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RESEARCH

Arsenic exposure from drinking water and mortality from cardiovascular disease in Bangladesh: prospective cohort study

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ABSTRACT

Objective To evaluate the association between arsenic exposure and mortality from cardiovascular disease and to assess whether cigarette smoking influences the association.

Design Prospective cohort study with arsenic exposure measured in drinking water from wells and urine.

Setting General population in Araihazar, Bangladesh.

Participants 11 746 men and women who provided urine samples in 2000 and were followed up for an average of 6.6 years.

Main outcome measure Death from cardiovascular disease.

Results 198 people died from diseases of circulatory system, accounting for 43% of total mortality in the population. The mortality rate for cardiovascular disease was 214.3 per 100 000 person years in people drinking water containing <12.0 µg/L arsenic, compared with 271.1 per 100 000 person years in people drinking water with ≥12.0 µg/L arsenic. There was a dose-response relation between exposure to arsenic in well water assessed at baseline and mortality from ischaemic heart disease and other heart disease; the hazard ratios in increasing quarters of arsenic concentration in well water (0.1-12.0, 12.1-62.0, 62.1-148.0, and 148.1-864.0 µg/L) were 1.00 (reference), 1.22 (0.65 to 2.32), 1.35 (0.71 to 2.57), and 1.92 (1.07 to 3.43) (P=0.0019 for trend), respectively, after adjustment for potential confounders including age, sex, smoking status, educational attainment, body mass index (BMI), and changes in urinary arsenic concentration since baseline. Similar associations were observed when baseline total urinary arsenic was used as the exposure variable and for mortality from ischaemic heart disease specifically. The data indicate a significant synergistic interaction between arsenic exposure and cigarette smoking in mortality from ischaemic heart disease and other heart disease. In particular, the hazard ratio for the joint effect of a moderate level of arsenic exposure (middle third of well arsenic concentration 25.3-114.0 µg/L, mean 63.5 µg/L) and cigarette smoking on mortality from heart disease was greater than the sum of the hazard ratios associated with their individual effect (relative excess risk for interaction 1.56, 0.05 to 3.14; P=0.010).

Conclusions Exposure to arsenic in drinking water is adversely associated with mortality from heart disease, especially among smokers.

INTRODUCTION

Arsenic is a natural element of the earth's crust, which can enter drinking water supplies from natural deposits. Raised concentrations of arsenic in groundwater pose a public health threat to millions of people worldwide, including 13 million residents in the United States.[1] Although the International Agency for Research on Cancer has classified arsenic as a group 1 human carcinogen, evidence of other effects on health, including cardiovascular effects, has not been well established.[2 3]

High levels of arsenic exposure (>500 µg/L) in drinking water have been related to increased risks in many cardiovascular diseases, including hypertension,[4 5] ischaemic heart disease,[6 7] and carotid atherosclerosis[8] in a series of retrospective cohort studies in south western Taiwan and Chile.[6 8-11] Several studies in the United States and Spain have reported positive associations between arsenic exposure at lower concentrations (<300 µg/L) and mortality from coronary heart disease, hypertension, hypertensive heart disease, or diseases of arteries, arterioles, and capillaries.[12-15] The use of ecological study designs[12-14] or exposure measured at the group level, however,

limits the causal inference in studies that investigated effects of lower concentrations. In addition, previous studies have reported that the risks associated with arsenic exposure for skin lesions,[16 17] bladder cancer,[18 19] and lung cancer[20] are higher among smokers. Studies examining the potential interaction between arsenic exposure and cigarette smoking in the risk of cardiovascular disease, however, are lacking. As cardiovascular disease leads to about a third of the mortality in the world, a small increased risk associated with arsenic exposure would translate into a large number of excess deaths in the exposed population and could be of major importance to public health. Prospective studies with multiple exposure measures and information on susceptible factors such as cigarette smoking at the individual level are needed.

The contamination of groundwater with arsenic in Bangladesh has been recognised as a massive public health hazard.[21 22] An estimated 57 million people have been chronically exposed to groundwater with arsenic concentrations exceeding the WHO standard.[23] To evaluate the health effects of such exposure, in 2000 we established a cohort study in Araihazar, Bangladesh. The study, with participants exposed to arsenic concentrations in water from drinking wells from 0.1 μg/L to 864 μg/L (mean 99 μg /L) at baseline, provides us with a unique opportunity to evaluate the cardiovascular effects of exposure at low to moderate concentrations.

We tested the hypothesis that exposure to arsenic, measured in both water and urine, is associated with mortality from cardiovascular disease. We also tested the hypotheses that cigarette smoking increases the susceptibility to the cardiovascular effects of arsenic exposure.

METHODS

The study is an ongoing prospective cohort study in Araihazar, Bangladesh. Details of the methods have been presented elsewhere.[24 25] Briefly, before participants were recruited, water samples and their geographical coordinates were collected for 5966 contiguous wells in a well defined area of 25 km^2. Between October 2000 and May 2002, we recruited 11 746 men and women who met the following eligibility criteria: married (to reduce loss to follow-up) and aged between 18-75; living in the study area for at least five years before recruitment; and primary user of one of the 5966 tube wells, designated as the "index" well, for at least three years.[24] The response rate was 97.5%.[24] Information on demographic and lifestyle variables was collected with a standardised questionnaire at baseline and follow-up visits. Trained clinicians measured blood pressure with an automatic sphygmomanometer.[26] Participants who said that a physician had given them a diagnosis of diabetes before baseline were retrospectively identified from data collected at the first follow-up.[27] The comparison between self reported diabetes status and test results for blood glycosylated haemoglobin and glucosuria suggested validity of the questionnaire data.[27] The cohort is being actively followed with a personal visit at two year intervals, which includes a physical examination and structured interview conducted by trained physicians following the same procedures used in the baseline interview. The present study includes data from the first (September 2002 to May 2004), second (June 2004 to August 2006), and third (January 2007 to March 2009) follow-up. In addition, a field clinic was established for cohort participants for follow-up between their biennial visits.[24]

Assessment of causes of deaths

Our outcome of interest was deaths from cardiovascular disease, defined as deaths from disease of circulatory system (ICD-10 (international classification of diseases, 10th revision) codes I00-I99) in cohort participants from baseline to 18 March 2009 (end of the third follow-up). Details of the methods for the assessment of causes of deaths are described elsewhere.[28] Briefly, we adapted a validated verbal autopsy procedure, developed by the International Centre for Diarrhea Disease Research, Bangladesh, in collaboration with the World Health Organization, to ascertain the causes of deaths. During the follow-up, on receipt of a report of death from family or neighbours, a study physician and a trained social worker administered the verbal autopsy form to the next of kin. Medical records from physicians who had treated the dead person were collected. For deaths in hospital, information on death certificates and biopsies was ascertained. Each month an outcome assessment committee, consisting of physicians and consulting medical specialists blinded to the exposure status, reviewed the data. Causes of deaths were coded according to the WHO classification[29] and ICD-10.[30] The International Centre for Diarrhea Disease Research, Bangladesh, has used this method to ascertain causes of deaths since 1971[31 32] and documented an overall 95% specificity, with a 85% sensitivity for deaths from cancer and up to 85% sensitivity for cardiovascular deaths.[33]

Measurements of arsenic exposure

At baseline, water samples from all 5966 tube wells in the study area were collected in 50 mL acid washed tubes after the well was pumped for five minutes.[34 35] Total arsenic concentration was determined by graphite furnace atomic absorption spectrometry with a Hitachi Z-8200 system.[36] Samples that fell below the detection limit (5 μg/L) were subsequently analysed by inductively coupled plasma mass spectrometry, with a detection limit of 0.1 μg/L.[37] Analyses for time series samples collected from a subset of tube wells in the study area showed little variation in arsenic concentration over time.[36] We therefore used the same arsenic concentration for a given well as the exposure level for its users who were recruited in the study, even if they were included at different time frames.

