Gender-Specific Protective Effect of Hemoglobin on Arsenic-Induced Skin Lesions

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Abstract

Chronic arsenic poisoning remains a public health crisis in Bangladesh. As arsenic has been shown to bind to human hemoglobin (Hb), hematologic mechanisms may play a role in the pathway through which arsenic exerts its toxicity. Two separate studies, a case-control and a cohort, were conducted to investigate the role of Hb in the development of arsenic-induced skin lesions. In the first, conditional logistic regression was used to investigate the effect of Hb on skin lesions among 900 case-control pairs from Pabna, Bangladesh, in which individuals were matched on gender, age, and location. In the second, mixed linear regression models were used to examine the association between toenail arsenic, urinary arsenic, and Hb within a cohort of 184 individuals from 50 families in the same region who did not have arsenic-induced skin lesions. Hb was significantly associated with skin lesions but this association was gender specific. In males, a 40% reduction in the odds of skin lesions occurred for every 1 g/dL increase in Hb (odds ratio, 0.60; 95% confidence interval, 0.49-0.73). No effect was observed for females (odds ratio, 1.16; 95% confidence interval, 0.92-1.46). In the cohort of 184 individuals, no associations between toenail arsenic or urinary arsenic species and Hb levels were observed. Low Hb levels may exacerbate the detrimental health effects of chronic arsenic poisoning. Whereas providing clean water remains the optimal solution to Bangladesh's problem of arsenic poisoning, improving nutrition and reducing iron-deficiency anemia may ameliorate negative health effects, such as skin lesions in individuals who have been exposed. (Cancer Epidemiol Biomarkers Prev 2006;15(5):902–7)

Introduction

Chronic arsenic poisoning is a major health concern in Bangladesh where an estimated 29 to 40 million people are at risk of ingesting arsenic-contaminated drinking water and developing related diseases (1, 2). Whereas chronic arsenic exposure has been studied extensively, the mechanisms of toxicity remain largely unknown, although there is evidence that hematologic variables may be involved (3, 4).

In vivo and *in vitro* studies of inorganic arsenic exposure have shown that inorganic arsenic can bind to animal and human hemoglobin (Hb) (5-7) and can change cell shape, morphology, heme metabolism, and Hb levels (8-10). Acute exposure to arsenite has been shown to cause anemia, leukopenia, and thrombocytopenia, secondary to bone marrow depression (3). Furthermore, arsine gas is known to induce hemolytic anemia (5, 11, 12).

Human studies provide further evidence that hematologic variables may be involved. Analysis of 102 human skin lesions found that cancerous skin lesions had less total Hb in skin cells than did benign lesions (13). Another study found that chronic ingestion of arsenic-contaminated drinking water altered heme metabolism by increasing porphobilinogen deaminase and uroporphyrinogen decarboxylase enzyme activities in peripheral blood erythrocytes and increasing urinary excretion of total porphyrins (14). However, the effect of chronic arsenic toxicity on the heme system remains relatively unexplored and no direct evidence suggests interactions between inorganic arsenic and hematologic variables later cause disease.

Hb is of particular interest because of its widespread use for assessing anemia, which is typically defined as a blood Hb level <12 g/dL (15, 16). In Bangladesh, the national prevalence of anemia has remained constant at 74% for the past 30 years (17). The main reason for this high prevalence is believed to be iron deficiency due to inadequate iron intake and low dietary bioavailability of iron. This continued high rate of anemia is itself a public health problem, causing loss of productivity totaling 1.9% of the national gross domestic product (17).

To investigate the relationship between Hb and the development of arsenic-induced skin lesions, we conducted two separate studies in different populations. First, we investigated whether Hb was associated with arsenic-induced skin lesions, typically the first indication of chronic arsenic toxicity, in a case-control study of 900 matched pairs. Potential interactions between Hb and arsenic and Hb and gender were evaluated. Interactions between Hb and the *GSTT1*, *GSTM1*, and *GSTP1* gene polymorphisms were also assessed because these genes are known to play central roles in normal cell housekeeping activities, have been associated with cancer susceptibility, and are important in the metabolism of environmental carcinogens including arsenic (18-20). Second, direct associations between toenail and urinary arsenic levels and Hb levels were analyzed in a disease-free population of 184 adults from 50 families in the same region in Bangladesh.

