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Arsenic in Drinking Water and Skin Lesions: Dose-Response Data from West Bengal, India

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Background. Over 6 million people live in areas of West Bengal, India, where groundwater sources are contaminated with naturally occurring arsenic. The key objective of this nested case-control study was to characterize the dose-response relation between low arsenic concentrations in drinking water and arsenic-induced skin keratoses and hyperpigmentation.

Methods. We selected cases (persons with arsenic-induced skin lesions) and age- and sex-matched controls from participants in a 1995–1996 cross-sectional survey in West Bengal. We used a detailed assessment of arsenic exposure that covered at least 20 years. Participants were reexamined between 1998 and 2000. Consensus agreement by four physicians reviewing the skin lesion photographs confirmed the diagnosis in 87% of cases clinically diagnosed in the field.

Results. The average peak arsenic concentration in drinking water was 325 μg/liter for cases and 180 μg/liter for controls. The average latency for skin lesions was 23 years from first exposure. We found strong dose-response gradients with both peak and average arsenic water concentrations.

Conclusions. The lowest peak arsenic ingested by a confirmed case was 115 μg/liter. Confirmation of case diagnosis and intensive longitudinal exposure assessment provide the basis for a detailed dose-response evaluation of arsenic-caused skin lesions.

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Key words: arsenic, keratoses, hyperpigmentation, drinking water, case-control, dose-response.

Over 6 million people live in areas of West Bengal, India where groundwater from tube wells is contaminated with naturally occurring arsenic.[1] Tube wells were dug during the 1950s and 1960s as part of a state-wide irrigation plan to provide clean water and thereby reduce deaths attributable to diarrheal diseases.[2] As residents switched to using the groundwater rather than water obtained from ponds and rivers, they inadvertently became exposed to arsenic. Reports of individuals with skin keratoses and hyperpigmentation, two hallmark signs of arsenic toxicity, first emerged in the mid-1980s.[2–3] Skin lesions pose an important public health problem because advanced forms of keratoses are painful and debilitating, and the subsequent disfigurement can lead to social isolation in the villages. The arsenic-induced skin lesions may be associated with increased risks of skin, bladder and lung cancers,[4–6] although increased cancer risks may result even without skin lesions being present.[7] Prior studies of skin lesions have been cross-sectional in nature, and have only focussed on recent or current exposures.[8,9,11–32]

We previously reported on the association of levels of arsenic in drinking water with the prevalence of keratoses and hyperpigmentation in West Bengal.[8] Clear exposure-response relations were found for water-arsenic levels and the prevalence of these arsenic-induced skin effects. We also identified cases who apparently consumed low levels of arsenic (<50 μg/liter). However, the survey examined only the participants' primary current drinking-water source. Here, we present a nested case-control study to examine the dose-response pattern

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for the arsenic-induced skin lesions using detailed exposure assessment. The exposure assessment incorporates arsenic concentration data from current and past water sources used in households and work sites.

Methods

Source Population

The source population included 7683 individuals who participated in a 1995–1996 population-based cross-sectional survey of the 24 South Parganas, a rural district located south of Calcutta.[8] The survey identified 415 individuals with signs of arsenic-induced skin lesions. Water samples were collected from the primary current drinking-water source of each participant. Arsenic concentrations in the tube wells ranged from nondetectable to 3400 μg/liter (mean = 185 μg/liter; standard deviation = 290). Because of our interest in examining effects at low doses, the case-control study was based on survey participants living in 21 villages whose primary drinking-water sources contained less than 500 μg/liter of inorganic arsenic (N = 4,185; 2160 females and 2025 males). The study protocol was approved by the Institutional Review Boards of both the Institute of Post Graduate Medical Education and Research, Calcutta, and the University of California, Berkeley. Informed consent was obtained before administering the questionnaire.

Case Criteria

Cases included all individuals from the cross-sectional survey who were diagnosed with arsenic-induced skin lesions and whose main water source contained less than 500 μg/liter of arsenic. Of the 265 identified cases, 174 had pigmentation changes, 15 had keratoses and 76 had both types of lesions. In advanced cases, hyperpigmentation and keratoses caused by arsenic are quite distinctive, but mild cases are difficult to diagnose.[8] Hyperpigmentation is marked by raindrop-shaped discolored spots, diffuse dark-brown spots, or diffuse darkening of the skin on the limbs and trunk. Simple keratoses usually appear as bilateral thickening of the palms and soles. In nodular keratosis, small protrusions emerge on the palms and soles, and also occasionally on the dorsum of the hands and feet, or on the legs.

