladesh, which could directly or indirectly influence the effect of As. In particular, poor nutritional status may be associated with lower intake of the antioxidants, folates and/or dietary proteins that are necessary for the metabolism and detoxification of As in the body (45, 46).

Several limitations of the study need to be discussed. First, many participants drank water from a single well, making well water As concentration a shared characteristic. However, we have employed the GEE method in estimating the effect of As to handle the correlated errors arising from shared wells. Second, the present study included prevalent cases and thus may be susceptible to the survival bias. However, since skin lesions themselves are not fatal it is unlikely that the study preferentially included skin diseases with prolonged survival. Third, the assessment of As exposure based on current well As concentration may have introduced non-differential measurement errors. However, analyses for time-series samples collected from 20 tube wells in the study area have showed that the standard deviation of groundwater As concentrations was <10 μg/L over 3 years (47). Although information on continuing As exposure was available for 9 years on average, differences in prior As exposure might have masked some of the underlying gradients in the observed dose-response relationship. Additionally, this study did not consider individual metabolites of As in urine or blood. We are addressing the possible role of As metabolism on disease risk in a nested case-control study and the findings of which will be reported in the future.

In conclusion, this study reports a strong dose-response effect of As exposure on skin lesion risk in Bangladesh. This dose-response effect was uniformly evident in several statistical models appropriate for analyzing cross-sectional data. There was an increased risk even among the population consuming water containing As less than 50 μg/L – the currently permissible limit in Bangladesh and other countries and in the US until very recently. This risk appears to be influenced by gender, age, and BMI, at least in a subset of individuals. These findings need to be taken into consideration for policy-making decisions.

References

1. The British Geological Survey. Groundwater studies for arsenic contamination in Bangladesh-Phase 1 findings, 1999. (http://www.bgs.ac.uk/arsenic/).

2. Rahman MM, Chowdhury UK, Mukherjee SC, et al. Chronic arsenic toxicity in Bangladesh and West Bengal, India--a review and commentary. J Toxicol Clin Toxicol 2001;39:683-700.

3. Arsenic Occurrence in Public Drinking Water Supplies (EPA-815-R-00-023 / December 2000), 2000. (http://www.epa.gov/safewater/ars/occurrence.pdf)

4. Fact sheet about the Janurary 2001 arsenic rule (EPA 815-F-00-015), 2001. (http://www.epa.gov/safewater/ars/ars_rule_factsheet.html)

5. Brown KG, Boyle KE, Chen CW, et al. A dose-response analysis of skin cancer from inorganic arsenic in drinking water. Risk Anal 1989;9:519-28.

6. Hopenhayn-Rich C, Biggs ML, and Smith AH. Lung and kidney cancer mortality associated with arsenic in drinking water in Cordoba, Argentina. Int J Epidemiol 1998;27:561-9.

7. Chen CJ and Wang CJ. Ecological correlation between arsenic level in well water and age-adjusted mortality from malignant neoplasms. Cancer Res 1990;50:5470-4.

8. Buchet JP and Lison D. Mortality by cancer in groups of the Belgian population with a moderately increased intake of arsenic. Int Arch Occup Environ Health 1998;71:125-30.

9. Hertz-Picciotto I and Smith AH. Observations on the dose-response curve for arsenic exposure and lung cancer. Scand J Work Environ Health 1993;19:217-26.

10. Chiou HY, Huang WI, Su CL, et al. Dose-response relationship between prevalence of cerebrovascular disease and ingested inorganic arsenic. Stroke 1997;28:1717-23.

11. Chen CJ, Chiou HY, Chiang MH, et al. Dose-response relationship between ischemic heart disease mortality and long-term arsenic exposure. Arterioscler Thromb Vasc Biol 1996;16:504-10.

12. Chen CJ, Hsueh YM, Lai MS, et al. Increased prevalence of hypertension and long-term arsenic exposure. Hypertension 1995;25:53-60.

13. Lai MS, Hsueh YM, Chen CJ, et al. Ingested inorganic arsenic and prevalence of diabetes mellitus. Am J Epidemiol 1994;139:484-92.

14. Tseng CH, Tai TY, Chong CK, et al. Long-term arsenic exposure and incidence of non-insulin-dependent diabetes mellitus: a cohort study in arseniasis-hyperendemic villages in Taiwan. Environ Health Perspect 2000;108:847-51.

