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Arsenic exposure at low-to-moderate levels and skin lesions, arsenic metabolism, neurological functions, and biomarkers for respiratory and cardiovascular diseases: Review of recent findings from the Health Effects of Arsenic Longitudinal Study (HEALS) in Bangladesh

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ABSTRACT

The contamination of groundwater by arsenic in Bangladesh is a major public health concern affecting 35–75 million people. Although it is evident that high levels (>300 μg/L) of arsenic exposure from drinking water are related to adverse health outcomes, health effects of arsenic exposure at low-to-moderate levels (10–300 μg/L) are not well understood. We established the Health Effects of Arsenic Longitudinal Study (HEALS) with more than 20,000 men and women in Araihazar, Bangladesh, to prospectively investigate the health effects of arsenic predominately at low-to-moderate levels (0.1 to 864 μg/L, mean 99 μg/L) of arsenic exposure. Findings to date suggest adverse effects of low-to-moderate levels of arsenic exposure on the risk of pre-malignant skin lesions, high blood pressure, neurological dysfunctions, and all-cause and chronic disease mortality. In addition, the data also indicate that the risk of skin lesion due to arsenic exposure is modifiable by nutritional factors, such as folate and selenium status, lifestyle factors, including cigarette smoking and body mass index, and genetic polymorphisms in genes related to arsenic metabolism. The analyses of biomarkers for respiratory and cardiovascular functions support that there may be adverse effects of arsenic on these outcomes and call for confirmation in large studies. A unique strength of the HEALS is the availability of outcome data collected prospectively and data on detailed individual-level arsenic exposure estimated using water, blood and repeated urine samples. Future prospective analyses of clinical endpoints and related host susceptibility will enhance our knowledge on the health effects of low-to-moderate levels of arsenic exposure, elucidate disease mechanisms, and give directions for prevention.

Introduction

Arsenic is abundant in the earth's crust and can be released into groundwater under certain conditions. In many parts of the world where groundwater is an important source of drinking water, long-term exposure to arsenic from drinking water has been considered a public health hazard. In addition to be classified as a Class I human carcinogen by the International Agency for Research on Cancer (IARC) for its association with an increase in skin cancer risk, arsenic exposure has also been linked to increased risks of internal cancers, diabetes, cardiovascular disease, adverse pregnancy outcomes, and a decrease in children's intellectual function.

However, epidemiologic evidence on many of these health effects of arsenic exposure has not been well-established, with uncertainties in latency, dose–response relationships and population differences. In particular, although it is evident that high levels of arsenic exposure (>300 μg/L) are related to internal cancer and cardiovascular disease, epidemiologic evidence of the effects of arsenic exposure from drinking water at low-to-moderate levels (<300 or <100 μg/L) remains inconclusive. Most existing studies include limited sample size (Chiou et al., 2001; Karagas et al., 2004; Steinmaus et al., 2003) and/or unreliable long-term measures of arsenic exposure (Engel and Smith, 1994; Lewis et al., 1999; Meliker et al., 2007; Zierold et al., 2004) with exposure measured ecologically or only cross-sectionally. Ecologic exposure measurement that uses the mean or median values in a county or community as the exposure level for individuals could

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result in considerable measurement errors. Since the effects of low-to-moderate levels of arsenic on disease are likely to be modest in magnitude, these studies are particularly susceptible to measurement errors in exposure ascertainment, which, in most cases, would lead to bias towards the null, but could also generate spurious associations under certain conditions (Greenland and Robins, 1994). In addition, the heterogeneity of drinking water resources, the limited exposure range, and the relatively high migration rates within the nation altogether pose a challenge for epidemiologic studies in the U.S. or other populations with surface water as the main source of drinking water that aim to investigate the impact of low-to-moderate levels of arsenic exposure to have a valid long-term arsenic measure at the individual level.

The contamination of groundwater by arsenic in Bangladesh is the largest poisoning of a population in history (Smith et al., 2000). As 95% of the country's 140 million population rely on well water, an estimated 57 million people have been chronically exposed to drinking water with arsenic levels exceeding the WHO standard of 10 μg/L, and 35 million people were exposed to arsenic levels above the country's government standard of 50 μg/L (British Geological Survey, 1999). Given the known health consequences of arsenic exposure, there is an imminent need to develop arsenic mitigation programs. Although there have been several case–control and cross-sectional studies published on arsenic exposure in Bangladesh (McCarty et al., 2007; Milton et al., 2005; Mitra et al., 2004; Tondel et al., 1999; Rahman et al., 1998), large epidemiologic studies are needed to assess the health effects of arsenic exposure in the population.

In Bangladesh, where 95% of the population exclusively drinks groundwater through wells and the population is remarkably stable, long-term arsenic exposure can be measured and tracked in large cohort at the individual level. We have established the Health Effects of Arsenic Longitudinal Study (HEALS) in Araihazar, Bangladesh, a prospective cohort with more than 20,000 participants, to investigate the health effects of arsenic exposure, avoiding many of the limitations in previous studies. In particular, the study population has been exposed to a wide range of arsenic exposure at low-to-moderate levels, providing us with a unique opportunity to evaluate health effects of arsenic exposure at these levels and avoiding many of the limitations of previous studies. We summarize findings from the HEALS to date and discuss the implications of our findings and future directions.