All participants were primary users of one of the 5966 tube wells; 89% of study participants shared tube wells with up to five other study participants, while the 14% remaining shared their wells with 6-13 others.[38] Using baseline data, we derived a time weighted arsenic

concentration as a function of drinking durations and well arsenic concentrations (see equation A in fig 1).[38] The average duration of use of wells for wells with a known arsenic concentration accounted for 25% of lifetime (>8 years) for both sexes.[38]

Spot urine samples were collected from 11 224 (95.6%) of 11 746 interviewed participants at baseline, 11 109 (98.1%) of 11 323 at the first follow-up, and 10 726 (98.1%) of 10 934 at the second follow-up. Total urinary arsenic concentration was measured by graphite furnace atomic absorption spectrometry, with a Perkin-Elmer AAnalyst 600 graphite furnace system with a detection limit of 2 µg/L, as previously described.[39] Urinary creatinine was analysed with a method based on the Jaffe reaction for adjustment of urinary total arsenic concentration.[40] We implemented an arsenic mitigation programme in the study area at baseline to promote switching to safe wells—that is, to wells yielding water with an arsenic concentration lower than the Bangladesh standard of 50 µg/L.[41] As exposure concentration might change in some participants from baseline, we calculated changes in urinary arsenic between visits using urinary creatinine adjusted arsenic.

Measurements of dietary intakes

Dietary intakes were measured at baseline with a validated semiquantitative food frequency questionnaire designed for the study population. Detailed information on the design and the validation of the food frequency questionnaire has been published elsewhere.[42] Briefly, to assess the validity of the questionnaire, trained interviewers completed two seven-day food diaries in two separate seasons for 189 participants. The results of the validation study indicate that the food frequency questionnaire provides reasonably valid measurements for long term dietary intakes of common foods, macronutrients, and common micronutrients.[42]

Statistical analysis

Primary objectives: association between arsenic exposure and mortality from cardiovascular disease

We computed person time from baseline to the date of death from any cause or the date of the third active follow-up visit, whichever came first. We used Cox proportional hazards regression to compute the hazard ratios for deaths from disease of the circulatory system, ischaemic heart disease, and cerebrovascular disease, in relation to quarters of baseline well arsenic concentration, time weighted arsenic concentration, baseline urinary arsenic concentration, and changes in urinary arsenic between visits. We also estimated the hazard ratios in relation to 1 SD increase in well arsenic or urinary arsenic for better interpretation of the effect estimates. The assumption of proportional hazards was examined by testing the cross product terms between covariate variables and log function of survival time, and P values for all the terms were >0.10. We examined the assumption of non-linear effect of arsenic exposure by including higher order polynomial terms for arsenic exposure variables in the models, and there was no indication of any non-linear relation. Because deaths from other forms of heart disease were often a consequence of previous ischaemic heart disease in our study population, and patterns of the hazard ratios were similar for ischaemic heart disease and other forms of heart disease, we estimated the hazard ratios for the combined category and for ischaemic heart disease separately.

Equation A

Time weighted arsenic concentration in µg/L=

$$\sum C_i T_i / \sum T_i$$

where C_i and T_i denote the well arsenic concentration and drinking duration for the ith well

Equation B

$$1 - \sum_{j=1}^{k} \frac{P_j}{HR_j}$$

where k is the number of exposure strata; P_j is the proportion of cause specific deaths in the jth exposure stratum; and HR_j is the adjusted hazard ratio associated with j exposure stratum

Fig 1 | Equations for time weighted arsenic concentration and population attributable proportion of mortality from cardiovascular disease

We first adjusted for age at baseline and sex. A second model was constructed to additionally adjust for a priori defined potential confounders, including baseline educational level (years), body mass index (BMI), and smoking status (never, past, and current), all of which are known risk factors for cardiovascular disease that might be related to the distribution of baseline arsenic exposure or influence health effects of arsenic exposure in our study population.[16 38 43] Appendix 1 on bmj.com shows the associations between potential confounders and well arsenic concentration. We included changes in urinary arsenic between visits as a time dependent variable. For instance, for deaths from cardiovascular disease that occurred between the first and second follow-up, we used the difference in urinary creatinine adjusted arsenic (urinary arsenic concentration with adjustment for creatinine in the urine) between the baseline and first follow-up as the relevant changes in urinary arsenic. Missing data (<2%) were coded by using dummy variables, allowing participants with one or more missing potential confounders to be included in the analyses under a "missing at random" assumption. In the same models, we also explored the association between changes in urinary arsenic between visits and the risk of mortality from cardiovascular disease.

We estimated the population attributable proportion of mortality from cardiovascular disease associated with higher concentrations of baseline well arsenic concentration (>12 µg/L) using adjusted hazard ratios estimated from Cox proportional hazards regression (see equation B in fig 1).[44]

Confidence intervals were estimated with the bootstrap method with 1000 bootstrap samples.

As fish can accumulate organic arsenic, which could influence total urinary arsenic concentration, rice can contain inorganic arsenic from the soil, and arsenic has been related to increased risk of hypertension and diabetes,[45 46] we performed a sensitivity analysis to evaluate whether the association between arsenic exposure and risk of cardiovascular disease can be attributable to arsenic from intakes of fish and rice, or to the association of arsenic exposure with diabetes and hypertension. The adjustment for creatinine might influence the relation between arsenic and disease outcomes related to creatinine in cross sectional studies.[47 48] Although our study is a prospective cohort study, we also conducted sensitivity analyses to assess whether the association between urinary arsenic and risk of cardiovascular disease was different when we did not adjust for urinary creatinine. Given the sample size, we had 80% power with α=0.05 to detect a hazard ratio of 1.22 for cardiovascular disease mortality associated with 1 SD difference in baseline well arsenic concentration.

Secondary objectives: synergy between arsenic exposure and cigarette smoking in mortality from heart disease

We assessed the presence of synergy (that is, epidemiological interaction or positive interaction on an additive scale) between arsenic exposure and cigarette smoking by testing whether the joint effect from exposure to both factors was greater than the sum of their independent effects. We considered similar analysis methods and classification of smokers used in previous studies assessing synergy between arsenic exposure and smoking in the risk of lung cancer and skin lesions.[16 20 49] Rothman has discussed the use of relative excess risk for interaction (RERI) in assessing additive interactions.[50] The additivity of risks associated with two exposures corresponds to $(R_{11}–R_{00}) = (R_{01}–R_{00}) + (R_{10}–R_{00})$, where R_{11} is the risk of disease associated with having both exposures, R_{10} and R_{01} are the risks of disease associated with one of the exposures along, and R_{00} is the risk of disease associated with absence of both exposures (background risk). The equation can be represented with risk ratios by dividing all the components by R_{00} and assessing whether the relative excess risk for interaction (relative excess risk for interaction, $RR_{11}–RR_{10}–RR_{01}+1$) is greater than zero and can be used to evaluate if there is a positive departure from additivity, or synergy, of the effects from two exposures.[50] We used adjusted hazard ratios as surrogates of risk ratios in the following equation: $RERI \approx HR_{11}–HR_{10}–HR_{01}+1$, where HR_{11} indicates the hazard ratio for disease associated with a higher level of arsenic exposure (moderate or high level) and tobacco smoking (ever, current, or past) in comparison to the a priori reference group with the lowest level of arsenic exposure and never smoking; HR_{10} indicates hazard ratio for a higher level of arsenic exposure alone; and HR_{01} denotes hazard ratio for tobacco smoking alone. We also estimated the attributable proportion attributable to interaction as $RERI/HR_{11}$.[50] To increase power for interaction analyses, we used thirds of arsenic exposure instead of quarters. We estimated the relative excess risk for interaction with arsenic exposure considered as a continuous variable as follows: $(e^{\beta 1+\beta 2+\beta 3})–(e^{\beta 1})–(e^{\beta 2})+1$, where β_1 is the coefficient of the effect of per SD increase in the arsenic exposure measure, β_2 is the coefficient of tobacco smoking, and β_3 is the coefficient of the cross product of per SD increase in arsenic exposure and tobacco smoking.[49 51 52] Confidence intervals of the relative excess risk for interaction were estimated for statistical inferences by using the standard delta method described by Hosmer and Lemeshow[51 53] (for categorical arsenic exposure) and bootstrap method with 1000 bootstrap samples (for continuous exposure measure).[52] As the relative excess risk for interaction is a measure of difference in excess relative risks, an estimate over zero indicates presence of synergy of two risk factors, and a 95% confidence interval that is positive and excludes zero corresponds to $P<0.05$.