Control Selection

We selected controls from survey participants who did not have skin keratoses or hyperpigmentation when seen during the 1995–1996 survey, and whose main tube well–water source, like the cases, contained <500 μg arsenic/liter. For each case, one control matched on age (±5 years) and sex was randomly identified from all eligible noncases. Replacement controls were selected for controls who had died, could not be located or did not wish to participate. Because controls and cases were investigated concurrently, we had a small group of "extra" controls at the end of the study. These "extra" controls were included in analyses that were not confined to the matched pairs.

Interviews

Participants were visited in their homes between April 1998 and January 2000. A physician interviewer who was blind to case or control status administered a structured questionnaire in Bengali. The questionnaire assessed the following information: lifetime residential history, current and past water sources at home and work sites, current and past (5 years prior) fluid consumption patterns, smoking habits and sociodemographic characteristics. In India, socioeconomic status is often measured by type of house, which is correlated with household economic status.[33] Houses were differentiated between those built of high-quality materials (concrete or brick) and those constructed of mud or thatched natural fibers.

Clinical Exam and Photographic Review

All participants underwent a full medical examination conducted according to a written protocol. A careful examination of the skin was conducted in a well-lit area outdoors, under natural light. Visible or palpable dermal lesions were documented noting the location, appearance and whether the patterns were characteristic of arsenic-induced skin toxicity.

Part way through the project, we purchased a camera to photograph participants with suspected arsenic-induced skin lesions. The interviewer photographed the most highly affected skin areas. Four project physicians later reviewed the slides. After joint review and discussion, the physicians classified the skin lesions (by consensus agreement) as definitely, probably, possibly or not related to arsenic. Dermal changes "definitely" or "probably" induced by arsenic were classified as a current skin lesion. Participants for whom slides were not available were classified as currently having a skin lesion if the physician interviewer recorded on the questionnaire that the dermal changes were of a type related to arsenic.

Water Sample Collection

The field team collected water samples from all functioning tube wells used by participants for at least 6 months in the last 20 years. Samples were also obtained from tube wells used in earlier years if they were still functioning. When available, such measurements were incorporated in the data analyses. Many wells were closed because of damaged filters, mechanical problems causing high amounts of suspended sand or scanty flow, or arsenic contamination. For some of these closed tube

wells, we obtained historical arsenic concentration measurements. For 12 closed wells, samples were obtained from the closest (proxy) tube wells that were of the same depth as the closed well, and located within the same hamlet (group of houses). We collected samples from approximately 800 functioning tube wells in the 21 villages combined. Private tube wells were sometimes used by just one household, whereas government tube wells were used by multiple families. Water samples were stored in a cooler containing an ice block and transported to the laboratory in Calcutta on the same day. The water samples were then kept frozen at −20°C until they were transported on dry ice to the University of Washington for arsenic analysis.

Total water arsenic was measured by flow injection analysis using atomic fluorescence detection with inline photooxidation and continuous hydride generation.[34] The lower limit of quantitation was <0.2 μg/liter. Each sample was assayed twice (mean percent relative standard deviation = 2.3%).

Statistical Methods

Information about tube well usage at each residence and work site and the results of the arsenic measurements were used to construct arsenic exposure histories. We estimated peak, average and cumulative arsenic exposure. Annual average water concentrations were first calculated for participants for each calendar year based on the measured water arsenic concentrations for each tube well used in that year, and the fraction of their drinking water participants obtained from that source in that year.

We defined peak arsenic water concentration (μg/liter) as the highest known annual average water concentration of arsenic ingested by a participant. The peak concentration was estimated from the year the participant first started using tube wells up to 1995–1996. We next calculated the cumulative arsenic exposure (μg/liter-year) for each participant by summing the annual average drinking-water arsenic concentration. In years for which arsenic measurements could not be obtained, the cumulative exposure estimation treated these missing years as zero. Average arsenic exposure (μg/liter) was estimated by dividing the cumulative arsenic exposure by the total number of years in which arsenic drinking-water concentrations were known. To calculate the cumulative and average exposures for closed tube wells, we used historical or proxy measurements if these data were available. A few participants reported they had at times used pond water (surface water) for drinking. Because the arsenic concentrations were very low or nondetectable in three pond samples (ranging from <0.2 to 4.2 μg/liter), we used zero as the concentration for all pond water sources.