Health Effects of Arsenic Longitudinal Study (HEALS)

The HEALS was established as part of the Columbia University's Superfund Basic Research Program (CU-SBRP). Details of the HEALS have been presented elsewhere (Ahsan et al., 2006a; Parvez et al., 2006). Briefly, prior to subject recruitment, water samples and geographic positional system data were collected for a set of 5966 contiguous wells in a well-defined geographic area of 25 km^2 in Araihazar. As a baseline measurement, well owners were interviewed to create an initial roster list of 65,876 regular well users and residents in the area. From October, 2000 to May, 2002, 12,050 potential participants meeting eligibility criteria were approached, and 11,746 of those eligible agreed to participate (97.5% response rate) (Ahsan et al., 2006a). In 2007, an additional 8000 participants were recruited using the same methodologies, with a roster established based on arsenic measurement of a different set of 5000 well with a response rate of 95%. In order to update changes in arsenic exposure and the detection of a variety of health outcomes of interest, all cohort members were actively followed every two years through in-person home visits by trained physicians and interviewers.

Water samples from all the tube wells in the study area were collected and tested for total arsenic concentrations first by graphite furnace atomic-absorption spectrometry (GFAA) with a Hitachi Z-8200 system at the Lamont-Doherty Earth Observatory (LDEO) of Columbia University. Samples that fell below the detection limit of GFAA (5 μg/L) were subsequently analyzed by inductively coupled plasma mass spectrometry (ICP-MS), with a detection limit of 0.1 μg/L. The study population has been exposed to a wide range of arsenic exposure at low-to-moderate levels, ranging from 0.1 to 864 μg/L, with a mean of 99 μg/L.

At baseline recruitment, venous whole blood samples and a spot urine sample were collected for >90% cohort participants. At follow-up visits, a spot urine sample is also collected. Both blood and urine samples were kept in portable coolers immediately after collection. All samples were kept frozen and shipped to USA on dry ice within 1–2 months. All urine samples collected at baseline and at follow-up visits were analyzed for total arsenic concentration by GFAA, using the Analyst 600 graphite furnace system at Columbia University, as previously described (Nixon et al., 1991).

At baseline and in each in-person biennial follow-up visit, participants undergo a comprehensive clinical examination conducted by trained physicians. Information on patterns and history of well use, demographics and lifestyle characteristics are also collected at every visit. At baseline, the questionnaire also included a validated food frequency questionnaire (FFQ) designed specifically for the study population (Chen et al., 2004b). In addition, a field clinic was established solely for cohort study participants and their family members to passively follow up participants between their biennial in-person visits to augment the detection of study outcomes through active follow-up.

Given its biological samples and its data on arsenic exposure, dietary factors and lifestyle habits, the HEALS provides a unique opportunity to study the health effects of arsenic exposure, host susceptibility and pre-clinical disease states measured using biomarkers. In addition, based on findings from observational studies, a series of intervention studies has been initiated, including those pertaining to health education and chemoprevention trials.

Key findings from heals, to date

Well arsenic concentration and biochemical measures of arsenic exposure

Relationships among different measures of arsenic exposure

Although total urinary and blood arsenic levels have been considered indicators of short-term internal dose of arsenic exposure, with chronic and continuing exposure, steady-state concentrations in blood and urine are can be achieved. Repeated measurements of these indices may be used to depict long-term exposure level and changes in exposure over time. Among 849 individuals randomly selected from the HEALS participants, baseline urinary arsenic was highly correlated with baseline blood arsenic ($r = 0.85$, p-value <0.01) and with water arsenic levels ($r = 0.76$, p-value <0.01) (Hall et al., 2006).

Using urinary arsenic data collected during the first three visits from 10,224 participants, we estimated that the pair-wise correlation between urinary arsenic measured at each visit was high (all ≥ 0.60, p-values <0.01). The correlation of baseline urinary arsenic levels with urinary arsenic levels at follow-up visits somewhat decreased from 0.66 for Visit 2 to 0.60 for Visit 3, suggesting a change in arsenic exposure among some participants; however, the change in arsenic exposure is not substantial in the overall cohort. On average, urinary arsenic levels decreased by 62 μg/g creatinine from baseline to the first follow-up visit (~ -20 μg/L in well arsenic), remained at the same level at Visit 3, with a high correlation (0.74, p-value <0.01) between urinary arsenic at Visits 2 and 3.

These data suggest that the level of urinary arsenic does not fluctuate greatly overtime, and urinary arsenic can serve as a long-term biomarker of arsenic exposure to track arsenic exposure levels during the follow-up time in the cohort.

Measures and determinants of arsenic metabolism

Arsenic in drinking water is present as inorganic arsenic (InAs), i.e., arsenite (As^{III}) and arsenate (As^{V}). In Bangladesh, As^{III} is the predominant form to which people are exposed from groundwater. Methylation of $InAs^{III}$, which is primarily hepatic, relies on folate-dependent, one-carbon metabolism; folate contributes methyl groups used in generating s-adenosylmethionine (SAM). Arsenic (+3 oxidation state) methyltransferase (AS3MT) transfers the methyl group from SAM to $InAs^{III}$ to generate monomethylarsonic acid (MMA^{V}). After the reduction of MMA^{V} to monomethylarsonous acid (MMA^{III}), AS3MT can catalyze a second methylation, generating dimethylarsinic acid (DMA^{V}) (Yang et al., 2002). The relative distribution of urinary arsenic metabolites varies from person to person and has been interpreted to reflect biologically effective doses of exposure to the various arsenic metabolites (Chen et al., 2005) as well as arsenic methylation efficiency, with some evidence suggesting that increased proportions of MMA and InAs species in urine may be associated with a higher risk of cancer (Chen et al., 2003a; Huang et al., 2008).