As some participants shared the same well, we used robust standard errors for the proportional hazards model[54] to account for the potential influence of correlated exposure data. We also carried out sensitivity analyses to exclude participants with any missing data or imputed using the median values of the covariates. All analysis was conducted with SAS 9.2 (SAS Institute, Cary, NC).

RESULTS

We observed 77 252 person years during the follow-up period. There were 460 deaths, of which 198 were from diseases of circulatory system (ICD-10 codes I00-I99), accounting for 43% of total mortality in the population and yielding a mortality rate of 256.3 per 100 000 person years. Among the 198 deaths from cardiovascular disease, 85 were from cerebrovascular disease (codes I60-I69); 104 were from ischaemic heart disease (codes I20-I25, n=71) or other forms of heart disease (codes I30-I52, n=33), which included mostly deaths from heart failure (codes I50.0-I50.9, n = 32) and ventricular tachycardia (code I47, n=1); and nine deaths were from pulmonary heart disease, hypertensive heart disease, or multiple valve diseases (codes I08, I11, and I27).

Higher BMI and presence of diabetes were associated with an increased risk of death from ischaemic heart disease and other heart disease (table 1). There was no association between educational level and mortality from either diseases of the circulatory system overall or any of the subtypes of cardiovascular disease. Diastolic and systolic hypertension were related to an increased risk of death from diseases of the circulatory system overall and all subtypes of cardiovascular disease, and the associations were stronger for cerebrovascular disease. Participants who were current smokers, had smoked for at least 20 years, or had accumulated at least 10 pack years at baseline were 1.6 to 1.9 times more likely to die from disease of circulatory system and 2.2 to 2.7 times more likely to die from ischaemic heart disease and other heart disease.

Table 1 | Relations between baseline risk factors and mortality from disease of circulatory system

Baseline risk factors†	Person years of follow-up	Disease of circulatory system		Ischaemic heart disease and other forms of heart disease*		Ischaemic heart disease*		Cerebrovascular disease	
		No of deaths	Hazard ratio	No of deaths	Hazard ratio	No of deaths	Hazard ratio	No of deaths	Hazard ratio
Body mass index (BMI):									
12.0-18.5	29 767	88	1.06 (0.75 to 1.44)	39	0.95 (0.60 to 1.50)	25	0.96 (0.53 to 1.73)	45	1.16 (0.71 to 1.92)
18.5-22.0	29 829	61	1.00	31	1.00	19	1.00	26	1.00
22.1-40.0	15 834	44	1.29 (0.88 to 1.87)	30	1.74 (1.06 to 2.86)	24	2.34 (1.27 to 4.31)	13	0.88 (0.45 to 1.68)
Education (years):									
0	34 070	93	1.00	49	1.00	27	1.00	40	1.00
1-5	23 060	45	0.80 (0.56 to 1.13)	24	0.79 (0.49 to 1.27)	18	1.04 (0.58 to 1.86)	19	0.80 (0.46 to 1.37)
6-16	20 094	60	1.18 (0.84 to 1.61)	31	1.12 (0.72 to 1.75)	26	1.58 (0.92 to 2.73)	26	1.21 (0.72 to 2.02)
Systolic blood pressure (mm Hg):									
62-140	71 218	131	1.00	80	1.00	54	1.00	42	1.00
141-224	6046	67	3.22 (2.27 to 4.18)	24	2.03 (1.25 to 3.28)	17	2.03 (1.14 to 3.63)	43	5.39 (3.48 to 8.35)
Diastolic blood pressure (mm Hg):									
60-90	70 240	151	1.00	90	1.00	62	1.00	53	1.00
91-139	7024	47	2.36 (1.70 to 3.27)	14	1.24 (0.70 to 2.20)	9	1.16 (0.57 to 2.37)	32	4.31 (2.82 to 6.61)
Diabetes:									
No	75 972	185	1.00	97	1.00	67	1.00	80	1.00
Yes	1292	13	2.13 (1.26 to 3.62)	7	2.46 (1.15 to 5.23)	4	1.93 (0.72 to 5.14)	5	1.64 (0.67 to 4.02)
Cigarette or bidi‡ smoking:									
Never	50 032	50	1.00	27	1.00	14	1.00	20	1.00
Past	4939	32	1.24 (0.71 to 2.18)	15	1.57 (0.66 to 3.71)	9	1.01 (0.35 to 2.90)	13	0.91 (0.41 to 2.01)
Current	22 250	115	1.74 (1.10 to 2.76)	61	2.23 (1.11 to 4.48)	47	1.87 (0.76 to 4.57)	52	1.65 (0.90 to 3.03)
Years of smoking cigarettes or bidi‡:									
0	50 032	50	1.00	27	1.00	14	1.00	20	1.00
1-20	11 192	21	1.16 (0.61 to 1.98)	10	1.16 (0.46 to 2.92)	7	0.95 (0.31 to 2.92)	9	1.08 (0.49 to 2.36)
21-60	15 923	126	1.92 (1.13 to 3.01)	66	2.65 (1.24 to 5.67)	49	2.02 (0.79 to 5.17)	56	1.55 (0.81 to 2.96)
Pack years of cigarettes or bidi‡ smoked:									
0	50 032	50	1.00	27	1.00	14	1.00	20	1.00
1-10	12 626	48	1.69 (1.00 to 2.62)	24	1.90 (0.90 to 4.05)	17	1.59 (0.61 to 4.15)	22	1.56 (0.82 to 2.97)
11-185	14 382	99	1.64 (1.00 to 2.59)	52	2.30 (1.06 to 4.95)	39	1.73 (0.70 to 4.30)	43	1.30 (0.69 to 2.43)

*Because deaths from other forms of heart disease were often consequence of previous ischaemic heart disease in our study population and patterns of hazard ratios were similar for ischaemic heart disease and other forms of heart disease, we present hazard ratios for combined category and also for ischaemic heart disease separately.
†Categories of BMI based on conventional classification for underweight (‹18.5), lower normal (18.5-22.0), and higher normal/overweight (›22.0). Categories of educational attainment based on primary level (1-5 years) and secondary level or greater (›6 years) according to Bangladeshi educational system. Blood pressure categories based on established definitions for systolic hypertension and diastolic hypertension. Categories of number of years smoked or pack years smoked based on cut points that were meaningful with easy interpretation.
‡Tobacco wrapped in tendu leaf.

Primary objectives: association between arsenic exposure and cardiovascular disease mortality

We found an increased risk of mortality from diseases of the circulatory system in people with high concentrations of well arsenic. The mortality rate for cardiovascular disease was 214.3 per 100 000 person years in people drinking water containing <12.0 µg/L arsenic compared with 271.1 per 100 000 person years in people drinking water with ≥12.0 µg/L arsenic. Participants exposed to >148 µg/L (mean 265.7 µg/L) of well arsenic were 1.47 (95% confidence interval 0.99 to 2.18) times more likely to die from diseases of the circulatory system compared with their counterparts who were exposed to <12 µg/L (model 2, table 2). There was an increased risk of mortality from ischaemic heart disease and other heart disease in relation to high concentrations of well arsenic, and a dose-response relation remained after adjustment for BMI, smoking status, educational attainment, and changes in arsenic concentration between visits adjusted for urinary creatinine in addition to age and sex (model 2, P=0.0019 for trend). The hazard ratio was 1.29 (1.10 to 1.52, model 2) for a 1 SD increase in well arsenic concentration (115 µg/L). A similar association was observed between baseline well arsenic and mortality from ischaemic heart disease; participants with >148 µg/L of arsenic in well water were 1.94 (0.99 to 3.84) times more likely to die from ischaemic heart disease compared with those with <12 µg/L (model 2, P=0.0294 for trend). The hazard ratio was 1.25 (1.03 to 1.52, model 2) for a 1 SD increase in well arsenic concentration. On the other hand, there was no association between well arsenic and mortality from cerebrovascular disease. Analysis results were similar when we used time weighted arsenic concentration as the exposure measure (see appendix 2 on bmj.com).

