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Original Contribution

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

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Millions of persons around the world are exposed to low doses of arsenic through drinking water. However, estimates of health effects associated with low-dose arsenic exposure have been extrapolated from high-dose studies. In Bangladesh, many persons have been exposed to a wide range of doses of arsenic from drinking water over a significant period of time. The authors evaluated dose-response relations between arsenic exposure from drinking water and premalignant skin lesions by using baseline data on 11,746 participants recruited in 2000–2002 for the Health Effects of Arsenic Longitudinal Study in Araihazar, Bangladesh. Several measures of arsenic exposure were estimated for each participant based on well-water arsenic concentration and usage pattern of the wells and on urinary arsenic concentration. In different regression models, consistent dose-response effects were observed for all arsenic exposure measures. Compared with drinking water containing <8.1 μg/liter of arsenic, drinking water containing 8.1–40.0, 40.1–91.0, 91.1–175.0, and 175.1–864.0 μg/liter of arsenic was associated with adjusted prevalence odds ratios of skin lesions of 1.91 (95% confidence interval (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 seemed to be influenced by gender, age, and body mass index. These findings provide information that should be considered in future research and policy decisions.

arsenic; Bangladesh; cross-sectional studies; environmental exposure; keratosis; melanosis; risk

Abbreviations: CAI, cumulative arsenic index; HEALS, Health Effects of Arsenic Longitudinal Study; POR, prevalence odds ratio; RERI, relative excess risk due to interaction; TWA, time-weighted arsenic concentration.

Millions of persons in the world—including more than 3 million in the United States and more than 70 million in Bangladesh and adjoining West Bengal, India—are chronically exposed to arsenic through drinking water (1–4). Chronic exposure to arsenic has been associated with a variety of health outcomes, including neoplastic (5–9), cardiovascular (10–12), endocrine (13, 14), and neurodevelopmental (15, 16) disorders. Several studies have shown that an elevated risk of cancers persists even decades after exposure has ceased (17–19).

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The International Agency for Research on Cancer has classified arsenic as a group 1 human carcinogen (20). Although health effects of arsenic have been studied extensively, many research questions remain unanswered. First, scientific evidence is sparse regarding health effects of low-level arsenic exposure. Our knowledge about the health effects of arsenic exposure at doses of less than 100 μg/liter is based primarily on extrapolations from high-dose studies (5). Second, most of the studies conducted to date, including cohort studies, have used retrospective ecologic exposure measurements in their dose-response analyses because either the exposure had ceased many years before the study was conducted or the population drank water from multiple sources, making assessment of individual-level exposure 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 epidemiologic study of chronic arsenic 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 arsenic 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 arsenic 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 arsenic 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 arsenic (0.1–864 μg/liter) (23) in a 25-km^2 area southeast of the capital city that had not been subject to prior arsenic testing or other arsenic-related research/mitigation activities. In 2000, following identification, enumeration, and arsenic 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 these wells. This source population consisted of 65,876 persons and was used to sample and recruit cohort participants (23). We identified 14,828 potential study participants who met the following study eligibility criteria: 1) married and between 18 and 75 years of age, 2) residing in the study area for at least 5 years prior to recruitment, and 3) primary user of one of the 5,966 tube wells, designated as the ''index'' well, for at least 3 years. We targeted married persons mainly to reduce potential loss to follow-up due to migration because they were less likely than unmarried persons 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. Biologic samples (blood and urine) were also collected. The physicians were blinded to the arsenic concentrations in the tube wells. Detailed arsenic exposure information was disseminated to the subjects, along with pertinent health education information (21).

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

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 the well was pumped for 5 minutes. Water arsenic concentrations were analyzed by graphite furnace atomic absorption. Details of the methods of sample analysis and quality control procedures have been published elsewhere (25). Since the standard method of graphite furnace atomic absorption has a detection limit of 5 μg/liter, water samples found to have arsenic concentration at or below the detection limit were reanalyzed by inductively coupled plasma–mass spectrometry, which has a detection limit of 0.1 μg/liter (26).