Among 98 HEALS cohort members participating in the placebo arm of a double-blind randomized folate supplementation trial (Gamble et al., 2006), the intraclass correlations (ICCs) of three urine samples collected over a three-month period were all >0.65 for urinary arsenic metabolites. Of particular note, the ICCs for %MMA, %DMA, and the ratio of MMA-to-DMA were 0.84 (95% CI, 0.78–0.89), 0.82 (95% CI, 0.74–0.87), and 0.82 (95% CI, 0.75–0.88), respectively (Ahsan et al., 2007). The data suggest that, in the absence of interventions, there is little within-subject variability of urinary arsenic metabolite profiles and thus justifies the use of single measurements in epidemiologic studies.

Factors that are related to the composition of urinary arsenic metabolites may contribute to the susceptibility to health effects of arsenic exposure. Table 1 summarizes several studies that utilized the resources of the HEALS with a focus on inter-individual variability in the distribution of urinary arsenic metabolites. In 1041 individuals randomly selected from the HEALS participants who were free of skin lesions at baseline, water arsenic concentration was positively associated with urinary %MMA and inversely associated with urinary %DMA (Ahsan et al., 2007), suggesting that either methylation of arsenic to DMA is saturable or, alternatively, that arsenic inhibits the arsenic methyltransferase enzyme. Body mass index (BMI) was positively related to urinary %MMA and inversely related to urinary %DMA, however the association was not statistically significant (Ahsan et al., 2007). Specific dietary food/nutrient intakes may be more relevant to arsenic metabolism. In the same study population, higher intakes of cysteine, methionine, calcium, protein and vitamin B-12 measured by the FFQ were associated with higher %MMA in urine. Additionally, higher intakes of niacin (beta = 0.22, p-value = 0.02) and choline (beta = 0.10, p-value = 0.02) were associated with higher DMA-to-MMA ratios (Heck et al., 2007).

In a sample of 1650 HEALS cohort members who were recruited into the Nutritional Influences of Arsenic Toxicity (NIAT) study, an unusually high prevalence of hyperhomocysteinemia was found, particularly among males (Gamble et al., 2005a). In subsequent analyses of 300 randomly selected participants from the NIAT study, urinary %DMA was positively associated with plasma folate ($r = 0.14$, $p = 0.02$) and negatively associated with total homocysteine (tHcys; $r = -0.14$, $p = 0.01$). Conversely, urinary %MMA was negatively associated with folate ($r = -0.12$, $p = 0.04$) and positively associated with tHcys ($r = 0.21$, $p < 0.01$) (Gamble et al., 2005b). An unanticipated finding was that urinary creatinine, a breakdown product of creatine, is negatively associated with %InAs and positively associated with % DMA in urine. Creatine is synthesized endogenously in a process that relies on one-carbon metabolism; it also consumes methyl groups, and is itself consumed in the diet, predominantly from meat. The finding warrants further investigation. Collectively, these findings indicate that nutritional factors involved in one-carbon metabolism contribute to the variability in arsenic methylation.

Recently, the CU-SBRP Trace Metals Laboratory has developed the technology to measure arsenic metabolites in blood. This has led to the observation that there is relatively more MMA and less DMA in blood than in urine both in adults (Gamble et al., 2007) and in children (Hall et al., 2007). This is not surprising, given that DMA has a shorter circulating half-life than InAs, and is consistent with the role of methylation in facilitating arsenic elimination. This finding has important implications with regard to risk assessment in epidemiologic studies that traditionally rely on measures of arsenic metabolites in urine.

Pre-malignant skin lesions

Cutaneous abnormalities are well-known early signs of chronic inorganic arsenic poisoning. Melanosis is considered as an early-stage skin lesions, while keratosis is the most frequent manifestation preceding the appearance of arsenic-related skin cancer (Tseng et al., 1968). They give rise to the majority of arsenic-induced basal and squamous cell skin cancers (Tseng et al., 1968). Unlike arsenic-related internal cancers that could have long latencies, these premalignant skin lesions may appear with shorter periods of arsenic exposure (Saha, 2003). We have conducted several cross-sectional, case–control, case-cohort, and nested case–control studies of skin lesions to evaluate the full dose–response relationship between arsenic exposure and the risk of skin lesions, as well as relevant nutritional and genetic susceptibility factors (Table 2).

Arsenic exposure at low-to-moderate levels and skin lesions

At baseline, we identified 714 cases of premalignant skin lesions. In cross-sectional analyses of baseline data comparing cases and non-cases of skin lesions, we observed a dose–response effect of arsenic on

Table 1
Findings from HEALS on determinants of urinary or blood arsenic metabolites

Reference	Study design	Characteristics of subjects	Main findings
Heck et al. (2007)	Cross-sectional	1041 randomly selected subjects free of skin lesions	Positive associations of intakes of cysteine, methionine, calcium, protein and vitamin B-12 with urinary %MMA
Ahsan et al. (2007)			Positive associations of intakes of niacin, choline with urinary DMA-to-MMA ratios
			Positive associations between water arsenic and urinary %MMA, and inverse association between water arsenic and urinary %DMA
Gamble et al. (2005b)	Cross-sectional	300 randomly selected participants	Positive correlation between plasma folate and urinary %DMA, between total homocysteine (tHcys) and urinary %MMA, and between urinary creatinine and urinary %DMA
			Inverse correlation tHcys and urinary %DMA, and between plasma folate and urinary %MMA
Gamble et al. (2005a, b)	Randomized, double-blind, placebo-controlled trial	200 folate-deficient cohort participants	Greater increase of urinary %DMA and reduction in urinary %MMA in the folic acid group than in the placebo group
Gamble et al. (2007)			Significant reduction in total blood arsenic and of MMA in the blood in folic acid group.