15. Tsai SY, Chou HY, The HW, et al. The effects of chronic arsenic exposure from drinking water on the neurobehavioral development in adolescence. Neurotoxicology 2003;24:747-53.

16. Wasserman GA, Xinhua L., Parvez F, et al. Water Arsenic Exposure and Children's Intellectual Function in Araihazar, Bangladesh. Environ Health Perspect 2004;112:1329-33.

17. Chiou HY, Chiou ST, Hsu YH, et al. Incidence of transitional cell carcinoma and arsenic in drinking water: a follow-up study of 8,102 residents in an arseniasis-endemic area in northeastern Taiwan. Am J Epidemiol 2001;153:411-8.

18. Chen CJ, Kuo TL, and Wu MM. Arsenic and cancers. Lancet 1988;1:414-5.

19. Chen CJ, Chuang YC, Lin TM, et al. Malignant neoplasms among residents of a blackfoot disease-endemic area in Taiwan: high-arsenic artesian well water and cancers. Cancer Res 1985;45:5895-9.

20. Arsenic in drinking-water. International Agency for Research on Cancer (IARC) - Summaries & Evaluations 2004;84:39.

21. Ahsan H, Chen Y, Parvez F, et al. Health Effects of Arsenic Longitudinal Study (HEALS): Description of a Multidisciplinary Epidemiologic Investigation. J Expo Anal Environ Epidemiol 2005; advance online publication (doi: 10.1038/sj.jea.7500449).

22. 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 2005; advance online publication (doi:10.1289/ehp.7903).

23. 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.

24. Chen Y, Ahsan H, Parvez F, et al. Validity of a food-frequency questionnaire for a large prospective cohort study in Bangladesh. Br J Nutr 2004;92:851-9.

25. van Geen A, Ahsan H, Horneman A, et al. Promotion of well-switching to mitigate the current arsenic crisis in Bangladesh. Bulletin of the World Health Organization 2002;80:732-7.

26. Cheng Z, Zheng Y, Mortlock R, et al. Rapid multi-element analysis of groundwater by high-resolution inductively coupled plasma mass spectrometry. Anal Bioanal Chem 2004;379:512-8.

27. Nixon DE, Mussmann GV, Eckdahl SJ, et al. Total arsenic in urine: palladium-persulfate vs nickel as a matrix modifier for graphite furnace atomic absorption spectrophotometry. Clin Chem 1991;37:1575-9.

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

29. Liu J, Zheng B, Aposhian HV, et al. Chronic arsenic poisoning from burning high-arsenic-containing coal in Guizhou, China. Environ Health Perspect. 2002;110:119-22.

30. Demling RH. Burn and other thermal injuries. In: Way LW, ed. Current surgical diagnosis and treatment 10th ed. Norwalk, CT, Appleton and Lange, 1994:205-15.

31. Barros AJ and Hirakata VN. Alternatives for logistic regression in cross-sectional studies: an empirical comparison of models that directly estimate the prevalence ratio. BMC Med Res Methodol 2003;3:21.

32. Hanley JA, Negassa A, Edwardes MD, et al. Statistical analysis of correlated data using generalized estimating equations: an orientation. Am J Epidemiol 2003;157:364-75.

33. Preston DL, Lubin JH, Pierce DA, McConney ME: EPICURE User's Guide. Seattle, WA: Hirosoft International Corporation, 1993.

34. Hosmer DW and Lemeshow S. Confidence interval estimation of interaction. Epidemiology 1992;3:452-6.

35. Pearce N. Effect measures in prevalence studies. Environ Health Perspect 2004;112:1047-50.

36. Mazumder DN, Haque R, Ghosh N, et al. Arsenic in drinking water and the prevalence of respiratory effects in West Bengal, India. Int J Epidemiol 2000;29:1047-52.

37. Waalkes MP, Liu J, Chen H, et al. Estrogen signaling in livers of male mice with hepatocellular carcinoma induced by exposure to arsenic in utero. J Natl Cancer Inst 2004;96:466-74.

38. Tondel M, Rahman M, Magnuson A, et al. The relationship of arsenic levels in drinking water and the prevalence rate of skin lesions in Bangladesh. Environ Health Perspect 1999;107:727-9.