Materials and Methods

Participant Selection. From 2001 to 2003, 900 case-control pairs were recruited by Dhaka Community Hospital Trust primary-care clinics from 23 villages within the Pabna district of Bangladesh. Pabna, located north of Dhaka on the Jamuna (Ganges) River in central Bangladesh, is a region considered to be moderately affected by arsenic contamination in the drinking water (21).

Individuals were invited to participate in the study through a series of community meetings held by Dhaka Community

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Hospital. Participants with arsenic-induced skin lesions were included if a physician diagnosed any of the following lesions: (*a*) keratosis of the extremities, (*b*) spotted melanosis, (*c*) Bowen's disease, or (*d*) squamous cell carcinoma. Controls were subjects without any visible arsenic-induced skin lesions selected from the Dhaka Community Hospital catchment area and matched one to one on gender, age (within 3 years), and area of residence. At the time of recruitment, all participants underwent a clinic visit during which a behavioral and demographic questionnaire was administered and a blood sample collected. Toenail clippings, urine samples, and water samples were also collected during this visit. To ensure a sufficient range of arsenic exposure and to reflect the background exposure distribution, up to 80% of controls were selected from ''low-exposure'' arsenic (<50 μg/L) areas and 20% of the subjects were from ''high exposure'' (≥50 μg/L) areas in Pabna based on the Bangladesh drinking water standard of 50 μg/L (22). Initial measurements of water arsenic levels for the purpose of control selection were made with Merck field test kits (23).

A separate study, conducted from September 2001 to September 2005, recruited 248 individuals from 50 families in three villages in Pabna to characterize biomarker response in a repeated measures study. Subjects were eligible for this study if they were long-term residents of Pabna, obtained their drinking water from tube wells, and received primary health care from the Pabna Community Clinic, an affiliate of Dhaka Community Hospital. During the initial clinic visit, a behavioral and demographic questionnaire was administered and a blood sample collected. Researchers visited participants at their homes every 3 months for 4 years to collect urine, toenail, and water samples.

The Institutional Review Boards at the Harvard School of Public Health and the Dhaka Community Hospital approved the protocols for both studies. Informed consent was obtained from all adult participants before participation.

Data Collection and Analysis. During the baseline clinic visit for both studies, Hb values were measured using Sahli's method (24), a visual acid hematin method that can be easily and economically implemented in the field. This measure was chosen to provide participants with immediate results about their anemia status.

Toenail clippings were collected from all toes and prepared as described by Chen et al. (25). Arsenic was analyzed in five replicate analyses using an inductively coupled plasma mass spectrometer (ICP-MS Model 6100 DRC, Perkin-Elmer, Norwalk, CT). Standard reference material water (NIST 1643d and NIST 1643e Trace Elements in Water; National Institute of Standards and Technology, Gaithersburg, MD) and certified human hair reference material (CRM hair; Shanghai Institute of Nuclear Research, Academia Sinica, China) were used to validate instrument performance and digestion method. The average percent recovery of NIST 1643 and CRM hair was 92.6% and 94.1%, respectively.

The reported total arsenic concentrations were corrected for any detectable blank concentrations and for systemic error by normalizing the sample concentrations against the measured average daily NIST 1643 inorganic arsenic concentration (20). This corrected value was used in all the statistical analyses.

Unfiltered drinking water was collected from the tube well each participant identified as their primary drinking water source. Each tube well was purged for several minutes before sample collection. Total arsenic analysis was done by Environmental Laboratory Services (North Syracuse, New York) following U.S. Environmental Protection Agency method 200.8. The limit of detection for this method was 1 μg As/L. PlasmaCAL multielement QC standard #1 solution (SCP Science, Canada) was used to validate analysis. The average inorganic arsenic percent recovery was 104.6%. Arsenic concentrations below the limit of detection were set to half the limit of detection.

First void urine was collected from participants and stored at −20°C until analysis. Urinary arsenic metabolites were measured as described by Hsueh et al. (26) using high-performance liquid chromatography-atomic absorption spectrophotometry (HPLC model Waters 501, Waters Associates, Milford, MA). This method quantifies arsenate (As V), arsenite (As III), monomethylarsonic acid (MMA), and dimethylarsenic acid (DMA). Total arsenic species in urine was defined as the sum of As III, As V, MMA, and DMA. Detection limits for As III, As V, MMA, and DMA were 0.036, 0.055, 0.054, and 0.056 μg/L, respectively.

Multiplex PCR amplifications were done from genomic DNA extracted from whole blood following the Puregene Protocol (Gentra Systems, Minneapolis, MN). Genotyping of *GSTM1, GSTT1*, and *GSTP1* followed the protocol described by Liu et al. (27). Genotyping procedures were validated by randomly selecting 5% of the samples and subjecting them to repeat analysis. Two researchers independently reviewed all genotyping results until 100% concordance was achieved.