Table 2
Findings from HEALS on the risk of arsenic-related pre-malignant skin lesions

Reference	Study design	Characteristics of subjects	Arsenic measurement	Main findings
Ahsan et al. (2006b)	Cross-sectional	714 cases of skin lesion and 10,724 non-skin lesion subjects	Water, urinary arsenic, time-weighted arsenic concentration	Dose–response relationship between arsenic exposure and risk of skin lesions Synergistic effect between high levels of arsenic exposure and male gender, low BMI, and old age.
Chen et al. (2006a)	Cross-sectional	714 cases of skin lesion and 10,724 non-skin lesion subjects	Water, urinary arsenic, time-weighted arsenic concentration	Synergistic effect between the highest level of arsenic exposure and tobacco smoking in men. Additive effects of sun exposure and arsenic exposure in men
Argos et al. (2007)	Cross-sectional	714 cases of skin lesion and 10,724 non-skin lesion subjects	Water, urinary arsenic	Effect-modification by land ownership on the multiplicative scale
Zablotska et al. (2008)	Cross-sectional	714 cases of skin lesion and 10,724 non-skin lesion subjects	Water, urinary arsenic	Effect-modification by high intake of riboflavin, pyridoxine, folate, and vitamins A, C and E
Ahsan et al. (2007)	Case–control	594 skin lesion cases and 1041 controls	Water, urinary arsenic, urinary %MMA, %DMA, MMA/DMA	Stronger dose–response relationship with urinary %MMA compared with other methylation indices Effect differs by *MTHFR* and *GSTO1* diplotypes
Chen et al. (2007a)	Prospective case-cohort	303 incident skin lesion cases and 849 subcohort members	Water, urinary arsenic	An inverse association between baseline blood selenium status and the incidence of skin lesions Additive effect of high arsenic exposure and low selenium status.
Hall et al. (2006)	Prospective case-cohort	303 incident skin lesion cases and 849 subcohort members	Water, urinary, and blood arsenic	Dose–response relationship between baseline blood and well arsenic and incidence of skin lesions
Pilsner et al. (2007)	Prospective nested case–control study	274 skin lesion cases 274 matched controls	Water, blood, urinary arsenic	Positive relationships of folate deficiency, hyperhomocysteinemia, low urinary creatinine, with skin lesion risk

the risk of skin lesions (Ahsan et al., 2006b). In particular, arsenic exposure appears to increase the risk of skin lesions, even at the low end of exposure in this population. Compared with drinking water containing <8.1 μg/L of arsenic, drinking water containing 8.1–40.0, 40.1–91.0, 91.1–175.0 and 175.1–864.0 μg/L of arsenic was associated with adjusted prevalence odds ratios of skin lesions of 1.91 (95% confidence interval (CI): 1.26, 2.89), 3.03 (95% CI: 2.05, 4.50), 3.71 (95% CI: 2.53, 5.44) and 5.39 (95% CI: 3.69, 7.86), respectively (Ahsan et al., 2006b). A prospective case-cohort analysis of skin lesion cases diagnosed during two years of follow-up also showed a similar dose–response relationship (Hall et al., 2006).

Modifiable determinants of effects of arsenic on skin lesions

In our analyses, we found that males and older participants were more likely to be affected by arsenic exposure (Ahsan et al., 2006b). Additionally, a synergistic effect between the highest level of arsenic exposure (>113 μg/L) and tobacco smoking on risk of skin lesions was observed in men (Chen et al., 2006a). Furthermore, the risk of skin lesions associated with any given level of arsenic exposure was greater in men with excessive sun exposure (Chen et al., 2006a). The effect of arsenic was also modified by land ownership on a multiplicative scale, with an increased risk among non-land owners associated with well water arsenic (Argos et al., 2007). Part of the modification effect due to socioeconomic status may be explained by nutritional status. In particular, participants with a comparatively high BMI (Ahsan et al., 2006b) or with comparatively high intake levels of riboflavin, pyridoxine, folate, and vitamins A, C and E were less likely to be affected by arsenic exposure (Zablotska et al., 2008). In a prospective case-cohort analysis of 303 incident skin lesion cases and 849 subcohort members, there was an inverse association between baseline blood selenium status and the incidence of skin lesions (Chen et al., 2007a).

In another nested case–control study of 274 skin lesion cases identified two years after recruitment and 274 controls matched to cases for gender, age, and water arsenic, we found that folate deficiency, hyperhomocysteinemia, low urinary creatinine–each associated with decreased arsenic methylation–are risk factors for arsenic-induced skin lesions. The positive association between DNA methylation and arsenic exposure that we previously observed in the NIAT study (Pilsner et al., 2007) was confirmed among the controls in this study. However, we believe that this may be an adaptive change, as hypomethylation of leukocyte DNA was found to be associated with an increased risk for skin lesions (Pilsner et al., in press). These data suggest that lifestyle and nutritional factors may modify the risk of arsenic-related skin lesions.