Additional control for systolic blood pressure, baseline diabetes, and dietary intake of fish and rice did not appreciably change effect estimates (see appendix 3 on bmj.com). When participants with missing values for BMI, education level, smoking status, and changes in urinary arsenic between visits were excluded or imputed by using the median level in the population, the results were similar (data not shown).

We observed similar patterns of hazard ratios when we used baseline urinary arsenic as the exposure variable in the analyses (table 3). Baseline urinary arsenic was related to an increased risk of mortality from diseases of the circulatory system (model 2, P=0.0065 for trend), and the association was stronger for the combined category of ischaemic heart disease and other heart disease (model 2, P=0.0001for trend). The effect estimates did not materially change when we did not adjust for urinary creatinine. For instance, the association between urinary arsenic and mortality from heart disease in increasing quarters of urinary arsenic concentrations were 1.00 (reference), 1.07 (0.57 to 2.03), 1.44 (0.81 to 2.57), and 2.08 (1.09 to 3.95) (P=0.0001for trend) (data not shown).

Spearman correlations of baseline urinary arsenic with urinary arsenic measured at first and second follow-up were both 0.65. Overall, total urinary arsenic decreased by an average of 57.1 μg/g of creatinine from baseline to first follow-up and then essentially remained stable with an average increase of 3.2 μg/g creatinine from first to second follow-up. The hazard ratio was 1.18 (1.03 to 1.36) for total cardiovascular disease mortality in relation to a 1 SD increase in changes in urinary arsenic (240 μg per g of creatinine) between visits, adjustment for age, sex, BMI, smoking status, and baseline well arsenic concentration. The number of deaths in extreme categories of changes in urinary arsenic, however, was not enough for us to evaluate the dose-response relation for specific cardiovascular disease subtypes.

Secondary objectives: synergy between arsenic exposure and cigarette smoking in mortality from heart disease

Although not significant, the risk of mortality from heart disease seemed to be higher for people with high level of arsenic exposure who had ever smoked (fig 2). The risk of dying from ischaemic heart disease and other heart disease associated with moderate (25.3-114.0 μg/L, mean 63.5 μg/L) or high levels of arsenic exposure (>114 μg/L, mean 228.8 μg/L) was consistently higher in those who had ever smoked and especially in current smokers at baseline compared with those who had never smoked. The joint effect of moderate or high levels of arsenic exposure and ever smoking was greater than the sum of their individual effects, with estimates of the relative excess risk due to interaction all greater than zero. In exploratory analyses, when we further classified ever smokers into past and current smokers, the synergistic effect between moderate or high level of arsenic exposure and current smoking was stronger (relative excess risk due to interaction 2.21 (0.11 to 4.31; P=0.036) and 1.43 (0.02 to 3.61; P=0.045), respectively) (fig 2). Figure 3 shows the survival curves by thirds of well arsenic and smoking status in men and women. The synergistic effect between smoking and arsenic exposure was similar in men and women. We found a similar pattern of estimates of relative excess risk due to interaction when we considered

Table 2 | Association between baseline concentrations of well arsenic (μg/L) and mortality from disease of circulatory system during follow-up

	Hazard ratio (95% CI) per 1 SD (115 μg/L) increase	Hazard ratio (95% CI) by mean (range) baseline concentrations				P for trend*
		3.7 (0.1-12.0)	35.9 (12.1-62.0)	102.5 (62.1-148.0)	265.7 (148.1-864.0)	
Person years of follow-up	77 252	20 064	19 109	18 699	19 380	—
Disease of circulatory system						
No of deaths	198	43	51	41	63	—
Model 1†	1.08 (0.96 to 1.22)	1.00	1.35 (0.91 to 2.00)	1.17 (0.76 to 1.79)	1.47 (0.99 to 2.18)	0.2153
Model 2‡	1.11 (0.97 to 1.26)	1.00	1.21 (0.80 to 1.84)	1.24 (0.80 to 1.93)	1.46 (0.96 to 2.20)	0.1340
Ischaemic heart disease and other forms of heart disease						
No of deaths	104	21	24	21	38	—
Model 1†	1.23 (1.05 to 1.43)	1.00	1.29 (0.71 to 2.34)	1.20 (0.65 to 2.22)	1.84 (1.06 to 3.17)	0.0072
Model 2‡	1.29 (1.10 to 1.52)	1.00	1.22 (0.65 to 2.32)	1.35 (0.71 to 2.57)	1.92 (1.07 to 3.43)	0.0019
Ischaemic heart disease						
No of deaths	71	14	16	15	26	—
Model 1†	1.19 (1.00 to 1.42)	1.00	1.31 (0.63 to 2.71)	1.31 (0.63 to 2.72)	1.89 (1.00 to 3.60)	0.0479
Model 2‡	1.25 (1.03 to 1.52)	1.00	1.22 (0.56 to 2.65)	1.49 (0.70 to 3.19)	1.94 (0.99 to 3.84)	0.0294
Cerebrovascular disease						
No of deaths	85	19	26	18	22	—
Model 1†	0.90 (0.73 to 1.11)	1.00	1.56 (0.88 to 2.79)	1.20 (0.64 to 2.26)	1.15 (0.59 to 2.22)	0.3099
Model 2‡	0.89 (0.71 to 1.13)	1.00	1.35 (0.75 to 2.43)	1.20 (0.63 to 2.27)	1.07 (0.54 to 2.12)	0.3427

*Estimated with arsenic exposure variable as continuous variable in model.
†Adjusted for sex and baseline age (years).
‡Adjusted for sex and baseline age (years), BMI, smoking status (never, past, current), educational attainment (years), and changes in arsenic concentration adjusted for urinary creatinine (μg per g of creatinine) between visits.

Table 3 | Association between baseline urinary creatinine adjusted arsenic (μg/g of creatinine) and mortality from disease of circulatory system during follow-up

	Hazard ratio (95% CI) per 1 SD (282 μg per g of creatinine) increase	Hazard ratio (95% CI) by mean (range) of baseline urinary creatinine adjusted of arsenic (μg/g of creatinine)				
		68.5 (6.6-105.9)	150.6 (106.0-199.0)	264.9 (199.1-351.8)	641.5 (351.9-1100)	P for trend *
Person years of follow-up	73 835	18 818	18 355	18 161	18 501	—
Disease of circulatory system:						
No of deaths	192	44	48	54	46	—
Model 1†	1.11 (0.99 to 1.24)	1.00 (ref)	1.07 (0.73 to 1.58)	1.35 (0.91 to 2.01)	1.30 (0.87 to 1.95)	0.0679
Model 2‡	1.18 (1.04 to 1.33)	1.00 (ref)	1.15 (0.77 to 1.72)	1.56 (1.03 to 2.38)	1.55 (1.01 to 2.37)	0.0065
Ischaemic heart disease and other forms of heart disease:						
No of deaths	101	22	25	25	29	—
Model 1†	1.16 (1.04 to 1.30)	1.00 (ref)	1.13 (0.66 to 1.95)	1.26 (0.70 to 2.25)	1.61 (0.93 to 2.78)	0.0071
Model 2‡	1.26 (1.12 to 1.42)	1.00 (ref)	1.29 (0.74 to 2.27)	1.53 (0.83 to 2.82)	2.06 (1.14 to 3.72)	0.0001
Ischaemic heart disease:						
No of deaths	69	17	18	17	17	—
Model 1†	1.08 (0.87 to 1.36)	1.00 (ref)	1.05 (0.56 to 2.00)	1.13 (0.58 to 2.21)	1.28 (0.65 to 2.51)	0.3878
Model 2‡	1.22 (0.99 to 1.49)	1.00 (ref)	1.29 (0.66 to 2.51)	1.47 (0.72 to 3.01)	1.90 (0.91 to 3.98)	0.0585
Cerebrovascular disease:						
No of deaths	82	20	20	27	15	—
Model 1†	1.03 (0.82 to 1.29)	1.00 (ref)	0.96 (0.52 to 1.77)	1.47 (0.82 to 2.65)	0.96 (0.49 to 1.88)	0.8062
Model 2‡	1.06 (0.84 to 1.35)	1.00 (ref)	0.96 (0.52 to 1.79)	1.60 (0.88 to 2.90)	1.03 (0.53 to 2.03)	0.6230

*Estimated with arsenic exposure variable as continuous variable in model.
†Adjusted for sex and baseline age (years).
‡Adjusted for sex and baseline age (years), BMI, smoking status (never, past, current), educational attainment (years), and changes in arsenic concentration adjusted for urinary creatinine (μg per g of creatinine) between visits.

well arsenic concentration as a continuous variable. There was a synergy between a 1 SD increase in well arsenic concentration and ever smoking (0.38, 0.02 to 1.35), and the synergy was stronger with current smoking status (0.67, 0.08 to 1.86).