Statistical Analysis

Case-Control Study. Physical and sociodemographic characteristics of the cases and controls were compared using the χ^2 test for categorical data and *t* test for comparison of means. Water arsenic and toenail arsenic concentrations were not normally distributed. Thus, median arsenic values were compared using the Wilcoxon rank sum test and concentrations were subsequently natural log transformed in all statistical models.

A conditional logistic regression model was used to evaluate the effect of Hb on skin lesions in the case-control data set. Penalized smoothing splines were initially incorporated for Hb, water arsenic, and toenail arsenic values to relax assumptions about the functional forms of these dose-response associations (28-30). Models were fit in R (version 2.0.1, R) using the *coxph* function available in the *survival* library (31). For each model, the value of the smoothing variable was chosen systematically by selecting the model with the lowest corrected AIC (32).

The spline function for toenail arsenic was essentially linear and was therefore replaced with a linear term in the final model. The Hb spline exhibited a threshold effect at 12 g/dL, above which value the function was approximately linear. To interpret the effect of Hb on skin lesions, the linear portion of the spline was evaluated by assigning the observed threshold value of 12 g/dL to all exposures below this concentration according to the following logistic regression model. The spline for logged drinking water concentrations was retained in the final model.

$$\ln\{p_{k_i}/(1-p_{k_i})\} = \alpha_k + f(\ln \text{ water As}_{k_i}) + \beta_{\text{As}} \ln \text{ toenail } As_{k_i} + \beta_{\text{Hb}}\text{Hb}_{k_i} + \beta z^T Z_{k_i}, \quad \text{(A)}$$

where p_{k_i} is the probability that the ith individual in the kth matched pair has skin lesions; α_k is a baseline intercept for matched pair k; Z_{k_i} is a vector of covariates; the β coefficients are estimated variables; and f is a function estimated nonparametrically.

Variables initially considered as potential confounders were body mass index (BMI), education, smoking biri or cigarettes, chewing tobacco or betel nuts, presence of smokers in the environment, tea drinking, and systolic and diastolic blood

pressure. Presence of smokers in the environment, tea drinking, and blood pressure were subsequently dropped from final models because none of these variables appreciably changed the effect estimate for Hb.

Finally, interactions between Hb and arsenic, Hb and gender, and Hb and the genotypes *GSTT1, GSTM1*, and *GSTP1* were evaluated. An interaction term for each of the five possibilities was incorporated into separate models using Eq. A. Hb and arsenic variables were treated as continuous linear variables whereas gender and the genotypes were categorical. The odds ratio (OR) for each interaction term was then evaluated and the 95% confidence intervals (95% CI) were calculated. Interactions were considered significant if their 95% CIs did not include one. If the interaction term was statistically significant, stratum-specific ORs were presented.

Cohort Study. To evaluate whether toenail arsenic levels were associated with subsequent Hb levels in the cohort of 50 families, a mixed regression model with a random family effect was used. Toenail samples taken from 6 to 12 months after baseline were chosen to best represent arsenic accumulation in the nail that would correspond to exposures accrued at the time of baseline Hb measurements and then averaged together. In a previous study in this cohort, we found that toenail arsenic levels were a good reflection of water arsenic ingested 6 to 12 months before the toenail collection (20).

Analyses were run according to the following model:

$$Y_{i_t} = \beta_0 + \beta_{\mathrm{As}} X_i + \beta_Z^T Z_i + b_t + \varepsilon_{it} \qquad \text{(B)}$$

where Y_{i_t} is the value of Hb for the ith individual in the tth family; X_i represents the natural logarithm of the 6- to 12-month averaged toenail arsenic concentration; Z_i is a vector of covariates age, BMI, gender, education, creatinine, and smoking; b_t represents a normally distributed random family-specific intercept; and ε_{i_t} represents a normal error.

It is possible that Hb levels could affect downstream formation of arsenic products excreted in the urine; thus, Eq. B was also used separately to evaluate the effect of Hb on total urinary arsenic, total inorganic arsenic, MMA, DMA, and the MMA/DMA ratio. In these cases, Y_{i_t} represented the natural logarithm of the arsenic value and X_i represented the Hb values that were not log transformed. The statistical programs SAS version 9.1.2 (SAS Institute, Cary, NC) and R version 2.0.1 (R Foundation for Statistical Computing, Vienna, Austria) were used for all analyses.