Genetic susceptibility to effects of arsenic on skin lesions

Interindividual variability in arsenic metabolism capacity may also contribute to the variation in susceptibility to the effect of arsenic. Glutathione S-transferase 1 (GSTO1) and methylenetetrahydrofolate reductase (MTHFR) are enzymes involved in arsenic metabolism pathways. In a case–control study of 594 skin lesion cases and 1041 controls, the dose–response relationship of skin lesion risk with urinary monomethylarsonous acid percentage (%MMA) was more apparent than those with other methylation indices (Ahsan et al., 2007). Individuals with the *MTHFR* 677TT/1298AA and 677CT/1298AA diplotypes were 1.66 (95% CI, 1.00–2.77) and 1.77 (95% CI, 0.61–5.14) times more likely to have skin lesions, compared with those carrying 677CC/1298CC diplotype. The OR for skin lesions in relation to the *GSTO1* diplotype containing all at-risk alleles was 3.91 (95% CI, 1.03–14.79) (Ahsan et al., 2007). These findings reiterate that arsenic-induced health effects may be especially deleterious in subsets of the population carrying susceptible variants of genes relevant to arsenic metabolism. Based on the risk estimates observed in this study, the proportion of skin lesions in our study population attributable to the *MTHFR* 677TT/1298AA and 677CT/1298AA diplotypes was estimated to be 7.5%. The corresponding estimated attributable proportion for the *GSTO1* at-risk diplotype was 8.9%.

Peripheral neuropathy and children's intellectual function

Among 137 HEALS participants randomly selected from those visited the field clinic over eight weeks, peripheral neuropathy was assessed by a vibration sensitivity tester (Vibratron II) (Hafeman et al., 2005). Arsenic exposure was associated with elevated toe vibration threshold (TVT). Specifically, urinary arsenic was significantly associated with elevated TVT (p-value <0.01) after adjustment for age and gender.

In cross-sectional analyses of 201 children at 10 years of age (whose parents participate in HEALS), children's intellectual function was assessed using tests drawn from the Wechsler Intelligence Scale for

Children, version III (WISC-III). Children provided urine specimens for the measurement of urinary As and creatinine. Information on the primary source of drinking water was obtained from the child's mother. Exposure to arsenic from drinking water was associated with reduced intellectual function after adjustment for sociodemographic covariates in a dose–response manner, such that children exposed to water arsenic levels >50 μg/L achieved significantly lower Performance and Full-Scale scores than did children exposed to lower water arsenic levels (Wasserman et al., 2004). In a similar investigation of 301 randomly selected six-year-olds, water arsenic exposure was significantly negatively associated with both Performance and Processing Speed raw scores of the Wechsler Preschool and Primary Scale of Intelligence, version III (WPPSI-III). Although, these associations were less strong than in our previously studied 10-year-olds, this second cross-sectional study of arsenic exposure expands the concerns about arsenic neurotoxicity to a younger age group (Wasserman et al., 2007).

Arsenic exposure at low-to-moderate levels and blood pressure

Using baseline data in 10,910 participants, we assessed the association between arsenic exposure from drinking water and blood pressure. We observed a positive association between low-to-moderate levels of arsenic exposure from drinking water and high pulse pressure (pulse pressure ≥55 mm Hg) (Chen et al., 2007b), an indicator of arterial stiffness, which is associated with an increased risk of atherosclerosis. In addition, among participants with lower-than-average dietary intake levels of B vitamins and folate, those with a higher well arsenic concentration were 1.83–1.89 times more likely to have a pulse pressure ≥55 mm Hg, compared with those in the bottom quintile of well arsenic concentration (<8 μg/L) (Chen et al., 2007b). These findings indicate that the effect of low-level arsenic exposure on blood pressure is nonlinear and may be more pronounced in persons with a lower intake of nutrients related to arsenic metabolism and cardiovascular health.

Intermediate biomarkers of early biological effect or altered structures and functions of target organs

Arsenic and pre-clinical markers of cardiovascular diseases

Previous publications have documented dose–response relationships between Carotid Artery Intima-Medial Thickness (IMT), which is measured using ultrasound imaging, stroke, angina pectoris, myocardial infarction (MI), intermittent claudication and essential hypertension (Bots et al., 1997). These relationships indicate that IMT is a valid surrogate marker for clinical endpoints.

In a pilot cross-sectional study of 66 healthy, normotensive, relatively young cohort members, the ORs for carotid IMT >0.75 mm were 1.61 (95% CI: 0.29–8.81) and 2.84 (95% CI: 0.39–20.86) comparing middle and highest levels with the lowest level of total urinary arsenic, respectively (Chen et al., 2006b). Although the observed associations were not statistically significant, the trend in effect estimates suggests a possible role of low-to-moderate levels of arsenic exposure in atherosclerosis, which warrants future investigation in a larger study.

Adhesion of circulating leucocytes to the endothelial cell and subsequent transendothelial migration is an important step in the initiation of atherosclerosis. In part, this process is mediated by cellular adhesion molecules (CAMs) (Cybulsky and Gimbrone, 1991; Adams and Shaw, 1994), expressed on the endothelial membrane, in response to inflammatory stimuli. Circulating markers of systemic inflammation and endothelial dysfunction, such as soluble intercellular adhesion molecule-1 (sICAM-1) and soluble vascular adhesion molecule-1 (sVCAM-1), have been shown to predict future cardiovascular disease (CVD) (Hwang et al., 1997). In a subgroup of 115 individuals with arsenic-related skin lesions, there was a positive association of urinary arsenic and well arsenic concentration with plasma levels of sICAM-1 and sVCAM-1 (Chen et al., 2007c). The positive associations of well arsenic with baseline and changes in plasma sVCAM-1 and sICAM-1 suggest a potential pathway underlying the effect of long-term arsenic exposure on CVD. Future larger studies are required to further examine the associations of low-level arsenic exposure with markers of vascular inflammation and endothelial dysfunction in healthy persons.