Descriptive analyses were conducted by comparing general characteristics, mean arsenic water concentrations, fluid consumption, and the number of tube wells used by case and control status. We stratified peak and average arsenic concentrations in drinking water and we estimated odds ratios (OR) with 95% confidence intervals (CI) for each level, using conditional logistic regression analysis incorporating the matching. Because 21 extra controls could not be included in the matched analyses, we also conducted unconditional logistic regression with all participants (inserting six indicator variables for age strata [<10, 10–19, 20–29, 30–39, 40–49, 50–59 and >60 years] and one for sex). Other covariates included smoking habits, body mass index and sociodemographic factors. Latency was first determined from the year each participant first consumed water containing a concentration greater than 100 μg/liter to the year in which they first noticed the appearance of hyperpigmentation and/or keratoses. We selected this cutoff for the latency analysis because the confirmed cases with complete water history data had all ingested more than 100 μg/liter of arsenic at some point in their lifetime. Latency was also evaluated from the beginning of the highest known peak arsenic concentration. If cases could not recall the year in which their dermal changes occurred, or if they had not noticed them previously, we used 1995 (year of the initial survey) to calculate latency.

Results

Participation

Of the 530 selected individuals (265 cases and controls each), 405 participants were recruited (192 cases and 213 controls). Because of their previous animosity towards the field team, 15 cases identified in the cross-sectional survey living in two adjacent hamlets were not invited to participate (no controls lived in these areas). Some individuals did not participate because they moved outside the study region (6 cases and 10 controls) or could not be located (26 cases and 28 controls). We calculated the response percentage using these individuals subtracted from the total selected as the denominator. Other individuals could not be recruited because they were too ill to participate, refused or died (26 cases and 14 controls). However, the response among those who were located and were invited to participate was excellent (88% in cases and 94% in controls).

General Characteristics

Cases and controls were similar in their sociodemographic characteristics (Table 1). The distribution of body mass indices showed minor differences, with the average of approximately 18 kg/m^2 in both groups.

Clinical Examination and Photographic Review of Cases

Of the total 192 interviewed "cases," 72 were thought not to have current arsenic-caused skin lesions as determined by the clinical examination in the field. Fifty-nine of these had initially been diagnosed with hyperpigmentation alone, whereas six had keratoses, and seven had both types of lesions. Between the cross-sectional survey and the present study, the majority of these 72 cases (N = 57, or 79%) consumed water with low arsenic concentrations (below 50 μg/liter on average). In contrast, 25 of the 213 controls were now found to have an arsenic-induced skin lesion (24 developed pigmentation changes, and one developed keratoses). Their water history revealed that they had been exposed to high levels in the past. The average known peak concentration for these 25 controls was 253 μg/liter. In the 5 years between the two studies, their average drinking-water arsenic concentration was 140 μg/liter.

Table 2 compares the evaluation of skin lesions by clinical examination in the field and the photographic review with consensus assessment by the four physicians. Ninety of the 192 cases had skin lesions photographed during the current investigation. Among the photographed cases, 77 had been classified in the field as definitely (N = 71) or probably (N = 6) having arsenic-caused skin lesions. After photographic review, 67 of the definite or probable clinical cases were confirmed as definite or probable (67/77, or 87% confirmed). In addition, 29 of the controls with newly developed lesions were photographed, and 18 of them were confirmed to have definite or probable arsenic-caused skin lesions based on photographic review. Photographs were not always clear or comprehensive enough to make a definitive diagnosis, and the consensus agreement was classified as "possible" for 32 (17 + 15) participants. In contrast, the clinical examination in the field yielded only two "possible" cases.