Results

The sociodemographic characteristics of 900 cases of skin lesions and 900 controls are presented in Table 1. Cases were more likely than controls to chew betel nuts and in general had a lower education level. No differences between cases and controls were found in smoking status, use of chewing tobacco, age, or gender. Because one individual had a computed BMI of 60, this individual was excluded from all analyses.

Overall, 12.1% of the study population was anemic, much less than generally occurs in the Bangladeshi population (74%) (17, 33). Cases were more likely than controls to be anemic (13.7% versus 10.5%, $P = 0.02$) and women were more likely than men to be anemic (18.2% versus 8.2%, $P < 0.0001$).

Differences in median drinking water arsenic levels and toenail arsenic levels were also observed although these may, in part, be a reflection of our control selection process. Of note, however, is that the median drinking water arsenic level among cases was below the current Banglandeshi health standard of 50 μg As/L but greater than the WHO recommended guideline of 10 μg As/L. Specifically, 51.7% of cases with skin lesions had water arsenic levels below the Bangladeshi standard and 36.3% had water arsenic levels below the WHO standard.

The effect of Hb on skin lesions among the case-control population was first evaluated using a conditional logistic regression model in which the matching factors were age, gender, and area of residence (Table 2, main effects model). The model included a nonparametric penalized spline for the concentration of arsenic in the participant's drinking water. After adjusting for toenail arsenic level, BMI, education, smoking biri, smoking cigarettes, chewing tobacco, and betel nut chewing, every 1 g/dL increase in Hb above a value of 12 g/dL was associated with a 21% decrease in the odds of having skin lesions (OR, 0.79; 95% CI, 0.69-0.91).

Interactions between Hb and arsenic, gender, and the genotypes *GSTT1, GSTM1*, and *GSTP1* were assessed. The only significant interaction was observed between Hb level and gender, in which an increased Hb level was associated with a decreased odds of skin lesions among males but not females (Table 2, interaction model). In males, a 40% reduction in the odds of skin lesions occurred for every 1 g/dL increase in Hb above a value of 12 g/dL (OR, 0.60; 95% CI, 0.49-0.73) whereas no effect was observed in females (OR, 1.16; 95% CI, 0.92-1.46).

Within the gender-Hb interaction model, betel nut chewing and arsenic levels in toenails were predictive of skin lesions, associations shown previously in this population (22). Higher education levels were generally protective against the presence of skin lesions, as were smoking biri and chewing tobacco. However, because nearly all women did not smoke biri or cigarettes in this population, the model estimates for biri and cigarettes reflect the effect among men only.

In the second study of 50 families, the population was restricted to skin lesion–free adults >18 years of age, which resulted in a total sample size of 184. A mixed linear regression model did not indicate an association between toenail arsenic levels and Hb levels (Table 3). No effects were found between Hb and urinary arsenic species including total urinary arsenic, MMA, DMA, total inorganic arsenic, and the methylation ratio MMA/DMA (Table 4). Male gender and BMI, on the other hand, were significant predictors of Hb level.

Discussion

In the present study, higher Hb levels were significantly protective against the presence of skin lesions among Bangladeshi males but no such effect was seen in females. This observed protective effect is consistent with results from Garcia-Uribe et al. (13) who reported that skin cells from nonarsenic-related cancerous skin lesions have less total Hb and a lower level of oxygen saturation than benign lesions. They suggest that Hb variables may be related to several factors in carcinogenesis such as tumor necrosis, intratumoral hemorrhage with blood stagnation, abnormal blood supply, and distribution and metabolic abnormalities, although it is important to note that Hb was measured in the skin cells and not the overall blood.

The associations between Hb and nonarsenic-related cancerous lesions found by Garcia-Uribe et al. may suggest hematologic defects are a common denominator of many types of skin disease. Whereas many arsenic-induced skin lesions such as keratosis and melanosis are not themselves cancerous, they can progress to include squamous cell carcinoma *in situ* (34). The presence of skin lesions is highly associated with further development of skin cancers, both squamous cell and basal cell carcinomas. Moreover, skin tumors associated with arsenic do not differ histologically from skin tumors unrelated to arsenic (34). Thus, it is possible that the protective effect of Hb may not be specific to arsenic-induced skin disease.