Arsenic and biomarkers of reno-vascular diseases

Microalbuminuria, a marker of glomerular hyperfiltration, has been correlated with and may be a manifestation of impaired endothelial function (Stehouwer et al., 2004). Impairment of endothelial function is recognized as one of the initial mechanisms that lead to atherosclerosis. In a study of arsenic and proteinuria (detected by dipstick analysis) in 11,121 persons in HEALS, we observed a dose–response relationship between well arsenic concentration and prevalence of proteinuria. In addition, cohort analysis with repeated measures of proteinuria and urinary arsenic concentrations revealed that a change in urinary arsenic was positively related to incidence of proteinuria during the four years of follow-up. The dose–response relationship between arsenic exposure and proteinuria provides evidence of the effect of low-to-moderate arsenic exposure levels on a common causal intermediate of CVD and kidney disease.

Arsenic and biomarkers of cancer

The development of skin lesions from arsenic exposure may be mediated by increases in the expression of various growth factors, including transforming growth factor-alpha (TGFα). To investigate this association in humans, levels of total urinary arsenic and urinary TGFα were determined in 41 individuals from HEALS, with and without arsenic-associated skin lesions; all individuals had chronic exposure to arsenic in their drinking water (Do et al., 2001). Linear regression analyses suggest that total urinary arsenic explained a substantial variation of urinary TGFα (R-squared value of the model = 0.40; p-value <0.01), particularly in individuals with arsenic-associated skin lesions (R-squared value of the model = 0.70; p-value<0.01). The R-squared values did not change appreciably with the adjustment for age and gender. There was also a trend of increasing odds ratios for the presence of arsenic-associated skin lesions with increasing urinary TGFα, although this was not significant (p-value = 0.15). However, the results were based on a small number of subjects.

In a follow-up study of 574 participants of the HEALS, the extracellular domain of the epidermal growth factor receptor (EGFR), to which TGFα binds to promote carcinogenesis, was measured by enzyme-linked immunosorbent assay in serum. Serum EGFR was found to be positively associated with three different measures of arsenic exposure (well water arsenic, urinary arsenic and a cumulative arsenic index) at statistically significant levels ($p \leq 0.034$) (Li et al., 2007). In addition, the risk of skin lesions for a given level of arsenic increased with increasing levels of EGFR. These results indicate that TGFα-/EGFR-dependent mechanisms may play a role in the development of arsenic-related skin lesions and skin cancer.

Arsenic and molecular and clinical biomarkers of pulmonary diseases

Serum level of Clara cell protein (CC16), one of the 20 proteins secreted by Clara cells in the lung's alveolar epithelium, has been indicated as a novel biomarker for respiratory illnesses, with a reduced CC16 concentration indicating damages in alveolar Clara cells. In cross-sectional analyses of 241 nonsmoking individuals randomly selected from the cohort, there was an inverse association between urinary arsenic and serum CC16 among persons with skin lesions ($\beta = -0.13$, $p = 0.01$) (Parvez et al., 2008). There was also a positive association between the ratio of DMA to MMA in the urine and CC16 levels ($\beta = 0.12$, $p = 0.05$). In a subsample of study participants undergoing spirometric measures ($n = 31$), urinary arsenic was inversely associated with lung function measured by predictive FEV1 (forced expiratory volume measured in 1 sec) ($r = -0.37$) (Parvez et al., 2008).

Arsenic and biomarkers of gene expressions in peripheral blood

In a microarray-based gene expression analysis comparing skin lesion cases with non-cases, Affymetrix HG-U133A GeneChip arrays were used to measure the expression of 22,000 transcripts, using RNA from peripheral blood lymphocytes (Argos et al., 2006). Down-regulating of several genes, including *superoxide dismutase 2* (*SOD2*) gene, *tumor necrosis factor* (*TNF*) gene and chemokine factor *CCL20* gene was observed in skin lesion cases. These findings suggest the involvement of inflammation, oxidative stress defense and chemokine response pathways in the development of skin lesions, or as a consequence of skin lesion manifestation.

In a separate comparison of samples collected from skin lesions cases before and after selenium supplementation, genes up-regulated by selenium supplementation included *TNF*, *IL1B*, *IL8*, *SOD2*, *CXCL2* and several other immunological and oxidative stress-related genes (Kibriya et al., 2007). These findings suggest that selenium supplementation may reverse some gene expression changes in individuals with pre-malignant skin lesions.

Prevention of health effects of arsenic exposure

Based on our findings of potential nutritional influence on arsenic toxicity (described above), we desired to assess whether dietary supplementation with specific micronutrients could prevent the occurrence of skin lesions, improve existing skin lesions and/or prevent skin and other cancers. In a pilot randomized, placebo-controlled, double-blind trial of 121 skin lesion cases, supplementation with vitamin E and selenium, either alone or in combination, slightly improved skin lesion status, although the improvement was not statistically significant (Verret et al., 2005). A large 2×2 factorial randomized placebo control trial is currently underway to evaluate whether supplementation with vitamin E or selenium can prevent the risk of skin cancer among 6000 patients with skin lesions.

In a randomized, double-blind, placebo-controlled folic acid-supplementation trial of 200 folate-deficient cohort participants, we tested the hypothesis that folic acid supplementation could increase arsenic methylation and lower blood arsenic concentrations (Gamble et al., 2006). In the trial, the increase of urinary %DMA in the folic acid group was significantly greater than that in the placebo group, as was the reduction in urinary %MMA (Gamble et al., 2006). Furthermore, the concentrations of total blood arsenic and of MMA in the blood were significantly reduced, by 14% and 22%, respectively (Gamble et al., 2007).