In addition to information on the index well, we also collected usage data on any other wells and at least one previous well. The average durations of well use for wells with a known arsenic concentration were 10.0 years for males and 8.3 years for females, accounting for an average of 25 percent of their lifetime for both genders. We derived a time-weighted arsenic concentration (TWA) as a function of drinking durations and well arsenic concentrations (TWA in μg/liter $= \sum C_i T_i / \sum T_i$, where C_i and T_i denote the well arsenic concentration and drinking duration for the ith 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 the TWA calculation. In addition, a ''cumulative arsenic index'' (CAI) was calculated to also incorporate the amount of water consumed [CAI in mg $= C$ (well water arsenic concentration in mg/liter) $\times$ Q (daily consumption of well water in liters/day) $\times$ D (duration of well use in days; 365.25 $\times$ 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 in the CAI calculation (CAI in mg $= \sum C_i Q_i D$, where C_i and Q_i denote the well arsenic concentration and daily water consumption for the ith 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. The 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 arsenic concentration was measured by graphite furnace atomic absorption using the Analyst 600 graphite furnace system, as previously described (27). This newer version of the graphite furnace atomic absorption system has a detection limit of 1 μg/liter; therefore, no additional inductively coupled plasma–mass spectrometry analyses were required for urine samples. Urinary creatinine levels were also assayed by using a colorimetric Sigma Diagnostics Kit (Sigma, St. Louis, Missouri) for adjustment of urinary total arsenic concentration.

Assessment and diagnosis of premalignant skin lesions at baseline recruitment

Nonmalignant 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 in arsenic-exposed persons are thought to develop from these lesions, which are considered precursors of skin cancer (28, 29).

To ensure uniformity in the clinical examination of skin lesions across the entire body, we instituted a structured protocol following the plan for 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) and assigning percentages to each of them based on their size relative to the whole body surface. This method requires a physician to not only record 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 premalignant 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 these participants were excluded. The present analysis included 714 confirmed cases of premalignant skin lesions; 421 of the participants (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 designed to estimate prevalence odds ratios (PORs) for skin lesions using unconditional logistic regression modeling. We also estimated the prevalence ratio by 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 to estimate effects while accounting for the correlated errors (32).

We also examined variations in the risk estimates for different types of premalignant skin lesions (i.e., melanosis vs. hyperkeratosis) by using polytomous logistic regression models. The models compared each of the different types of skin lesions by using nondiseased cohort members as a common referent group.

In addition, to assess the linear relation between arsenic exposure and risk of skin lesions, we estimated excess relative risk and excess absolute risk measures (33). The general model for the linear excess relative risk takes the following 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 excess relative risk, and D is the estimate of arsenic 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 relation between risk and dose is linear. The general model for the linear excess absolute risk takes this form: $R_D = R_0 + \beta_1 D$, where β_1 is the estimate of excess absolute risk, 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 analysis, we estimated the excess absolute risk per 10,000 persons.

Finally, we explored the joint effects of arsenic and key host characteristics (gender, age, and body mass index) on risk of skin lesions. The statistical significance of the joint effect of arsenic exposure and host characteristics was assessed by estimating relative excess risk due to interaction (RERI) and its 95 percent confidence intervals, as suggested by Hosmer and Lemeshow (34). RERI is estimated as follows: RERI $\approx \mathrm{POR}_{1k} - \mathrm{POR}_{10} - \mathrm{POR}_{0k} + 1$, where POR_{1k} indicates the POR for skin lesion comparing participants with arsenic exposure at k level and a hypothesized more susceptible attribute (e.g., male gender) with the reference group, that is, participants with the lowest arsenic exposure level and a less susceptible attribute (e.g., female gender); POR_{0k} indicates the POR for skin lesion comparing participants with arsenic exposure at k level alone with the reference group; and POR_{10} denotes the POR for skin lesion comparing participants with a more susceptible attribute (e.g., male gender) alone with the reference group.