DISCUSSION

Interpretation of the results

In this prospective cohort study exposure to arsenic from drinking water, as measured in well water and urine, was associated with an increased risk of cardiovascular disease, in particular ischaemic heart disease and other heart disease. Based on our observed estimates, 28.9% (1.4% to 60.0%) of deaths from heart disease in this population can be attributable to arsenic concentrations over 12 μg/L in well water. We found a synergistic effect between arsenic exposure and cigarette smoking on mortality from ischaemic heart disease and other heart disease, and this effect was apparent even when arsenic exposure was moderate (25.3-114.0 μg/L, mean 63.5 μg/L).

Comparison with other studies

Previous studies on mortality from cardiovascular disease in areas with low or moderate levels of arsenic exposure from drinking water generated inconsistent findings,[12-15] probably because the exposure range was limited in detecting the association and the limitation of using group level exposure as exposure level for the individuals. For instance, using mean arsenic level at the county level and National Center for Health Statistics data, an ecological study in US counties found raised standardised mortality ratios for mortality from diseases of the arteries, arterioles, and capillaries associated with water arsenic concentration >20 μg/L but no associations for mortality from any other cardiovascular diseases.[12] The mortality from hypertensive heart disease was raised in female but not in male members of the Mormons in Millard County, Utah, with <200 μg/L of arsenic in drinking water, according to historical records of arsenic measurements at the community level.[13] Ecological measures of arsenic exposure are subject to large measurement errors when there is variation in water concentrations within a study region. As the effects of low or moderate levels of arsenic exposure on cardiovascular disease risk are likely to be modest in magnitude, studies of such levels are particularly susceptible to measurement errors in ascertainment of exposure, which, in most cases, would lead to bias towards the null but could also generate spurious associations under certain conditions.[55] More recently, a cohort study in Bangladesh with well arsenic concentrations measured at household level reported a dose-response relation and an increased risk at exposures of 50-149 μg/L for death from the combined category of cardiovascular disease (hazard ratio 1.16, 0.96 to 1.40).[33] A recent ecological study in Spain reported raised mortality rates for cardiovascular disease and coronary heart disease associated with arsenic exposure at 10-118 μg/L.[15] Our study confirmed and extended observations in previous reports in that the validity of the findings is

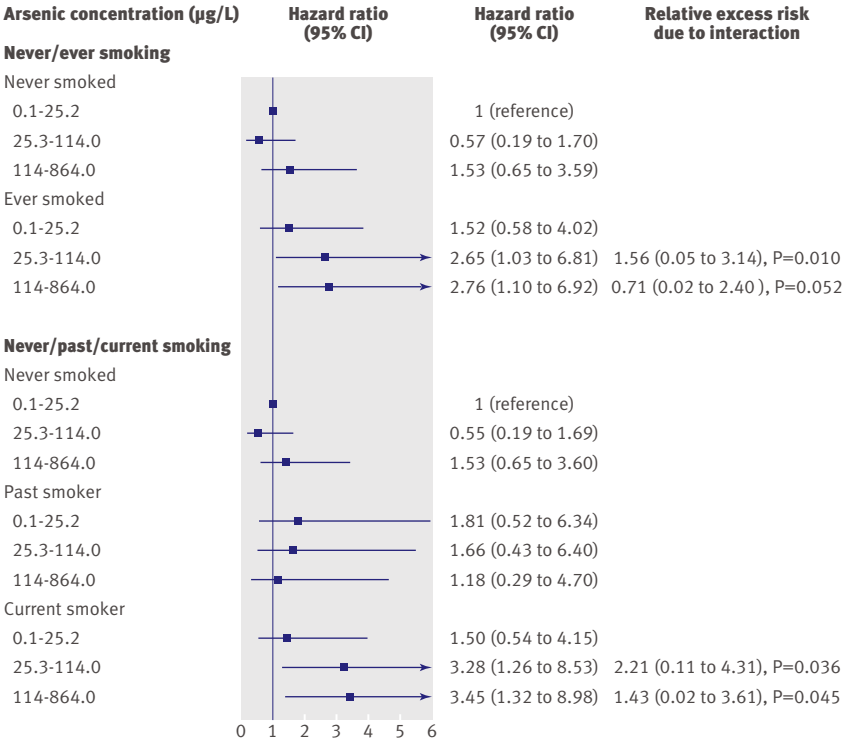

Fig 2 | **Joint effect of cigarette smoking and concentrations of well arsenic at baseline on mortality from ischaemic heart disease and other heart disease. Hazard ratios adjusted for sex and baseline age (years), BMI, education, and changes in urinary creatinine adjusted arsenic (μg/g of creatinine) between visits**

strengthened by the prospective study design and the fact that the outcome, potential confounders, effect modifiers, and well arsenic and urinary arsenic were all measured at the individual level.

Consistent with retrospective cohort and ecological studies of mortality from cerebrovascular disease in Chile, Taiwan, and Spain,[11 15 56] we found no association between arsenic exposure and mortality from cerebrovascular disease. A cross sectional study in Taiwan, however, reported a positive association between arsenic exposure and the prevalence of cerebrovascular disease.[57] The conflicting results in the studies could be partly because of the heterogeneity of the causes of cerebrovascular disease. The distribution of ischaemic stroke and haemorrhagic stroke might be different in studies of mortality and prevalence of cerebrovascular disease. Future larger studies with subtypes of cerebrovascular disease are needed to further assess the association.

We found that changes in urinary arsenic over time were positively associated with the risk of mortality from total cardiovascular disease. A study in Chile has suggested that the latency required for arsenic exposure to have an influence on the risk of cardiovascular disease was relatively shorter than that for the risk of cancer.[11] With longer follow-up, we will be able to characterise this association and study the influence of changes in arsenic exposure on the risk of subtypes of cardiovascular disease in the future.

Interaction between arsenic exposure and cigarette smoking

The hypothesis that cigarette smoking increases susceptibility to the cardiovascular effects of arsenic is well supported by previous studies on cancer and skin lesions.[16-20 58] Cigarette smoking has been associated with a lower methylation capacity of arsenic, as indicated by a higher ratio of urinary monomethylarsonate to dimethylarsinate in smokers.[59] Moreover, tobacco smoking can increase the requirement of folate, a critical cofactor in one-carbon metabolism, a process through which arsenic is enzymatically methylated. Taken together, cigarette smoking is likely to influence arsenic toxicity and should be taken into consideration in studies of lower levels of arsenic exposure. The consideration of smoking is more important for outcomes such as lung cancer and cardiovascular disease, for which arsenic might not be a necessary cause, as opposed to skin lesions. The synergistic effect observed in our study provides evidence of the presence of individuals who would experience a high risk of ischaemic heart disease and other heart disease only if they are exposed to both cigarette smoking and arsenic exposure at concentrations as low as 25.3-114.0 μg/L. The corresponding estimate for the attributable proportion due to interaction is 59% (13.8% to 100%), indicating that as much as 59% of deaths from heart disease among smokers with moderate level of arsenic exposure might be attributable to the synergistic effect of these two exposures. This finding has important public health implications in that smoking cessation or reduction in exposure to arsenic can lead to a greater than expected reduction in mortality from heart disease.