TABLE 1. General Characteristics of Cases and Controls, West Bengal, India

	Cases (N = 192)		Controls (N = 213)	
	N	%	N	%
Sex				
Female	73	38	75	35
Male	119	62	138	65
Age				
<10	3	2	5	2
10–19	22	12	28	13
20–29	27	14	31	15
30–39	46	24	41	19
40–49	38	20	42	20
50–59	25	13	32	15
≥60	31	16	34	16
Smoking habits				
Never	120	63	119	56
Exsmoker	16	8	17	8
Current	56	29	77	36
Type of dwelling				
Concrete/brick	30	16	28	13
Mixed quality materials	63	33	75	35
Mud/thatched	98	51	106	50
Missing	1	1	4	2
Participant's education				
College	9	4	11	5
Secondary	27	14	41	19
Primary	96	50	103	48
No formal education	59	31	54	25
Missing	1	1	4	2
Education of household head				
College	14	7	16	8
Secondary	31	16	25	12
Primary	88	46	121	57
No formal education	54	28	42	20
Missing	5	3	9	4
Occupation				
Farmer	51	27	53	25
Work at home (housewife, maid)	58	30	57	27
Service (laborer, vendor, office)	43	22	56	26
Student	15	8	24	11
Unemployed	25	13	23	11
BMI (kg/m^2)				
<16.5	49	26	52	24
16.5–18.3	53	28	46	22
18.4–20.8	36	19	63	30
≥20.8	42	27	47	22
Missing	2	1	5	2

Latency Patterns

We estimated latency for 42 of the 49 cases who currently had skin lesions and for whom we had collected water samples from all known sources. For 31 of these 42 cases, skin lesions had been confirmed by photographs. All 42 cases had hyperpigmentation and 12 also had keratoses. These 42 cases ranged in age from 13 to 66 years.

Average latency was 19 years (range = 3 to 42 years) based on the first year a case was exposed to their peak arsenic concentration (range = 115 to 1113 μg/liter). Latency from peak exposure could not be estimated for seven of the 42 cases because the lesion occurred before peak exposure.

We also estimated latency from the first year the participants started consuming water with greater than 100 μg/liter of arsenic. Average latency was 23 years (range = 10 to 42 years), with an average duration of 21 years (range = 9 to 42 years) of exposure to >100 μg/liter.

Exposure History Information

Table 3 shows the number of cases and controls with and without water samples from all known tube wells. Complete exposure histories were obtained from an equal proportion of interviewed cases and controls (49% and 48%). For many cases and controls,

TABLE 2. Rating of the Presence of Hyperpigmentation and/or Keratoses Based on the Photographic Assessment or Clinical Examination

	Clinical Exam									
	Cases					Controls				
	Definite	Probable	Possible	None	Total	Definite	Probable	Possible	None	Total
All participants										
Total	110	10	1	71	192	16	9	3	185	213
With photograph										
Definite	38	2	0	2	42	2	2	0	2	6
Probable	25	2	1	1	29	4	0	0	1	5
Possible	8	2	0	7	17	3	5	1	6	15
None	0	0	0	2	2	1	1	0	1	3
Total with photograph	71	6	1	12	90	10	8	1	10	29

the reason we could not collect water samples from some of their sources was because the wells were now closed or located in distant villages (98 cases and 111 controls). However, the average number of closed wells per participant was similar in cases (2.2 wells; range = 1 to 8) and controls (2.3 wells; range = 1 to 7). The majority of the closed wells had been closed because of mechanical problems. A few wells were dismantled because of elevated arsenic levels, but we could not determine the exact number because records were not available.

Arsenic Exposure and Water Consumption Patterns

Table 4 presents the mean arsenic water concentrations by case and control status. The water consumed by cases had known peak arsenic concentrations that were twice that of controls (mean = 325 *vs* 183 μg/liter). The mean duration of exposure to the peak concentrations was similar in cases and controls (about 12.5 years). The average and cumulative arsenic concentrations ingested were also about twice as high in cases compared with controls. On average, cases had fewer years of missing arsenic concentration data compared with controls, although the difference was not substantial (4.4 *vs* 5.5 years, respectively).

Nearly all liquid consumed was water. The mean volumes of water consumed in the last 24 hours were also similar in cases and controls (2.6 and 2.5 liters, respectively). There was no evidence of change in the volume of recent water consumption compared with consumption 5 years earlier. The number of tube wells used by cases and controls ranged from one to eight tube wells, with a median of four wells. The total number of wells ever used by all participants combined was about 1,600.