Arsenic exposure reduction by mitigation program

Since the baseline recruitment of the HEALS, we implemented interventions including: 1) person-to-person reporting of well test results and health education; 2) well labeling and village-level health education; and, 3) installations of 50 deep, low-arsenic community wells in villages with the highest levels of arsenic exposure. Two years after these interventions, 58% of the 6512 participants with unsafe wells at baseline (arsenic $\geq$50 μg/L) had responded by switching to other wells (Chen et al., 2007d). Urinary arsenic levels in participants who switched to a well identified as safe (arsenic <50 μg/L) dropped from an average of 375 to 200 μg arsenic/g creatinine, a 46% reduction towards the average urinary arsenic content of 136 μg arsenic/g creatinine for participants that used safe wells throughout (Chen et al., 2007d). These findings suggest that interventions such as these can effectively encourage switching to safe wells and lower arsenic exposure.

All-cause mortality and chronic disease mortality

A total of 219 participants have passed on from 2000 to 2006 in HEALS, with ages ranging from 30–70 years and a mean of 51 years. Major causes of chronic disease death within the cohort included diseases of the cardiovascular system ($n=87$), which primarily included hypertensive diseases, ischemic heart diseases, and cerebrovascular diseases; neoplasms ($n=32$); and diseases of the respiratory system ($n=18$), which primarily included asthma, chronic obstructive pulmonary disease, bronchiectasis, and respiratory infections. Other causes of death were related to the nervous system ($n=3$), the digestive system ($n=14$), the genitourinary system ($n=8$), pregnancy complications ($n=5$), diabetes ($n=1$), musculoskeletal disorders ($n=1$), or infectious disease ($n=18$).

We estimated hazard ratios for all-cause mortality and chronic disease mortality, the latter category included deaths due to cardiovascular disease, cancers, and non-infectious diseases, in relation to baseline well arsenic levels. The adjusted hazard ratios for all-cause mortality are 1.00 (ref), 1.23 (95% CI: 0.80, 1.88), 0.98 (95% CI: 0.65, 1.49) and 1.71 (95% CI: 1.16, 2.53) in increasing levels of well arsenic (<10, 10–50, 51–150, and 151–864 μg/L), respectively. The adjusted hazard ratios for chronic disease mortality are 1.00 (ref), 1.23 (95% CI: 0.80, 1.88), 0.98 (95% CI: 0.65, 1.49) and 1.71 (95% CI: 1.16, 2.53) in increasing levels of well arsenic, respectively. The hazard ratios for chronic disease mortality associated with a baseline well arsenic concentration >50 and >100 μg/L were 1.40 (0.93–2.10) and 1.61 (1.06–2.44), respectively.

Discussion

Recent findings from the HEALS provide valuable information on the effects of arsenic exposure at low-to-moderate levels on arsenic-related skin lesions, children's intelligence function, blood pressure, all-cause and chronic disease mortality, and an array of biomarkers for early biological effects or altered structures and functions of target organs.

Consistent with studies of bladder and lung cancer (Karagas et al., 2004; Steinmaus et al., 2003; Chen et al., 2004a), we also found a synergistic effect between the use of tobacco products and arsenic exposure on the risk of skin lesions. Cigarette smoking is likely to influence arsenic toxicity and should be taken into consideration in any studies on low-to-moderate levels of arsenic exposure. In addition, the data strongly suggest effect-modification roles of nutritional factors (i.e., folate and selenium) that are either involved in arsenic metabolism or have an antagonistic relationship with arsenic. While mounting evidence suggests that folate has an effect in arsenic metabolism, epidemiologic data on the mechanisms through which selenium may influence arsenic toxicity is limited. Similar to arsenic, methylation of selenium also uses SAM as the methyl donor. In addition to continue the investigation of gene expression changes due to selenium, future studies are needed to assess whether the effect of arsenic on DNA methylation is modifiable by selenium status. Intervention trials with clinical disease endpoints may be the next step in evaluating the potential of using some of these agents as treatments or preventive measures. In this regard, a large randomized clinical trial of 6000 cases of skin lesions investigating the effects of selenium and/or vitamin E supplementation with five years of follow-up is ongoing. Future studies are needed to evaluate whether the associations of arsenic exposure with cancer, cardiovascular disease or other arsenic-related clinical and intermediate endpoints also differ by these nutritional factors.

Our observation that at a given level of As exposure, %MMA was most strongly associated with increased risk of As-induced skin lesions is consistent with studies of skin cancer (Hsueh et al., 1997; Chen et al., 2003b; Yu et al., 2000), urothelial carcinoma (Pu et al., 2007), and bladder cancer (Chen et al., 2003c). These data suggest a role of interindividual variability that may be regulated by arsenic methylation capacity. There is also evidence that genes coding the enzymes that catalyze the metabolism processes of arsenic can influence the risk of arsenic-related skin lesions. Beside *MTHFR* and