39. Haque R, Mazumder DN, Samanta S, et al. Arsenic in drinking water and skin lesions: dose-response data from West Bengal, India. Epidemiology 2003;14:174-82.

40. Rossman TG, Uddin AN, Burns FJ, et al. Arsenite is a cocarcinogen with solar ultraviolet radiation for mouse skin: an animal model for arsenic carcinogenesis. Toxicol Appl Pharmacol 2001;176:64-71.

41. 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; In press.

42. Anisimov VN. The relationship between aging and carcinogenesis: a critical appraisal. Crit Rev Oncol Hematol 2003;45:277-304.

43. Wei Q. Effect of aging on DNA repair and skin carcinogenesis: a minireview of population-based studies. J Investig Dermatol Symp Proc 1998;3:19-22.

44. Guha Mazumder DN, Haque R, Ghosh N, et al. Arsenic levels in drinking water and the prevalence of skin lesions in West Bengal, India. Int J Epidemiol 1998;27:871-7.

45. Gamble MV, Liu X, Ahsan H, et al. Folate, homocysteine, and arsenic metabolism in arsenic-exposed individuals in Bangladesh. Environ Health Perspect 2005;113:1683-8.

46. Steinmaus C, Carrigan K, Kalman D, et al. Dietary intake and arsenic methylation in a U.S. population. Environ Health Perspect 2005;113:1153-9.

47. Cheng Z, van Geen A, Seddique AA, et al. Limited temporal variability of arsenic concentrations in 20 wells monitored for 3 years in Araihazar, Bangladesh. Environ Sci Technol 2005;39:4759-66.

Table 1. Distributions of Demographic, Anthropometric, and Lifestyle Variables by Status of Skin Lesions

	Skin lesions		Adjusted Prevalence Odds Ratios*
	Yes (N=714) n (%)	No (N=10724) n (%)	
Sex			
Female	130 (18.2)	6432 (60.0)	1.00
Male	584 (81.8)	4292 (40.0)	4.15 (3.27-5.26)
Age			
< 30	43 (6.0)	2852 (26.6)	1.00
30-39	178 (24.9)	3848 (35.9)	2.15 (1.50-3.07)
40-49	256 (35.9)	2670 (24.9)	3.74 (2.59-5.41)
50-59	187 (26.2)	1150 (10.7)	4.52 (3.03-6.73)
60+	50 (7.0)	204 (1.9)	4.99 (3.04-8.19)
Mean (SD)	44.3 (9.8)	36.6 (10.0)	
Body Mass Index			
<17.2	197 (27.8)	2075 (19.4)	1.00
17.2-18.5	168 (23.7)	2106 (19.8)	0.94 (0.76-1.18)
18.6-19.9	146 (20.6)	2127 (20.0)	1.01 (0.79-1.27)
20.0-22.2	113 (15.9)	2160 (20.3)	0.82 (0.64-1.06)
22.3+	85 (12.0)	2187 (20.5)	0.76 (0.57-1.02)
Missing	5	69	
Mean (SD)	18.9 (2.7)	19.8 (3.2)	
Education (years)			
0	371 (52.0)	4702 (43.9)	1.00
1-5	210 (29.4)	3180 (29.7)	0.94 (0.78-1.13)
6-9	72 (10.1)	1638 (15.3)	0.72 (0.54-0.96)
10-16	61 (8.5)	1198 (11.1)	0.70 (0.51-0.97)
Missing	0	6	
Mean (SD)	2.7 (3.6)	3.5 (3.9)	
Land ownership (acres)			
0	382 (53.6)	5387 (50.3)	1.00
<1	232 (32.5)	3351 (31.3)	0.95 (0.79-1.15)

≥1	89 (12.5)	1742 (16.3)	0.67 (0.50-0.88)
Don't know how much	10 (1.4)	228 (2.1)	1.51 (0.72-3.21)
Missing	1	16	
Cigarettes or bidi Smoking			
Non-smokers	208 (29.1)	7197 (67.2)	1.00
Past-smokers	110 (15.4)	645 (6.0)	1.01 (0.77-1.32)
Current smokers ≤10 sticks/day	219 (30.7)	1734 (16.2)	1.17 (0.86-1.59)
Current smokers >10 sticks/day	177 (24.8)	1137 (10.6)	1.08 (0.80-1.45)
Missing	0	11	
Hukka smoking			
Non-smokers	380 (53.3)	9127 (85.2)	1.00
Past-smokers	295 (41.3)	1463 (13.7)	1.44 (1.16-1.79)
Current users <5 times/day	21 (2.9)	69 (0.6)	2.30 (1.33-3.98)
Current users 5+ times/day	18 (2.5)	54 (0.5)	1.62 (0.91-2.90)
Missing	0	11	

* Prevalence Odds Ratios were adjusted for all other variables on the table as well as well arsenic concentration (in quintiles).