TABLE 3. Water Samples Collected from Known Tube Wells

	Cases (N = 192)		Controls (N = 213)	
	N	%	N	%
Interviewed participants with arsenic measurements for all known tube wells	94	49	102	48
Samples collected from all known water sources	69		89	
Collected samples + proxy well samples	2		3	
Collected samples + historical measurements	23		10	
Interviewed participants with some closed or distant wells	98	51	111	52

Peak and Average Arsenic Concentrations

Table 5 presents the distribution of the 405 total cases and controls by the highest known (peak) arsenic concentration consumed stratified by sex. The right half of Table 5 presents a subset of 158 participants with complete water histories (69 cases and 89 controls). Of these, all cases who currently had a skin lesion had peak arsenic water concentrations of 100 μg/liter or higher. Eight of the cases with current skin lesions had ingested peak arsenic concentrations between 100 and 199 μg/liter. These eight cases comprised four men (ages 31 to 75 years) and four women (ages 21 to 66 years). All eight cases had hyperpigmentation, and four also had keratoses. One male case was exposed to 115 μg/liter, whereas the others had ingested known peak concentrations above 150 μg/liter. Of the 49 cases who currently had skin lesions and for whom samples were collected from all known sources, 31 cases were confirmed by photograph, including the case who was exposed to a peak of 115 μg/liter.

Table 6 summarizes the dose-response findings for skin lesions for all interviewed participants (N = 405), and those for whom we had collected samples from all known water sources (N = 158). Estimates from the conditional logistic analyses based on 192 age- and sex-matched pairs yielded results similar to those from the unconditional analyses, which included all 405 participants. Both conditional and unconditional analyses demonstrated a clear trend of increasing risk by peak and average arsenic water concentrations (tests for trend,[35] $P < 0.001$). Odds ratios were also adjusted for age, sex, smoking status, body mass index and indicators for so-

TABLE 4. Mean Concentrations of Inorganic Arsenic in Known Tube Wells Used, and Patterns of Water Consumption (192 Cases and 213 Controls)

	Mean	SD	Interquartile Range
Highest known tube well concentration (peak, μg/L)			
Cases	325	183	211–405
Controls	180	159	45–260
Duration of exposure to peak concentrations (years)			
Cases	12.7	12.1	2–21
Controls	12.4	12.9	1–18
Average of known years (μg/L)			
Cases	179	139	86–233
Controls	83	923	9–133
Cumulative of known years (μg/L—years)			
Cases	4,923	4,808	1,784–6,824
Controls	2,445	3,825	32–3,550
Years ingested arsenic concentrations could not be ascertained			
Cases	4.4	7.9	0–35
Controls	5.5	10.3	0–61
Average 24-hour water consumption (L)			
Cases	2.6	1.4	1.6–3.5
Controls	2.5	1.3	1.5–3.2
Average 24-hour water consumption (L), 5 years earlier			
Cases	2.7	1.5	1.6–3.5
Controls	2.5	1.4	1.5–3.3
Number of tubewells used			
Cases	4.3	1.5	3–5
Controls	4.6	1.8	3–5

TABLE 5. Distribution of Cases and Controls by Highest Known (Peak) Concentrations of Inorganic Arsenic

	All Participants (N = 405)			Participants with Samples from All Known Water Sources (N = 158)		
	Cases			Cases		
	Skin Lesions			Skin Lesions		
Peak Arsenic (μg/L)	1995–1996	1998–2000*	Controls	1995–1996	1998–2000*	Controls
Women						
<50	2	0	21	0	0	6
50–99	1	0	8	0	0	3
100–199	14	6	17	5	4	6
200–299	14	8	13	7	4	6
300–399	20	15	7	7	0	2
400–499	14	5	8	0	6	2
≥500	8	8	2	5	5	1
Men						
<50	4	2	36	1	0	16
50–99	5	0	17	2	0	9
100–199	18	13	29	7	4	14
200–299	31	21	31	9	6	18
300–399	32	25	11	13	10	3
400–499	16	5	10	5	2	3
≥500	13	12	3	8	8	0
Both sexes						
<50	6	2	57	1	0	22
50–99	6	0	25	2	0	12
100–199	32	19	46	12	8	20
200–299	45	29	44	16	10	24
300–399	52	40	18	20	16	5
400–499	30	10	18	5	2	5
≥500	21	20	5	13	13	1
Total	192	120	213	69	49	89