In all analyses, we adjusted for the following confounding variables defined a priori: age, gender, cigarette smoking, socioeconomic status indicators, sun exposure, and body mass index. Variables measured on a continuous scale, including arsenic exposure, were categorized on the basis of

TABLE 1. Distribution of demographic, anthropometric, and lifestyle variables by status of skin lesions, baseline data from the Health Effects of Arsenic Longitudinal Study, Araihazar, Bangladesh, October 2000–May 2002

Variable	Skin lesions				Adjusted prevalence odds ratio*	95% CI†
	Yes ($n = 714$)		No ($n = 10,724$)			
	No.	%	No.	%		
Gender						
Female	130	18.2	6,432	60.0	1.00	
Male	584	81.8	4,292	40.0	4.15	3.27, 5.26
Age (years)						
<30	43	6.0	2,852	26.6	1.00	
30–39	178	24.9	3,848	35.9	2.15	1.50, 3.07
40–49	256	35.9	2,670	24.9	3.74	2.59, 5.41
50–59	187	26.2	1,150	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 (9.77)		36.6 (10.0)			
Body mass index‡						
<17.2	197	27.8	2,075	19.4	1.00	
17.2–18.5	168	23.7	2,106	19.8	0.94	0.76, 1.18
18.6–19.9	146	20.6	2,127	20.0	1.01	0.79, 1.27
20.0–22.2	113	15.9	2,160	20.3	0.82	0.64, 1.06
≥22.3	85	12.0	2,187	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	4,702	43.9	1.00	
1–5	210	29.4	3,180	29.7	0.94	0.78, 1.13
6–9	72	10.1	1,638	15.3	0.72	0.54, 0.96
10–16	61	8.5	1,198	11.1	0.70	0.51, 0.97
Missing	0		6			
Mean (SD)	2.7 (3.6)		3.5 (3.9)	3.9		
Land ownership (acres§)						
0	382	53.6	5,387	50.3	1.00	
<1	232	32.5	3,351	31.3	0.95	0.79, 1.15
≥1	89	12.5	1,742	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			
Cigarette or bidi smoking						
Nonsmoker	208	29.1	7,197	67.2	1.00	
Past smoker	110	15.4	645	6.0	1.01	0.77, 1.32
Current smoker of ≤10 sticks/day	219	30.7	1,734	16.2	1.17	0.86, 1.59
Current smoker of >10 sticks/day	177	24.8	1,137	10.6	1.08	0.80, 1.45
Missing	0		11			
Hukka smoking¶						
Nonsmoker	380	53.3	9,127	85.2	1.00	
Past smoker	295	41.3	1,463	13.7	1.44	1.16, 1.79
Current smoker <5 times/day	21	2.9	69	0.6	2.30	1.33, 3.98
Current smoker ≥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 in the table as well as for well arsenic concentration (in quintiles).
† CI, confidence interval; SD, standard deviation.
‡ Weight (kg)/height $(m)^2$.
§ One acre = 4,047 m^2.
¶ Tobacco smoking using water pipes.

TABLE 2. Prevalence odds ratios for skin lesions by levels of arsenic exposure, baseline data from the Health Effects of Arsenic Longitudinal Study, Araihazar, Bangladesh, October 2000–May 2002