Potential underlying mechanisms

The mechanisms by which arsenic leads to cardiovascular disease are not clear. Several animal studies have suggested that arsenic can induce atherosclerosis.[60-62] The induction of oxidative stress by arsenic can influence gene expression, inflammatory responses, and endothelial nitric oxide homoeostasis,[63] which play an important role in maintaining vascular tone.[64] In a cross sectional study of the baseline data, we observed a positive association between arsenic exposure and high pulse pressure,[45] a consequence of arteriosclerosis and arterial stiffness.[65 66] Several in vitro studies suggest that arsenic promotes inflammatory activity[61 67] and endothelial cell remodelling.[68] Among cases of arsenic induced skin lesions in our study population, we found a positive association between arsenic exposure and plasma concentrations of soluble cell adhesion molecules, markers of endothelial dysfunction for risk of cardiovascular disease.[69] Future studies in healthy individuals with preclinical phenotypes for vascular inflammation and endothelial dysfunction relevant for cardiovascular disease are needed to clarify the underlying mechanisms.

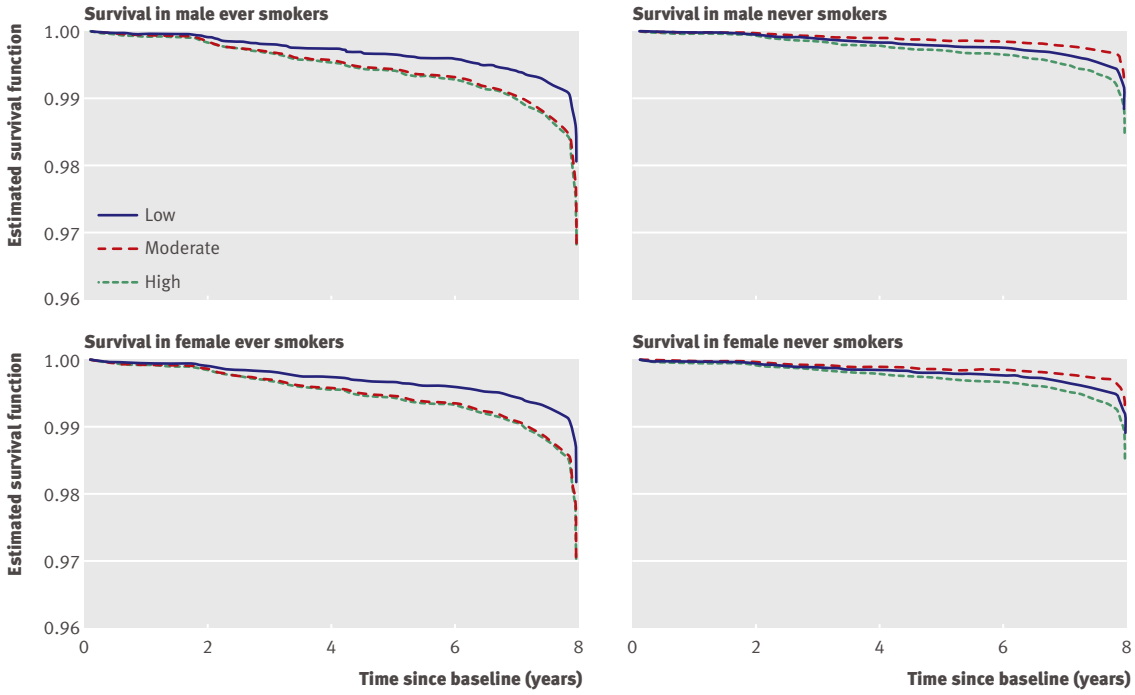

Fig 3 | Multivariate adjusted (education, age, BMI, changes in urinary arsenic over time) survival curves for ischaemic heart disease and other heart disease by baseline concentrations of well arsenic (low, medium, and high), sex, and baseline smoking status. Drop in survival curves around year 8 was because all participants who were alive at the end of third (final) follow-up visit were censored at date of that visit

Strengths and limitations

Unique features of the present study include the availability of data on arsenic exposure measured in both urine and water, the use of repeat measurements of total urinary arsenic to measure internal dose of arsenic exposure and to track changes in exposure levels over time, and the information on cigarette smoking. In a random 10% of participants, inorganic arsenic (As^{V} and As^{III}) and its metabolites monomethylarsonate and dimethylarsinate accounted for 96% of total urinary arsenic, whereas arsenobetaine and arsenocholine, derived mainly from dietary intakes of certain marine fish, together accounted for 3%.[70] Thus well arsenic, which has a correlation of 0.70, 0.61, and 0.57 with total urinary arsenic, urinary dimethylarsinate, and urinary monomethylarsonate concentration, respectively,[70] was clearly the main source of arsenic in the urine.

Several potential limitations, however, should also be noted. Firstly, we did not consider individual metabolites of arsenic in urine or blood and therefore could not assess susceptibility from arsenic methylation capacity. We are currently assessing the role of arsenic metabolites in modifying the risk of disease in a case-cohort study. Secondly, the study results might not be generalisable to other populations with a different profile of risk factors for cardiovascular disease that might interact with arsenic exposure in risk. In particular, the study population consisted of married men and women, who were mostly lean, with a mean BMI of 18.9. The choice of recruiting married men and women, however, helped the retention of the participants in follow-up and should enhance internal validity of the findings. Future studies are needed to evaluate potential effect modifications by specific nutritional factors that are related to arsenic metabolism, such as folate and selenium. Lastly, our study is an observational study and might be susceptible to unmeasured confounding. Individuals' choice of well was largely based on geographical convenience, however, and well arsenic concentration was not well known among the study population before recruitment.[24 25 38] Though we did not collect information on hyperlipidaemia, available literature does not suggest a positive association between arsenic exposures and cholesterol profile.[62 71] Adjustment for other established risk factors for cardiovascular disease did not change results appreciably. We would therefore expect the impact of residual confounding to be relatively minor.

Conclusions

In conclusion, in this prospective cohort analysis, we found a dose-response relation between arsenic exposure and mortality from cardiovascular disease, especially heart disease, at a much lower level of arsenic exposure than previously reported. There was a synergistic effect between cigarette smoking and arsenic exposure at moderate or high levels on mortality from ischaemic heart disease and other heart disease. These findings suggest the cardiovascular effects of arsenic exposure at moderate levels, which is further potentiated by smoking.

WHAT IS ALREADY KNOWN ON THIS TOPIC

High levels of exposure to arsenic (>500 µg/L) in drinking water have been related to an increased risk of cardiovascular disease

WHAT THIS STUDY ADDS

There was a dose-response relation between arsenic exposure, measured at baseline in both water and urine, and subsequent mortality from cardiovascular disease, especially heart disease, in a Bangladeshi population with a moderate level of exposure

There was a synergistic effect between cigarette smoking and arsenic exposure on mortality from heart disease

Contributors: YC and HA designed the study. HA, YC, and JHG obtained funding. MA, TK, DL, and GS maintained and supervised the HEALS cohort database. FP, TI, AA, MR-Z, and RH supervised the fieldwork. VS and JHG supervised measurement of urinary arsenic. LvG supervised the measurement of water arsenic. YC and ML analysed the data. YC, HA, JG, and FP helped to interpret the results. YC, HA, and JG wrote the paper. HA is guarantor.

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Competing interest: All authors have completed the Unified Competing Interest form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare: no support from any organisation for the submitted work; no financial relationships with any organisations that might have an interest in the submitted work in the previous three years; no other relationships or activities that could appear to have influenced the submitted work.

Ethical approval: The study procedures were approved by the ethics committee of the Bangladesh Medical Research Council and the institutional review boards of Columbia University and the University of Chicago. Verbal consent was obtained from study participants.