Table 2. Prevalence Odds Ratios for Skin Lesions by Levels of Arsenic Exposure

As exposure measures (Quintiles)	Overall (n = 10951)				Drank water exclusively from the index well (n =9468)
	Median in each category	Total n	Cases	PORs (95% CI) †	PORs (95% CI) †
Time-weighted well arsenic concentration (μg/l) ‡ §					
0.1-8.0	1.8	2259	57	1.00	1.00
8.1-40.0	23.0	2122	90	1.91 (1.26-2.89)	1.88 (1.20-2.94)
40.1-91.0	62.0	2202	144	3.03 (2.05-4.50)	3.32 (2.18-5.05)
91.1-175.0	125.0	2185	162	3.71 (2.53-5.44)	3.78 (2.50-5.71)
175.1-864.0	255.0	2183	242	5.39 (3.69-7.86)	5.70 (3.80-8.55)
Cumulative As exposure index (mg) ‡ **					
0.1-48.1	10.5	2191	53	1.00	1.00
48.2-226.4	119.7	2190	90	1.83 (1.25-2.69)	1.83 (1.21-2.77)
226.5-582.6	373.6	2190	122	2.53 (1.72-3.71)	2.46 (1.62-3.72)
582.7-1485.8	925.7	2190	162	3.62 (2.50-5.23)	3.84 (2.57-5.74)
1485.9-9609.0	2727.5	2190	268	5.49 (3.82-7.90)	5.73 (3.87-8.47)
Urinary creatinine-adjsuted arsenic (μg/g creatitine) ‡ #					
6.6-90.1	62.5	2129	60	1.00	1.00
90.2-158.4	122.8	2126	99	1.75 (1.23-2.48)	1.65 (1.14-2.41)
158.5-243.4	197.1	2128	129	2.33 (1.67-3.26)	2.43 (1.68-3.51)
243.5-396.5	303.7	2128	153	3.08 (2.19-4.35)	2.90 (2.00-4.20)
396.6-4306.0	590.7	2127	239	5.29 (3.78-7.41)	5.49 (3.81-7.92)
Unavailable		298	15		

† Prevalence odds ratios were estimated with GEE methods and adjusted for age (<30, 30-39, 40-49, 50-59, and 60+), gender, BMI (quintiles), education (0, 1-5, 6-9, 10+ years), cigarettes smoking (never, past, current), hukka usage (never, past, current), sun exposure in males (yes/no), and land ownership (0, <1, 1+ acres, don't know how much).

‡ Cut points were determined according to quintile values among the overall study population.

Table 3. Estimates of Excess Relative Risks (ERR) and Excess Absolute Risks (EAR) of Skin Lesions in Relation to Arsenic Exposure[†]

Arsenic exposure measure	Adjusted ERR (95% CI) [‡]	Adjusted EAR (95% CI) [‡] (excess cases per 10,000 person-years)
Time-weighted water arsenic levels, per 10 μg/L	0.122 (0.087, 0.171)	13.970 (9.737, 18.680)
Time-weighted water arsenic levels, per decile (86.4 μg/L)	1.053 (0.748, 1.473)	--
Urinary creatinine-adjusted As, per 10 μg/g of creatinine *	0.416 (0.217, 0.105)	10.110 (6.791, 13.790)
Urinary creatinine-adjusted As, per decile (129.3 μg/g of creatinine) *	5.37 (2.803, 13.630)	--
Cumulative As index, per 10 mg	0.008 (0.006, 0.011)	1.630 (1.218, 2.089)
Cumulative As index, per decile (1987.5 mg)	1.600 (1.141, 2.209)	--

[†] excluding those with missing occupation and urinary arsenic information; all analyses are based on 10,604 subjects.
[‡] Adjusted for gender, age at risk, BMI, education, smoking, and occupation.
* Additionally adjusted for categories of urinary creatinine.