Although it remains unclear whether exposure to arsenic specifically causes hematologic abnormalities that lead to

Table 1. Physical, social, and demographic characteristics of cases with skin lesions and matched controls in Pabna, Bangladesh

	Cases (*n*)	Controls (*n*)	*P*
Physical			
% Male	61.7 (555)	61.8 (556)	0.96*
Mean BMI	20.0 (900)	20.2 (900)	0.05†
Social			
Education			<0.0001*
% no school (illiterate)	20.5 (184)	15.0 (135)	
% no school (able to write)	30.4 (273)	24.7 (222)	
% primary	12.2 (110)	13.0 (117)	
% secondary	32.6 (293)	41.4 (372)	
% college +	4.3 (39)	5.9 (53)	
% Chew tobacco	14.8 (133)	14.8 (131)	0.96*
% Chew betel nuts	28.6 (256)	23.9 (215)	0.03*
% Ever smoked	28.9 (254)	31.6 (281)	0.17*
Demographic			
Hb categories			0.0175*
% Hb <12 g/dL (anemic)	13.7 (123)	10.5 (94)	
% Hb 12.1-14.0 g/dL	71.0 (638)	70.1 (629)	
% Hb 14.1-18.0 g/dL	15.4 (138)	19.4 (174)	
Median water arsenic (μg/L)	39.0 (868)	11.4 (872)	<0.0001‡
Median toenail arsenic (μg/g)	3.70 (899)	1.6 (896)	<0.0001‡

*The null hypothesis that the variable did not vary by case-control status was tested using the χ^2 test.
†The null hypothesis that the mean value did not differ by case-control status was tested using the *t* test.
‡The null hypothesis that the median value did not differ by case-control status was tested using the Wilcoxon rank sum test.

development of arsenic induced skin lesions, in our results from the cohort of 184 individuals, Hb levels were not associated with any arsenic variables measured in toenails or in urine. Our results suggest that there is little role for direct involvement of Hb in the mechanism of arsenic toxicity.

Hb could play a less direct role in preventing the development of arsenic-induced skin lesions by inhibiting angiogenesis. Kao et al. (35) showed *in vitro* that low concentrations of sodium arsenite preferentially enhanced angiogenesis by stimulating cell growth, up-regulating the expression of constitutive nitric oxide synthase, increasing von Willebrand factor antigen expression, and increasing vascular tubular formation in human umbilical vein endothelial cells. The vascular tubular formation was abolished by the presence of Hb, indicating a possible protective role for Hb against angiogenesis in tumor formation.

Alternatively, Hb could act as a scavenger molecule. Hb may either bind to free arsenic in the circulatory system or Hb may bind to oxidative free radicals formed in response to arsenic exposure. Laboratory experiments have shown that inorganic arsenite can bind to cysteine residues of Hb in the circulating blood of rats and humans (6) and in rabbits 20% of the total erythrocyte arsenite burden was associated with the Hb after 24-hour exposure (7). Reactive oxidative species can also attack oxygenated Hb, resulting in oxidation of the heme iron (3). Inorganic arsenic has been associated with increased generation of reactive oxygen species, which can lead to DNA damage that has been associated with the development of skin lesions and skin cancer (36-39). If arsenic generates enough excess oxidative stress to overwhelm the system, as Szymanska-Chabowska et al. suggest, reactive oxygen species may target Hb, oxidizing the heme groups. Individuals with sufficient or excess levels of Hb may be able to trap greater amounts of reactive oxygen species produced by arsenic.

It is also possible that the Hb measured in this study reflects the participants' general nutritional status because we observed that BMI was a significant predictor of Hb and BMI can be a good indicator of chronic undernutrition in developing countries (40). Furthermore, several nutritional factors and undernutrition in general have been associated with increased risk for skin lesions and other arsenic-related illnesses (3, 33, 41). Vitamin E and selenium supplementation may

Table 2. Results from two conditional logistic regression spline models with the outcome skin lesions (*n* = 1708)