Arsenic exposure measure (quintiles)	Overall ($n = 10,951$)					Study participants who drank water exclusively from the index well ($n = 9,468$)	
	Median in each category	Total no.	No. of cases	POR*	95% CI†	POR*	95% CI
Time-weighted well arsenic concentration (μg/liter)‡							
0.1–8.0	1.8	2,259	57	1.00	1.00	1.00	
8.1–40.0	23.0	2,122	90	1.91	1.26, 2.89	1.88	1.20, 2.94
40.1–91.0	62.0	2,202	144	3.03	2.05, 4.50	3.32	2.18, 5.05
91.1–175.0	125.0	2,185	162	3.71	2.53, 5.44	3.78	2.50, 5.71
175.1–864.0	255.0	2,183	242	5.39	3.69, 7.86	5.70	3.80, 8.55
Cumulative arsenic index (mg)‡							
0.1–48.1	10.5	2,191	53	1.00	1.00	1.00	
48.2–226.4	119.7	2,190	90	1.83	1.25, 2.69	1.83	1.21, 2.77
226.5–582.6	373.6	2,190	122	2.53	1.72, 3.71	2.46	1.62, 3.72
582.7–1,485.8	925.7	2,190	162	3.62	2.50, 5.23	3.84	2.57, 5.74
1,485.9–9,609.0	2,727.5	2,190	268	5.49	3.82, 7.90	5.73	3.87, 8.47
Urinary creatinine-adjusted arsenic (μg/g of creatinine)‡							
6.6–90.1	62.5	2,129	60	1.00	1.00	1.00	
90.2–158.4	122.8	2,126	99	1.75	1.23, 2.48	1.65	1.14, 2.41
158.5–243.4	197.1	2,128	129	2.33	1.67, 3.26	2.43	1.68, 3.51
243.5–396.5	303.7	2,128	153	3.08	2.19, 4.35	2.90	2.00, 4.20
396.6–4,306.0	590.7	2,127	239	5.29	3.78, 7.41	5.49	3.81, 7.92
Unavailable		298	15				

* Prevalence odds ratios (PORs) were estimated by using generalized estimating equation methods and were adjusted for age (<30, 30–39, 40–49, 50–59, ≥60 years), gender, body mass index (quintiles), education (0, 1–5, 6–9, ≥10 years), cigarette smoking (never, past, current), hukka smoking (tobacco smoking using water pipes: never, past, current), sun exposure in males (yes/no), and land ownership (0, <1, ≥1 acres (1 acre = 4,047 m^2), don't know how much).

† CI, confidence interval.

‡ Cutpoints were determined according to quintile values for the overall study population.

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 in the model 10,951 participants for whom data on duration of well water use and all other covariates were complete. Distributions of arsenic exposure and skin lesion status were similar between the 487 participants for whom data on any of the covariates were missing and the overall study population (data not shown). We used the GMBO module of the EPICURE software (33) program to conduct linear analysis of the data. PORs and prevalence ratios were estimated by using Statistical Analysis Software, version 8.0 (SAS Institute, Inc., Cary, North Carolina).

RESULTS

As shown in table 1, males were more than four times more likely than females to have skin lesions (POR = 4.15, 95 percent confidence interval: 3.27, 5.26). Older age was positively associated with risk of skin lesions in the study population. Compared with that for participants in the youngest age group (<30 years), the risk of skin lesions increased nearly fivefold for participants in the oldest age group (≥60 years). We found a general inverse trend regarding the association between body mass index and skin lesion risk. Cigarette smoking, hukka smoking (tobacco smoking using water pipes), 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 arsenic exposure was held constant in the analysis.

The POR estimates increased monotonically with levels of arsenic exposure, and the dose-dependent increases were evident for all three measures of arsenic 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/liter TWA than for the lowest exposure group (<8.1 μg/liter of TWA). Although the creatinine-adjusted urinary arsenic and CAI categories do not directly correspond to TWA categories, the elevated risks were also statistically significant for the second lowest category of these two measures.