* Determined by photographic evaluation or clinical examination in the field.

cioeconomic status in the unconditional multivariate logistic analyses. None of these factors were appreciably associated with skin lesions. Adjusted odds ratios increased from 2.5 in the 50–99 μg/liter peak category (CI = 0.7–8.9) to a nearly 30-fold increase in the >300 μg/liter peak category (OR = 29.4; CI = 11.1–77.5). A similar trend was found with average arsenic concentrations.

The right half of Table 6 presents unconditional ORs for a subset of participants for whom samples were collected from all known water sources (N = 158). Because of small numbers, we pooled the two lowest peak categories. The overall odds ratio increased from 6.8 in the 100–199 μg/liter category (CI = 1.8–25.1) to a nearly 40-fold increased risk in the highest peak category (OR = 39.2; CI = 10.5–142). The lower half of the table presents results using the average arsenic water concentrations. Odds ratios rose to 51.3, although again the confidence intervals were wide.

Discussion

We have characterized the dose-response pattern for skin lesions in relation to arsenic ingestion, based on detailed lifetime exposure assessment. Previous studies have mainly focused on current or recent exposures from primary drinking-water sources.[8,9,11–32] Only one other study on lung cancer ascertained longitudinal arsenic exposure data.[36]

Among participants with confirmed skin lesions for whom we had complete water histories, the lowest known peak arsenic concentration ingested by a case was 115 μg/liter. There were seven additional cases with peak concentrations between 150 and 200 μg/liter. This contrasts with our earlier study, which measured arsenic concentration in only the current primary drinking-water source.[8] In that study, we found 12 patients with keratoses and 29 patients with hyperpigmentation whose main drinking-water source contained less than

TABLE 6. Relation of the Highest-Known (Peak) Arsenic Concentration and Average Concentration (μg/L) Ingested with Skin Lesions

	All Interviewed Participants (N = 405)									Participants with Samples from All Known Water Sources (N = 158)			
				Overall*		Adjusted† Conditional (Matched) Analysis		Adjusted‡ Unconditional Analysis				Overall§	
As (μg/L)	Category Midpoint	Cases N	Controls N	OR	95% CI	OR	95% CI	OR	95% CI	Cases N	Controls N	OR	95% CI
Peak categories													
<50‖	13	6	57	1.0		1.0		1.0		1	22		
50–99	74	6	25	2.4	0.7–8.2	1.5	0.5–5.0	2.5	0.7–8.9	2	12	1.0	NR
100–199	155	32	46	6.7	2.6–17.5	7.8	2.7–22.8	7.4	2.8–20.0	12	20	6.8	1.8–25.1
200–299	248	45	44	9.9	3.9–25.2	9.3	3.4–25.5	11.1	4.2–29.6	16	24	7.6	2.1–26.8
≥300	405	103	41	24.3	9.7–60.6	26.9	9.4–76.9	29.4	11.1–77.5	38	11	39.2	10.5–142.4
Average categories													
<50‖	12	28	104	1.0		1.0		1.0		6	41	1.0	
50–99	74	32	38	3.1	1.7–5.8	3.1	1.5–6.3	3.3	1.7–6.4	12	17	4.8	1.6–14.5
100–199	147	66	49	5.0	2.9–8.7	6.1	3.1–12.0	5.6	3.1–10.2	24	22	7.5	2.7–20.4
200–299	242	40	14	10.6	5.1–22.1	10.8	4.4–23.1	13.2	6.0–29.4	12	7	11.7	3.9–40.6
≥300	404	26	8	12.1	5.0–29.1	16.3	5.6–48.0	12.2	4.7–31.3	15	2	51.3	10.0–NR

NR = Not reported.
* Test for trend with peak arsenic categories, $P < 0.001$; test for trend with average arsenic categories, $P < 0.001$; based on χ^2 distribution using the midpoints of the categories.
† Based on 192 age- and sex-matched pairs.
‡ Adjusted for age, sex, smoking status, type of dwelling, participant's education, education of household head, occupation and body mass index.
§ Two lower peak categories were pooled because of small numbers.
‖ Reference group.