Table 4. Prevalence Odds Ratios[†] for Skin Lesions by Levels of Arsenic Exposure and Gender

As exposure measures (Quintiles)	Women			Men			Dose-specific RERI
	Total N /cases	Median As level[‡]	Prevalence Odds Ratios	Total N /cases	Median As level[‡]	Prevalence Odds Ratios	
Time-weighted water arsenic (μg/L)							
0.1-8.0	1287/12	1.8	1.00	980/47	1.8	3.61 (1.79-7.28)	
8.1-40.0	1218/15	23.0	1.59 (0.65-3.89)	897/72	23.0	6.88 (3.09-15.32)	2.68 (-0.04-5.40)
40.1-91.0	1269/27	63.0	2.82 (1.20-6.61)	923/118	62.0	11.30 (5.11-24.99)	5.87 (0.83-10.91)*
91.1-175.0	1245/24	125.0	2.53 (1.07-5.97)	946/141	126.0	14.04 (6.39-30.87)	8.90 (1.72-16.08)*
175.1-864.0	1248/48	256.7	4.81 (2.12-10.88)	938/191	254.0	19.04 (8.70-41.65)	11.62 (2.24-21.00)*
Cumulative As exposure index (mg)							
0.1-48.1	1226/9	10.6	1.00	965/44	10.4	4.28 (2.10-8.72)	
48.2-226.4	1249/8	119.7	1.17 (0.50-2.75)	941/82	119.7	8.45 (3.93-18.18)	4.01 (0.45-7.57)*
226.5-582.6	1268/23	376.3	2.78 (1.20-6.41)	922/99	369.5	10.79 (4.97-23.41)	4.74 (0.36-9.11)*
582.7-1485.8	1308/35	934.9	3.92 (1.74-8.84)	882/127	904.9	15.07 (6.95-32.71)	7.87 (1.19-14.56)*
1485.9-9609.0	1216/51	2612.7	5.26 (2.36-11.71)	974/217	2912.6	24.31 (11.35-52.09)	15.78 (3.47-28.08)*

[†] Prevalence odds ratios and prevalence ratios were adjusted for age (<30, 30-39, 40-49, 50-59, and 60+), BMI (quintiles), education (0, 1-5, 6-9, 10+ years), cigarettes smoking (never, past, current), hukka usage (never, past, current), sun exposure in males (yes/no), and land ownership (0, <1, 1+ acres, don't know how much).
[‡] Median values of time-weighted water arsenic levels or cumulative As exposure index within each category.
* $p < 0.05$ for RERI estimates.

Table 5. Prevalence Odds Ratios[†] for Skin Lesions by Levels of Arsenic Exposure and Age

As exposure measures (Quintiles)	Age ≤36			Age >36			Dose-specific RERI
	Total N /cases	Median As level[‡]	Prevalence Odds Ratios	Total N /cases	Median As level[‡]	Prevalence Odds Ratios	
Time-weighted water arsenic levels (μg/L)							
0.1-8.0	1146/13	2.0	1.00	1121/45	1.6	1.96 (1.01-3.79)	
8.1-40.0	1122/12	23.0	0.89 (0.38-2.10)	993/75	22.3	4.08 (2.09-8.00)	2.23 (0.66-3.80)*
40.1-91.0	1147/26	63.0	2.13 (1.02-4.45)	1045/119	62.0	6.42 (3.34-12.37)	3.34 (1.01-5.66)*
91.1-175.0	1133/36	125.0	3.01 (1.48-6.13)	1058/129	126.0	7.30 (3.81-14.01)	3.33 (0.93-5.73)*
175.1-864.0	1130/68	256.3	5.61 (2.83-11.11)	1056/171	255.4	9.67 (5.06-18.47)	3.10 (0.41-5.78)*
Cumulative As exposure index (mg)							
0.1-48.1	1134/9	11.7	1.00	1057/44	9.5	3.14 (1.46-6.75)	
48.2-226.4	1140/12	120.2	1.82 (0.77-4.33)	1050/78	118.8	5.77 (2.65-12.56)	1.80 (-0.29-3.90)
226.5-582.6	1180/19	373.4	2.13 (0.89-5.05)	1010/103	373.7	8.26 (3.84-17.78)	4.00 (0.74-7.25)*
582.7-1485.8	1172/42	927.9	5.15 (2.30-11.51)	1018/120	922.3	10.05 (4.68-21.55)	2.76 (0.42-5.93)*
1485.9-9609.0	1052/73	2609.8	8.19 (3.74-17.95)	1138/195	2792.1	15.92 (7.48-33.88)	5.58 (0.62-10.55)*