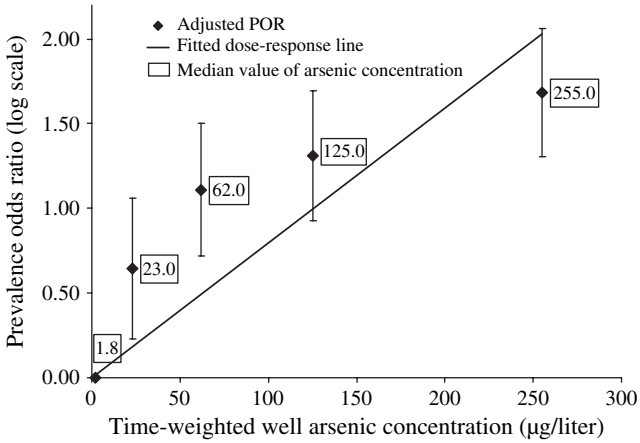

FIGURE 1. Adjusted prevalence odds ratios (PORs) from categorical analysis of time-weighted well arsenic concentrations and a fitted dose-response line, baseline results from the Health Effects of Arsenic Longitudinal Study, Araihazar, Bangladesh, October 2000–May 2002. The PORs were adjusted for gender, age, education (0, 1–5, 6–9, ≥10 years), cigarette smoking (never, past, current), hukka smoking (tobacco smoking using water pipes: never, past, current), sun exposure in males (yes/no), and land ownership (0, <1, ≥1 acres (1 acre = 4,047 m^2), don't know how much). Vertical bars, 95% confidence intervals.

PORs are considered a closer estimate than prevalence ratios for incidence rate ratios (35). Prevalence ratio estimates based on log-binomial and Poisson regression models, although slightly toward the null (as expected), were very similar to POR estimates (differences were <10 percent); therefore, results are not shown here. When we evaluated the dose-dependent effect of arsenic separately for early-stage (melanosis) and late-stage (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/liter increase in arsenic concentration in the tube well water was associated with an excess relative risk of 0.122 (95 percent confidence interval: 0.087, 0.171); that is, those exposed to arsenic doses of 10 μg/liter had a 1.22 times higher risk of developing skin lesions compared with those whose dose was zero (table 3). We estimated excess relative risks of 0.416 and 0.008 per 10-μg/g increase in urinary arsenic adjusted for creatinine and per 10-mg increase in CAI, respectively. On the basis of estimates from linear models of excess absolute risk in a cohort of 10,000 persons, in one year, exposure to 10 μg/liter of arsenic from well water may lead to 14 excess cases of nonmalignant skin lesions above the background occurrence typical for this population. The corresponding excess numbers for 10 μg of arsenic/g creatinine and CAI were 10 cases and two cases of skin lesions, respectively, per year per 10,000 persons.

Joint effects of arsenic and host factors (gender, age, and body mass index) on the risk of premalignant skin lesions are presented in tables 4–6. Patterns of PORs and RERIs were similar when we used urinary arsenic; therefore, results are not shown. Because RERI is a measure of the differences in risk ratios, if the 95 percent 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 arsenic exposure and male gender status was stronger for categories of higher levels of arsenic exposure.

TABLE 3. Estimates of excess relative risks and excess absolute risks of skin lesions in relation to arsenic exposure,* baseline data from the Health Effects of Arsenic Longitudinal Study, Araihazar, Bangladesh, October 2000–May 2002

Arsenic exposure measure	Adjusted excess relative risk†	95% CI‡	Adjusted excess absolute risk† (excess cases per 10,000 person-years)	95% CI
Time-weighted water arsenic concentration per 10 μg/liter	0.12	0.09, 0.17	13.97	9.74, 18.68
Time-weighted water arsenic concentration per decile (86.4 μ/liter)	1.05	0.75, 1.47		
Urinary creatinine-adjusted arsenic per 10 μg/g of creatinine§	0.42	0.22, 0.11	10.11	6.79, 13.79
Urinary creatinine-adjusted arsenic per decile (129.3 μg/g of creatinine)§	5.37	2.80, 13.63		
Cumulative arsenic index per 10 mg	0.01	0.01, 0.01¶	1.63	1.22, 2.09
Cumulative arsenic index per decile (1,987.5 mg)	1.60	1.14, 2.21		

* Excluding those study participants for whom occupation and urinary arsenic information were missing; all analyses are based on 10,604 subjects.