Data sharing: No additional data available.

1 United States Environmental Protection Agency. Fact sheet: drinking water standard for arsenic. EPA 815-F-00-015. 2009. www.epa.gov/safewater/arsenic/regulations_factsheet.html.
2 Wang CH, Hsiao CK, Chen CL, Hsu LI, Chiou HY, Chen SY,et al. A review of the epidemiologic literature on the role of environmental arsenic exposure and cardiovascular diseases. *Toxicol Appl Pharmacol* 2007;222:315-26.
3 Navas-Acien A, Sharrett AR, Silbergeld EK, Schwartz BS, Nachman KE, Burke TA,et al. Arsenic exposure and cardiovascular disease: a systematic review of the epidemiologic evidence. *Am J Epidemiol* 2005;162:1037-49.
4 Chen CJ, Hsueh YM, Lai MS, Shyu MP, Chen SY, Wu MM,et al. Increased prevalence of hypertension and long-term arsenic exposure. *Hypertension* 1995;25:53-60.
5 Rahman M, Tondel M, Ahmad SA, Chowdhury IA, Faruquee MH, Axelson O. Hypertension and arsenic exposure in Bangladesh. *Hypertension* 1999;33:74-8.
6 Chen CJ, Chiou HY, Chiang MH, Lin LJ, Tai TY. Dose-response relationship between ischemic heart disease mortality and long-term arsenic exposure. *Arterioscler Thromb Vasc Biol* 1996;16:504-10.
7 Tseng CH, Chong CK, Tseng CP, Hsueh YM, Chiou HY, Tseng CC,et al. Long-term arsenic exposure and ischemic heart disease in arseniasis-hyperendemic villages in Taiwan. *Toxicol Lett* 2003;137:15-21.
8 Wang CH, Jeng JS, Yip PK, Chen CL, Hsu LI, Hsueh YM,et al. Biological gradient between long-term arsenic exposure and carotid atherosclerosis. *Circulation* 2002;105:1804-9.
9 Tseng CH, Chong CK, Chen CJ, Tai TY. Dose-response relationship between peripheral vascular disease and ingested inorganic arsenic among residents in blackfoot disease endemic villages in Taiwan. *Atherosclerosis* 1996;120:125-33.
10 Hsueh YM, Wu WL, Huang YL, Chiou HY, Tseng CH, Chen CJ. Low serum carotene level and increased risk of ischemic heart disease related to long-term arsenic exposure. *Atherosclerosis* 1998;141:249-57.
11 Yuan Y, Marshall G, Ferreccio C, Steinmaus C, Selvin S, Liaw J,et al. Acute myocardial infarction mortality in comparison with lung and bladder cancer mortality in arsenic-exposed region II of Chile from 1950 to 2000. *Am J Epidemiol* 2007;166:1381-91.
12 Engel RR, Smith AH. Arsenic in drinking water and mortality from vascular disease: an ecologic analysis in 30 counties in the United States. *Arch Environ Health* 1994;49:418-27.
13 Lewis DR, Southwick JW, Ouellet-Hellstrom R, Rench J, Calderon RL. Drinking water arsenic in Utah: a cohort mortality study. *Environ Health Perspect* 1999;107:359-65.
14 Meliker JR, Wahl RL, Cameron LL, Nriagu JO. Arsenic in drinking water and cerebrovascular disease, diabetes mellitus, and kidney disease in Michigan: a standardized mortality ratio analysis. *Environ Health* 2007;6:4.
15 Medrano MA, Boix R, Pastor-Barriuso R, Palau M, Damian J, Ramis R, et al. Arsenic in public water supplies and cardiovascular mortality in Spain. *Environ Res* 2010;110:448-54.
16 Chen Y, Graziano JH, Parvez F, Hussain I, Momotaj H, van Geen A, et al. Modification of risk of arsenic-induced skin lesions by sunlight exposure, smoking, and occupational exposures in Bangladesh. *Epidemiology* 2006;17:459-67.
17 Lindberg AL, Sohel N, Rahman M, Persson LA, Vahter M. Impact of smoking and chewing tobacco on arsenic-induced skin lesions. *Environ Health Perspect* 2010;118:533-8.
18 Steinmaus C, Yuan Y, Bates MN, Smith AH. Case-control study of bladder cancer and drinking water arsenic in the western United States. *Am J Epidemiol* 2003;158:1193-201.
19 Karagas MR, Tosteson TD, Morris JS, Demidenko E, Mott LA, Heaney J, et al. Incidence of transitional cell carcinoma of the bladder and arsenic exposure in New Hampshire. *Cancer Causes Control* 2004;15:465-72.
20 Chen CL, Hsu LI, Chiou HY, Hsueh YM, Chen SY, Wu MM,et al. Ingested arsenic, cigarette smoking, and lung cancer risk: a follow-up study in arseniasis-endemic areas in Taiwan. *JAMA* 2004;292:2984-90.
21 Chowdhury UK, Biswas BK, Chowdhury TR, Samanta G, Mandal BK, Basu GC,et al. Groundwater arsenic contamination in Bangladesh and West Bengal, India. *Environ Health Perspect* 2000;108:393-7.
22 Mudur G. Half of Bangladesh population at risk of arsenic poisoning. *BMJ* 2000;320:822.
23 British Geological Survey. Groundwater studies for arsenic contamination in Bangladesh—phase 1 findings. 1999. www.bgs.ac.uk/arsenic/.
24 Ahsan H, Chen Y, Parvez F, Argos M, Hussain AI, Momotaj H,et al. Health Effects of Arsenic Longitudinal Study (HEALS): description of a multidisciplinary epidemiologic investigation. *J Expo Sci Environ Epidemiol* 2006;16:191-205.
25 Parvez F, Chen Y, Argos M, Hussain AZ, Momotaj H, Dhar R,et al. Prevalence of arsenic exposure from drinking water and awareness of its health risks in a Bangladeshi population: results from a large population-based study. *Environ Health Perspect* 2006;114:355-9.
26 Chen Y, Factor-Litvak P, Howe GR, Graziano JH, Brandt-Rauf P, Parvez F,et al. Arsenic exposure from drinking water, dietary intakes of B vitamins and folate, and risk of high blood pressure in Bangladesh: a population-based, cross-sectional study. *Am J Epidemiol* 2007;165:541-52.
27 Chen,Y, Ahsan H, Slavkovich V, Peltier GL, Gluskin RT, Parvez F, et al. No association between arsenic exposure from drinking water and diabetes mellitus: a cross-sectional study in Bangladesh: *Environ Health Perspect* 2010;118:1299-305.
28 Pierce BL, Kalra T, Argos M, Parvez F, Chen Y, Islam T,et al. A prospective study of body mass index and mortality in Bangladesh. *Int J Epidemiol* 2010;39:1037-45.
29 Aho K, Harmsen P, Hatano S, Marquardsen J, Smirnov VE, Strasser T. Cerebrovascular disease in the community: results of a WHO collaborative study. *Bull World Health Organ* 1980;58:113-30.
30 World Health Organization. Manual of the international classification of diseases, injuries and causes of death. WHO, 2007.
31 Ronsmans C, Vanneste AM, Chakraborty J, Van Ginneken J. A comparison of three verbal autopsy methods to ascertain levels and causes of maternal deaths in Matlab, Bangladesh. *Int J Epidemiol* 1998;27:660-6.
32 Baqui AH, Black RE, Arifeen SE, Hill K, Mitra SN, al Sabir A. Causes of childhood deaths in Bangladesh: results of a nationwide verbal autopsy study. *Bull World Health Organ* 1998;76:161-71.
33 Sohel N, Persson LA, Rahman M, Streatfield PK, Yunus M, Ekstrom EC,et al. Arsenic in drinking water and adult mortality: a population-based cohort study in rural Bangladesh. *Epidemiology* 2009;20:824-30.
34 Van Geen A, Ahsan H, Horneman AH, Dhar RK, Zheng Y, Hussain I, et al. Promotion of well-switching to mitigate the current arsenic crisis in Bangladesh. *Bull World Health Organ* 2002;80:732-7.