100 μg/liter of arsenic. However, based on the present study with detailed exposure assessment, all confirmed cases had ingested water containing >100 μg/liter of arsenic.

There are substantial differences in opinion concerning the diagnosis of skin lesions in mild cases.[37] Therefore, this study included an evaluation of skin lesions by photographs with a consensus assessment by experienced physicians. A total of 72 cases no longer had an arsenic-induced skin lesion at the time of the case-control interview. This suggests that these cases may have improved to the degree that their skin lesions are no longer visible to the naked eye, although observer differences between the two studies may have also played a role. Using the diagnoses made in 1995 alone, there were three skin lesion cases who never drank water >100 μg/liter of arsenic, and one of them never drank water containing >50 μg/liter (Table 5, fifth column). However, because of the difficulty in diagnosing mild cases, and because they were not confirmed to be cases in the present study, it is possible that they had not been correctly diagnosed in 1995. Thus, establishing the dose-response relation at low doses for arsenic-caused skin lesions requires careful attention to case diagnosis.

We determined that the average latency for skin lesions varied from 19 to 23 years, with the shortest being 10 years from first known exposure to >100 μg/liter. Conversely, prior case reports have suggested that arsenic-induced skin lesions typically occur 5 to 10 years after exposure, and even shorter latencies have been observed.[28,38] However, the previous studies involved individuals exposed to very high arsenic levels, and they may have underestimated latency because of failure to identify earlier arsenic-contaminated water sources.

The mean values for peak, average and cumulative exposures for known years were markedly higher in cases than controls. These differences could not be attributed to an increased water intake among cases, as both groups consumed equivalent amounts of water currently and in the past (Table 4).

As expected, we found strong dose-response trends with both peak and average arsenic water concentrations. These trends were not affected by adjustment for sex, age, smoking status, socioeconomic factors and body mass index (a crude indicator of nutritional status). In our earlier cross-sectional study, we reported that the risk of skin lesions was somewhat greater among those with low body weights.[8] In that study, we determined that individuals below 80% of standard weight had a slightly increased risk of keratoses (standardized morbidity ratio = 1.6; CI = 0.9–2.4). This finding related to a subgroup of individuals who were mainly exposed to high arsenic concentrations. These persons were not a part of the present study, which excluded the most highly exposed individuals. Consequently, we did not find an association with body mass index in this study. Nonetheless, it is possible that some dietary factors may affect susceptibility. We are currently analyzing data to determine whether overall nutrition or certain micronu-

trients (such as those involved in methylation pathways or in the development of dermal effects) differ between cases and controls.

The following potential limitations of this study need to be considered. We assumed that the arsenic levels of tube wells at the time of sampling were the same as in the past. Few data exist about the seasonal and annual variation of arsenic in groundwater sources in West Bengal. However, a recent report suggested that tube well arsenic concentrations in neighboring Bangladesh have not changed much over time.[39]

Despite the care we took to collect longitudinal arsenic data, the exposure assessment was incomplete. Assessment of drinking-water sources was based on recall. However, recall bias may be minimal because we collected water samples from all known sources for an equal percent of cases and controls (49% in both groups), and the average number of years of missing tube well arsenic concentrations was not appreciably different in cases and controls (4.4 *vs* 5.5 years, respectively).

Estimating the duration of tube well use at each residence was complicated because calendar years are not widely used in West Bengal, and years could have been inaccurately recalled. We tried to minimize this problem by asking exposure questions in relation to momentous life events.

Some evidence suggests that keratoses and hyperpigmentation are early biomarkers of other outcomes, including both nonmelanoma skin cancer and cancer of the internal organs.[4–6] Therefore, dose-response assessments for nonmalignant skin lesions may be relevant to later cancer risks. We found that arsenic concentrations above 100 μg/liter may be necessary to cause sufficient toxic skin effects that can be identified by the naked eye; however, this does not mean that cancer risks are absent below such exposures. It is likely that cancers would occur at lower exposures than those required to generate visible organ toxicity involving multiple cells, such as these skin lesions.

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