GSTO1, it is likely that there are many other candidate genes that may influence susceptibility to arsenic toxicity. Other GSTs may play a role in cellular antioxidant defense mechanisms by catalyzing the reduction of potentially harmful peroxides. Several studies have indicated that SNPs in genes for GST mu 1 (*GSTM1*) and theta 1 (*GSTT1*) were associated with urinary arsenic metabolite profiles (Schlawicke et al., 2007; Steinmaus et al., 2007). A case–control of 600 cases (with average exposure level at 174 μg/L) and 600 controls in Bangladesh (McCarty et al., 2007), and a cross-sectional study of IMT with 605 subjects in Taiwan (Wang et al., 2006), have found that effects of arsenic on these outcomes were modifiable by polymorphisms in *glutathione S-transferase P1* gene. However, the studies did not have enough sample size to evaluate the effect-modification at low levels of exposure (<50 or <100 μg/L). More recently, arsenic (+III) methyltransferase (AS3MT) was characterized in rodents as an arsenic methyltransferase capable of methylating inorganic arsenic to its monomethyl form, and monomethylarsenic to its dimethylarsenic form. In a study of 147 individuals in northern Argentina, three intronic polymorphisms in the *AS3MT* gene were associated with a lower %MMA and a higher %DMA in urine (Schlawicke et al., 2007). Although studies of arsenic-related skin lesions and urinary arsenic metabolites point to the role of genetic susceptibility, case–control studies of skin or bladder cancer generated mixed results, most probably due to the limited sample sizes (Karagas et al., 2005; Moore et al., 2004; Chen et al., 2004c) (number of cases <400). Taken together, additional studies with larger sample size and comprehensive genomic approach are needed to systemically evaluate gene-arsenic interactions, not only the risk of skin lesions but also on other arsenic-related disease conditions.

The studies of biomarkers for early biological effects or altered structures may aid in the recognition of early effects of arsenic exposure, as well as the elucidation of underlying pathogenic mechanisms. For instance, the study of serum CC16 shows promise as a biomarker for assessing early respiratory damage induced by arsenic. Consistent with studies that have shown arsenic to be an inducer of TNF (Das et al., 2005; Germolec et al., 1997), a trigger of chemokine signal responses including chemokine factor CCL20 (Spiekstra et al., 2005), we observed down-regulation of *CCL20* and *TNF* genes in skin lesion cases. ICAM-1 and VCAM-1 expression in human umbilical vein endothelial cells (HUVEC) was higher after stimulation with arsenic (Hou et al., 2005). In mice tumors, treatment with As trioxide was associated with a clear increase in expression of ICAM-1 and VCAM-1 (Griffin et al., 2000). To our knowledge, our studies on EGFR and CC16 are the first published studies that link arsenic exposure with these biomarkers. However, aspects of the data are consistent with other reports in the literature. For example, individuals at risk for cancer from carcinogen exposures are shown to have increased levels of serum EGFR ECD years prior to their clinical diagnosis of malignancy (Partanen et al., 1994). Serum CC16 concentrations are decreased in individuals with compromised lung condition induced by chronic environmental exposures such as cigarette smoking or ozone (Bernard et al., 1994; Lagerkvist et al., 2004). Reliable biomarkers may also serve as intermediate endpoints for intervention trials, arsenic mitigation programs, screening and/or large observational epidemiologic studies. Analyses of participants with available data for both biomarkers and disease endpoints may be useful to further support biological plausibility of the putative causal pathways linking arsenic exposure to disease.

The observed positive associations between arsenic exposure and biomarkers relevant to cardiovascular disease and respiratory illness underscore the potentially adverse effects of arsenic exposure at low-to-moderate levels on these conditions. In a study of 463 subjects from the high-exposure area in southwestern Taiwan, a dose–response relationship was observed between arsenic exposure and carotid atherosclerosis assessed by duplex ultrasonography (Wang et al., 2002). The only epidemiologic evidence of low-to-moderate levels of arsenic exposure on cardiovascular disease came from the results of a cross-sectional study of >8000 subjects in northeastern coast of Taiwan that showed an elevated risk of cerebrovascular disease associated with arsenic exposure level at <50 and 50–300 μg/L (Chiou et al., 1997). Because IMT is a reliable marker of atherosclerosis, which is a strong risk factor of both coronary heart disease and stroke, larger studies of IMT are needed to assess the influence of the low-to-moderate levels of arsenic.

Using the hazard ratios of chronic disease mortality for a baseline well arsenic concentration >50 and >100 μg/L and the prevalence of these exposure levels, we estimated population attributable risk (PAR %); 18.1% and 13.1% of chronic disease mortality in the population can be attributable to arsenic exposure >50 and >100 μg/L, respectively. Apparently, arsenic exposure from drinking water is associated with a significant proportion of chronic disease mortality in the overall population, especially in those with comparatively high exposure levels. In future, with a longer follow-up of the HEALS, we will be able to further assess the dose–response relationship between arsenic exposure and cause-specific mortality. The data will be valuable, given that existing studies of arsenic exposure at low-to-moderate levels and disease endpoints mostly include limited sample size (Chiou et al., 2001; Karagas et al., 2004; Steinmaus et al., 2003) and unreliable or group-level long-term measures of arsenic exposure (Engel and Smith, 1994; Lewis et al., 1999; Meliker et al., 2007; Zierold et al., 2004).

Importantly, the use of multiple exposure measures, including well arsenic, urinary arsenic and blood arsenic, with repeated measurements in HEALS, improves the ascertainment of long-term exposure levels. The availability of detailed exposure data also provides a unique opportunity to evaluate the health effects of changes in exposure status over time and the joint status of difference exposure measures. In light of complex data, future analyses will need to employ modern epidemiology techniques, such as longitudinal analyses methods, survival analyses methods with time-dependent variables and/or marginal structural models to enhance causal inferences.

In conclusion, findings from the HEALS to date indicate that arsenic exposure has a profound influence on the health of the arsenic-exposed population in Bangladesh. Our findings on skin lesions, biomarkers and susceptibility altogether indicate that health effects of arsenic exposure at low-to-moderate levels may be cause for further investigation. Future analyses of clinical endpoints of cancer and cardiovascular disease are likely to provide useful knowledge on dose–response relationships. The HEALS will continue to be a valuable resource for the investigation of the health effects of arsenic exposure for years to come.

Conflict of interest

The authors declare that there are no conflicts of interest.

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