35 Van Geen A, Zheng Y, Versteeg R, Stute M, Horneman A, Dhar R,et al. Spatial variability of arsenic in 6000 tube wells in a 25 km² area of Bangladesh. *Water Resour Res* 2003;39:1140.
36 Cheng Z, van Geen A, Seddique AA, Ahmed KM. Limited temporal variability of arsenic concentrations in 20 wells monitored for 3 years in Araihazar, Bangladesh. *Environ Sci Technol* 2005;39:4759-66.
37 Cheng Z, Zheng Y, Mortlock R, van Geen A. Rapid multi-element analysis of groundwater by high-resolution inductively coupled plasma mass spectrometry. *Anal Bioanal Chem* 2004;379:512-8.
38 Ahsan H, Chen Y, Parvez F, Zablotska L, Argos M, Hussain I,et al. Arsenic exposure from drinking water and risk of premalignant skin lesions in Bangladesh: baseline results from the Health Effects of Arsenic Longitudinal Study. *Am J Epidemiol* 2006;163:1138-48.
39 Nixon DE, Mussmann GV, Eckdahl SJ, Moyer TP. Total arsenic in urine: palladium-persulfate vs nickel as a matrix modifier for graphite furnace atomic absorption spectrophotometry. *Clin Chem* 1991;37:1575-9.
40 Slot C. Plasma creatinine determination. A new and specific Jaffe reaction method. *Scand J Clin Lab Invest* 1965;17:381-7.
41 Chen Y, van Geen A, Graziano JH, Pfaff A, Madajewicz M, Parvez F, et al. Reduction in urinary arsenic levels in response to arsenic mitigation efforts in Araihazar, Bangladesh. *Environ Health Perspect* 2007;115:917-23.
42 Chen Y, Ahsan H, Parvez F, Howe GR. Validity of a food-frequency questionnaire for a large prospective cohort study in Bangladesh. *Br J Nutr* 2004;92:851-9.
43 Argos M, Parvez F, Chen Y, Hussain AZ, Momotaj H, Howe GR,et al. Socioeconomic status and risk for arsenic-related skin lesions in Bangladesh. *Am J Public Health* 2007;97:825-31.
44 Bruzzi P, Green SB, Byar DP, Brinton LA, Schairer C. Estimating the population attributable risk for multiple risk factors using case-control data. *Am J Epidemiol* 1985;122:904-14.
45 Chen Y, Factor-Litvak P, Howe GR, Graziano JH, Brandt-Rauf P, Parvez F,et al. Arsenic exposure from drinking water, dietary intakes of B vitamins and folate, and risk of high blood pressure in Bangladesh: a population-based, cross-sectional study. *Am J Epidemiol* 2007;165:541-52.
46 Tseng CH, Tai TY, Chong CK, Tseng CP, Lai MS, Lin BJ,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.
47 Gamble MV, Liu X. Urinary creatinine and arsenic metabolism. *Environ Health Perspect* 2005;113:A442-3.
48 Steinmaus C, Yuan Y, Liaw J, Smith AH. On arsenic, diabetes, creatinine, and multiple regression modeling. *Epidemiology* 2009;20:e1-2.
49 Melkonian S, Argos M, Pierce BL, Chen Y, Islam T, Ahmed A,et al. A prospective study of the synergistic effects of arsenic exposure and smoking, sun exposure, fertilizer use, and pesticide use on risk of premalignant skin lesions in Bangladeshi men. *Am J Epidemiol* 2011;173:183-91.
50 Rothman KJ, Greenland S. Modern epidemiology. Little, Brown, 1986.
51 Li R, Chambless L. Test for additive interaction in proportional hazards models. *Ann Epidemiol* 2007;17:227-36.
52 Knol MJ, van der Tweel I, Grobbee DE, Numans ME, Geerlings MI. Estimating interaction on an additive scale between continuous determinants in a logistic regression model. *Int J Epidemiol* 2007;36:1111-8.
53 Hosmer DW, Lemeshow S. Confidence interval estimation of interaction. *Epidemiology* 1992;3:452-6.
54 Wei L, Lin D, Weissfeld L. Regression analysis of multivariate incomplete failure time data by modeling marginal distributions. *J Am Stat Assoc* 1989;84:1065-73.
55 Greenland S, Robins J. Invited commentary: ecologic studies—biases, misconceptions, and counterexamples. *Am J Epidemiol* 1994;139:747-60.
56 Wu MM, Kuo TL, Hwang YH, Chen CJ. Dose-response relation between arsenic concentration in well water and mortality from cancers and vascular diseases. *Am J Epidemiol* 1989;130:1123-32.
57 Chiou HY, Huang WI, Su CL, Chang SF, Hsu YH, Chen CJ. Dose-response relationship between prevalence of cerebrovascular disease and ingested inorganic arsenic. *Stroke* 1997;28:1717-23.
58 Ferreccio C, Gonzalez C, Milosavjlevic V, Marshall G, Sancha AM, Smith AH. Lung cancer and arsenic concentrations in drinking water in Chile. *Epidemiology* 2000;11:673-9.
59 Hopenhayn-Rich C, Biggs ML, Smith AH, Kalman DA, Moore LE. Methylation study of a population environmentally exposed to arsenic in drinking water. *Environ Health Perspect* 1996;104:620-8.
60 Bunderson M, Brooks DM, Walker DL, Rosenfeld ME, Coffin JD, Beall HD. Arsenic exposure exacerbates atherosclerotic plaque formation and increases nitrotyrosine and leukotriene biosynthesis. *Toxicol Appl Pharmacol* 2004;201:32-9.
61 Simeonova PP, Hulderman T, Harki D, Luster MI. Arsenic exposure accelerates atherogenesis in apolipoprotein E(−/−) mice. *Environ Health Perspect* 2003;111:1744-8.
62 Srivastava S, Vladykovskaya EN, Haberzettl P, Sithu SD, D'Souza SE, States JC. Arsenic exacerbates atherosclerotic lesion formation and inflammation in ApoE−/− mice. *Toxicol Appl Pharmacol* 2009;241:90-100.
63 Simeonova PP, Luster MI. Arsenic and atherosclerosis. *Toxicol Appl Pharmacol* 2004;198:444-9.
64 Willerson JT, Ridker PM. Inflammation as a cardiovascular risk factor. *Circulation* 2004;109:II2-10.
65 Dart AM, Kingwell BA. Pulse pressure—a review of mechanisms and clinical relevance. *J Am Coll Cardiol* 2001;37:975-84.
66 Safar ME, Levy BI, Struijker-Boudier H. Current perspectives on arterial stiffness and pulse pressure in hypertension and cardiovascular diseases. *Circulation* 2003;107:2864-9.
67 Bunderson M, Coffin JD, Beall HD. Arsenic induces peroxynitrite generation and cyclooxygenase-2 protein expression in aortic endothelial cells: possible role in atherosclerosis. *Toxicol Appl Pharmacol* 2002;184:11-8.
68 Straub AC, Klei LR, Stolz DB, Barchowsky A. Arsenic requires sphingosine-1-phosphate type 1 receptors to induce angiogenic genes and endothelial cell remodeling. *Am J Pathol* 2009;174:1949-58.
69 Chen Y, Santella RM, Kibriya MG, Wang Q, Kappil M, Verret WJ,et al. Association between arsenic exposure from drinking water and plasma levels of soluble cell adhesion molecules. *Environ Health Perspect* 2007;115:1415-20.
70 Ahsan H, Chen Y, Kibriya MG, Slavkovich V, Parvez F, Jasmine F,et al. Arsenic metabolism, genetic susceptibility, and risk of premalignant skin lesions in Bangladesh. *Cancer Epidemiol Biomarkers Prev* 2007;16:1270-8.
71 Tseng CH, Chong CK, Chen CJ, Tai TY. Lipid profile and peripheral vascular disease in arseniasis-hyperendemic villages in Taiwan. *Angiology* 1997;48:321-35.

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