[†] Prevalence odds ratios and prevalence ratios were adjusted for gender, BMI (quintiles), education (0, 1-5, 6-9, 10+ years), cigarettes smoking (never, past, current), hukka usage. (never, past, current), sun exposure in males (yes/no), and land ownership (0, <1, 1+ acres, don't know how much).

[‡] Median values of time-weighted water arsenic levels or cumulative As exposure index within each category.

* $p < 0.05$ for RERI estimates.

Table 6. Prevalence Odds Ratios[†] for Skin Lesions by Levels of Arsenic Exposure and Body Mass Index

As exposure measures (Quintiles)	BMI > 20.4			BMI 18.1-20.4			BMI < 18.1			Dose-specific RERI BMI < 18.1 vs. > 20.4
	Total N /cases	Median As level[‡]	Prevalence Odds Ratios	Total N /cases	Median As level[‡]	Prevalence Odds Ratios	Total N /cases	Median As level[‡]	Prevalence Odds Ratios	
Time-weighted water arsenic levels (μg/L)										
0.1-8.0	837/23	1.8	1.00	701/14	1.7	0.77 (0.39-1.55)	729/22	1.9	0.71 (0.38-1.32)	
8.1-40.0	719/21	23.0	1.25 (0.64-2.44)	715/30	24.0	1.63 (0.88-3.02)	681/36	22.0	1.84 (1.03-3.32)	0.88 (0.01-1.77)
40.1-91.0	762/40	61.9	2.40 (1.34-4.29)	753/48	64.2	2.53 (1.42-4.49)	677/57	62.0	2.67 (1.52-4.69)	0.57 (-0.55-1.68)
91.1-175.0	727/32	126.0	2.25 (1.25-4.07)	714/55	126.0	3.26 (1.87-5.69)	750/78	124.2	3.58 (2.07-6.19)	1.62 (0.36-2.88)*
175.1-864.0	679/45	259.0	2.96 (1.63-5.37)	731/82	257.0	4.75 (2.76-8.17)	776/112	252.0	5.25 (3.07-8.99)	2.59 (0.75-4.42)*
Cumulative As exposure index (mg)										
0.1-48.1	798/20	10.5	1.00	678/14	10.4	0.93 (0.46-1.90)	715/19	10.9	0.75 (0.40-1.41)	
48.2-226.4	769/22	122.7	1.40 (0.76-2.57)	718/25	118.3	1.43 (0.77-2.63)	703/43	118.7	2.08 (1.17-3.70)	0.93 (0.04-1.83)
226.5-582.6	723/32	370.9	2.18 (1.18-4.00)	759/38	368.8	2.10 (1.16-3.82)	708/52	382.2	2.55 (1.44-4.52)	0.63 (-0.51-1.77)
582.7-1485.8	753/41	920.4	2.58 (1.43-4.65)	718/60	933.5	3.86 (2.20-6.77)	719/61	923.5	3.14 (1.78-5.52)	0.80 (-0.45-2.07)
1485.9-9609.0	681/46	2691.4	3.24 (1.79-5.88)	741/92	2834.9	5.14 (3.00-8.80)	768/130	2669.3	6.17 (3.61-10.55)	3.18 (1.07-5.29)*

[†] Prevalence odds ratios and prevalence ratios were adjusted for gender, age, education (0, 1-5, 6-9, 10+ years), cigarettes smoking (never, past, current), hukka usage. (never, past, current), sun exposure in males (yes/no), and land ownership (0, <1, 1+ acres, don't know how much).

[‡] Median values of time-weighted water arsenic levels or cumulative As exposure index within each category.

*$p < 0.05$ for RERI estimates.

Figure 1. Adjusted prevalence odds ratios (POR) from the categorical analysis of time-weighted well arsenic concentration and a fitted dose-response line.