† Adjusted for gender, age at risk, body mass index, education, smoking, and occupation.

‡ CI, confidence interval.

§ Additionally adjusted for categories of urinary creatinine.

¶ Point estimate of 0.008 and 95% CI of 0.006, 0.011 were rounded to two decimal places.

TABLE 4. Prevalence odds ratios for skin lesions by levels of arsenic exposure and gender, baseline data from the Health Effects of Arsenic Longitudinal Study, Araihazar, Bangladesh, October 2000–May 2002

Arsenic exposure measure (quintiles)	Women					Men					Dose-specific RERI	95% CI†
	Total no.	No. of cases	Median arsenic level‡	POR§	95% CI	Total no.	No. of cases	Median arsenic level‡	POR§	95% CI		
Time-weighted water arsenic concentration (μg/liter)												
0.1–8.0	1,287	12	1.8	1.00		980	47	1.8	3.61	1.79, 7.28		
8.1–40.0	1,218	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	1,269	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	1,245	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	1,248	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 arsenic index (mg)												
0.1–48.1	1,226	9	10.6	1.00		965	44	10.4	4.28	2.10, 8.72		
48.2–226.4	1,249	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	1,268	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*
582.7–1,485.8	1,308	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*
1,485.9–9,609.0	1,216	51	2,612.7	5.26	2.36, 11.71	974	217	2,912.6	24.31	11.35, 52.09	15.78	3.47, 28.08*

* $p < 0.05$ for relative excess risk due to interaction (RERI) estimates.

† CI, confidence interval.

‡ Median values of time-weighted water arsenic concentrations or cumulative arsenic index within each category.

§ Prevalence odds ratios (PORs) were adjusted for age (<30, 30–39, 40–49, 50–59, ≥60 years), body mass index (quintiles), education (0, 1–5, 6–9, ≥10 years), cigarette smoking (never, past, current), hukka smoking (tobacco smoking using water pipes: never, past, current), sun exposure in males (yes/no), and land ownership (0, <1, ≥1 acres (1 acre = 4,047 m^2), don't know how much).

TABLE 5. Prevalence odds ratios for skin lesions by levels of arsenic exposure and age, baseline data from the Health Effects of Arsenic Longitudinal Study, Araihazar, Bangladesh, October 2000–May 2002

Arsenic exposure measure (quintiles)	Age ≤36 years					Age >36 years					Dose-specific RERI	95% CI†
	Total no.	No. of cases	Median arsenic level‡	POR§	95% CI	Total no.	No. of cases	Median arsenic level‡	POR§	95% CI		
Time-weighted water arsenic concentration (μg/liter)												
0.1–8.0	1,146	13	2.0	1.00		1,121	45	1.6	1.96	1.01, 3.79		
8.1–40.0	1,122	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	1,147	26	63.0	2.13	1.02, 4.45	1,045	119	62.0	6.42	3.34, 12.37	3.34	1.01, 5.66*
91.1–175.0	1,133	36	125.0	3.01	1.48, 6.13	1,058	129	126.0	7.30	3.81, 14.01	3.33	0.93, 5.73*
175.1–864.0	1,130	68	256.3	5.61	2.83, 11.11	1,056	171	255.4	9.67	5.06, 18.47	3.10	0.41, 5.78*
Cumulative arsenic index (mg)												
0.1–48.1	1,134	9	11.7	1.00		1,057	44	9.5	3.14	1.46, 6.75		
48.2–226.4	1,140	12	120.2	1.82	0.77, 4.33	1,050	78	118.8	5.77	2.65, 12.56	1.80	−0.29, 3.90
226.5–582.6	1,180	19	373.4	2.13	0.89, 5.05	1,010	103	373.7	8.26	3.84, 17.78	4.00	0.74, 7.25*
582.7–1,485.8	1,172	42	927.9	5.15	2.30, 11.51	1,018	120	922.3	10.05	4.68, 21.55	2.76	0.42, 5.93*
1,485.9–9,609.0	1,052	73	2,609.8	8.19	3.74, 17.95	1,138	195	2,792.1	15.92	7.48, 33.88	5.58	0.62, 10.55*

* $p < 0.05$ for relative excess risk due to interaction (RERI) estimates.

† CI, confidence interval.

‡ Median values of time-weighted water arsenic concentrations or cumulative arsenic index within each category.

§ Prevalence odds ratios (PORs) were adjusted for gender, body mass index (quintiles), education (0, 1–5, 6–9, ≥10 years), cigarette smoking (never, past, current), hukka smoking (tobacco smoking using water pipes: never, past, current), sun exposure in males (yes/no), and land ownership (0, <1, ≥1 acres (1 acre = 4,047 m^2), don't know how much).

TABLE 6. Prevalence odds ratios for skin lesions by levels of arsenic exposure and body mass index,† baseline data from the Health Effects of Arsenic Longitudinal Study, Araihazar, Bangladesh, October 2000–May 2002

Arsenic exposure measure (quintiles)	Body mass index >20.4					Body mass index 18.1–20.4					Body mass index <18.1					Dose-specific RERI‡	95% CI§
	Total no.	No. of cases	Median arsenic level¶	POR#	95% CI	Total no.	No. of cases	Median arsenic level¶	POR#	95% CI	Total no.	No. of cases	Median arsenic level¶	POR#	95% CI		
Time-weighted water arsenic concentration (μg/liter)																	
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 arsenic 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
582.7–1,485.8	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
1,485.9–9,609.0	681	46	2,691.4	3.24	1.79, 5.88	741	92	2,834.9	5.14	3.00, 8.80	768	130	2,669.3	6.17	3.61, 10.55	3.18	1.07, 5.29*

* $p < 0.05$ for relative excess risk due to interaction (RERI) estimates.

† Weight (kg)/height $(m)^2$.

‡ Body mass index <18.1 vs. >20.4.

§ CI, confidence interval.

¶ Median values of time-weighted water arsenic concentrations or cumulative arsenic index within each category.

Prevalence odds ratios (PORs) were adjusted for gender, age, education (0, 1–5, 6–9, ≥10 years), cigarette smoking (never, past, current), hukka smoking (tobacco smoking using water pipes: never, past, current), sun exposure in males (yes/no), and land ownership (0, <1, ≥1 acres (1 acre = 4,047 m^2), don't know how much).

At each level of TWA/CAI, older participants were more susceptible than their younger counterparts to skin lesions (table 5). The synergistic effects between higher levels of TWA and older age were statistically significant. Analysis results based on CAI showed a similar pattern of PORs and RERIs. The calculation of CAI incorporated exposure time. Therefore, when we used CAI as the measure of arsenic exposure to evaluate the influence of older age, exposure time was accounted for. When we analyzed data for participants in the highest tertile of body mass index and the 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 body mass index than for participants with the highest levels of TWA/CAI and the highest level of body mass index (table 6). The synergistic effects of a very low level of body mass index (<18.1) with the highest two quintiles of TWA and the highest quintile of CAI were statistically significant. We also assessed joint effects of arsenic exposure with age and body mass index in men and women separately. Patterns of PORs and RERIs were similar in men and women; therefore, the results for only the overall study population are given.

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 arsenic exposure in the HEALS study population and the relatively large sample size, we were able to estimate and report dose-response relations even at the very low end of the arsenic exposure range.

We observed a dose-response effect of arsenic on the risk of skin lesions based on all statistical models. In particular, arsenic exposure seems to increase the risk of skin lesions even at the low end of exposure in this population. Of the three measures of arsenic exposure we used, well water arsenic 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 currently exposed to well water arsenic concentrations of less than 50 μg/liter is at risk for skin lesions. Previous studies in other countries, including Bangladesh and West Bengal, India, have failed to show any increased risk at the lower arsenic dose range, partly because they lacked sufficient sample size at low levels of arsenic exposure (36–38). An obvious difference between the rural population of Bangladesh and other studied populations is that this population consumes a large amount of water (2.5–3 liters per day on average vs. <1 liter in the United States). Moreover, almost 100 percent of the drinking water for this population comes from one or two wells with relatively stable concentrations of arsenic, while, in the United States, people usually drink water from multiple sources.

We found that male, older, and/or thinner participants were more likely to be affected by arsenic exposure. The finding of a more pronounced effect of arsenic exposure in men is consistent with other studies conducted in Bangladesh and elsewhere (36, 38, 39). It is possible that hormonal and other biologic differences between men and women could be responsible for part of the gender differences in the skin lesion risks. Although, to our knowledge, this line of evidence has yet to be examined in humans, animal data have shown that arsenic interacts with steroid hormones (37). Women in rural Bangladesh tend to cover their bodies more extensively than men do. Although female physicians examined female participants in our study, 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 on the body, the increased risk of skin lesions on 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 arsenic-induced skin disease, then women, because of their reduced exposure to sun, would have a lower risk of skin lesions. While the evidence of interactions between sun exposure and arsenic 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 arsenic exposure on skin lesion risk among older participants has also been reported in other studies in Bangladesh and other countries (36, 38, 39). Our 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 persisted when arsenic was measured by CAI, it is possible that biologic factors associated with aging, rather than longer exposure time per se, are related to susceptibility to arsenic-induced skin lesions. Perhaps the enzyme systems responsible for detoxification of arsenic are less active in older persons. Other potential mechanisms responsible for age-related susceptibility to arsenic toxicity include decreased immune function and decreased DNA repair (42, 43). The biologic reactions to arsenic 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 a higher body mass index were at lower risk of skin lesions than participants with a lower body mass index (table 6). Previous studies in West Bengal found that prevalence of skin lesions was higher among people with a lower body weight. However, body mass index was not considered, and the joint effect of arsenic exposure and body weight on risk of skin lesions was not formally evaluated (44). Lower body mass index reflects poorer nutritional status in rural Bangladesh, which could directly or indirectly influence the effect of arsenic. In particular, poor nutritional status may be associated with lower intake of the antioxidants, folates, and/or dietary proteins necessary for metabolism and detoxification of arsenic in the body (45, 46).

Several limitations of this study need to be discussed. First, many participants drank water from a single well, making well water arsenic concentration a shared characteristic. However, we used the method of generalized estimating equations to assess the effect of arsenic to handle the

correlated errors arising from shared wells. Second, the present study included prevalent cases and thus may be susceptible to survival bias. However, since skin lesions themselves are not fatal, it is unlikely that the study preferentially included skin diseases associated with prolonged survival. Third, assessment of arsenic exposure based on current well arsenic concentration may have introduced nondifferential measurement errors. However, analyses for time-series samples collected from 20 tube wells in the study area have shown that the standard deviation of groundwater arsenic concentrations was less than 10 μg/liter over 3 years (47). Although information on continuing arsenic exposure was available for 9 years on average, differences in prior arsenic exposure might have masked some of the underlying gradients in the observed dose-response relation. Additionally, this study did not consider individual metabolites of arsenic in urine or blood. We are currently addressing the possible role of arsenic metabolism in disease risk in a nested case-control study.

In conclusion, this study reports a strong dose-response effect of arsenic 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 less than 50 μg/liter of arsenic—the currently permissible limit in Bangladesh and other countries, and in the United States until very recently. This risk appears to be influenced by gender, age, and body mass index, at least in a subset of persons. These findings need to be considered when formulating policy-making decisions.

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