	Main effects model	Interaction model
	OR (95% CI)	OR (95% CI)
Hb (threshold at 12 g/dL)	0.79 (0.69-0.91)	
Males	— (—)	0.60 (0.49-0.73)
Females	— (—)	1.16 (0.92-1.46)
BMI	1.01 (0.97-1.05)	1.01 (0.96-1.05)
Education		
No school (able to write)	1.18 (0.80-1.73)	1.24 (0.83-1.85)
Primary	0.81 (0.50-1.30)	0.84 (0.52-1.38)
Secondary	0.48 (0.30-0.74)	0.45 (0.28-0.72)
College+	0.90 (0.44-1.85)	0.35 (0.17-0.73)
Smoked cigarettes	0.80 (0.53-1.22)	0.77 (0.49-1.19)
Smoked biri	0.48 (0.31-0.74)	0.45 (0.28-0.71)
Chewed betel nuts	1.44 (1.00-2.09)	1.50 (1.02-2.19)
Chewed tobacco	0.49 (0.31-0.78)	0.48 (0.30-0.77)
Logged toenail arsenic	1.79 (1.52-2.10)	1.82 (1.54-2.15)
Spline (logged water arsenic) linear	1.14 (1.05-1.23)	1.13 (1.04-1.23)
	Nonlinear ($P < 0.0001$)	Nonlinear ($P < 0.0001$)

NOTE: The first model presents main effects and the second an interaction between gender and Hb. The estimates for Hb are valid above a level of 12 g/dL.

Table 3. Summary statistics and results from a mixed linear regression model evaluating the outcome Hb (g/dL) in the repeated measures population (*N* = 184)

	Summary characteristics	*n*	β coefficient	SE	*P*
Logged average toenail arsenic (median)	0.879	175	−0.107	0.146	0.465
Age (mean)	35.7	184	−0.006	0.008	0.490
BMI (mean)	20.8	184	0.069	0.033	0.040
Sex (male), %	45.1	83	2.234	0.267	<.0001
Education					
No school (able to write), %	31.5	53	0.074	0.323	0.820
Primary, %	20.1	37	−0.231	0.367	0.531
Secondary, %	25	46	0.001	0.367	0.998
College+, %	6.5	12	−1.049	0.524	0.048
Smoked cigarettes, %	24.5	45	−0.157	0.312	0.620

protect against skin lesion development (42). Mitra et al. (33) observed an increased risk of skin lesions with the lowest quintiles of intake of calcium, animal protein, folate, and fiber intake. If the primary source of dietary iron comes from animal protein, Hb could be an indicator of low protein intake or a more general marker for overall undernutrition. Conversely, Hb could be a proxy for other nutritional factors highly correlated with iron intake that protect against skin lesions. More specific nutritional data would be necessary to understand the relationship between Hb, nutritional factors, and skin lesions.

In a similar fashion, Hb could be a proxy for an unmeasured coexposure in males and not, in itself, have any protective benefits. Arsenic alone may not be sufficient to cause Hb changes but arsenic plus a coexposure might. Thus, arsenic plus a second exposure may independently increase the risk for skin lesions and also affect Hb levels.

It is unclear whether Hb exerts a causal protective effect on skin lesions or whether it is a proxy for some other factor. However, our results do suggest it is unlikely that arsenic directly affects Hb levels or that Hb alters metabolism of arsenic into its metabolites. Nevertheless, arsenic may still affect other hematologic variables. Laboratory studies and some epidemiologic evidence support a hematologic pathway for arsenic toxicity. A small epidemiologic study among a Mexican population exposed to arsenic-contaminated drinking water observed that chronic arsenic exposure increased certain enzyme activity in the heme biosynthesis pathway, although the biological significance of this alteration is not known (14). Arsenite can inactive the Janus-activated kinase/signal transducers and activators of transcription pathway, which plays a critical role in the function of hematopoietic cells, cell proliferation, and differentiation (43). Arsenic-contaminated drinking water has been associated with increased microvascular disease (44) and deficits of capillary blood flow and permeability in clinically normal skin of patients with chronic arsenical poisoning (45).

Table 4. Results of regression models evaluating the effect of Hb on urinary arsenic variables adjusted for age, gender, BMI, creatinine, education, and smoking

Model*	β coefficient	SE	*P*
Model 1: Total arsenic			
Hb	0.0186	0.0443	0.6762
Model 2: As III + As V			
Hb	0.0196	0.0716	0.7849
Model 3: MMA			
Hb	0.0369	0.1068	0.7302
Model 4: DMA			
Hb	−0.0007	0.0464	0.9888
Model 5: MMA/DMA ratio			
Hb	0.0347	0.0968	0.7208

*All outcomes are natural log transformed.

Several limitations of the present study warrant consideration. Hb was measured after the manifestation of skin lesions; therefore, we cannot rule out the possibility that Hb levels decreased as a result of disease. Similarly, the analysis in the cohort population was cross-sectional in nature with Hb only measured at baseline, making temporal separation of cause and effect difficult. Nevertheless, even if the association between Hb and skin lesions cannot be interpreted as causal, the association indicates the presence of systemic toxicity in persons with skin lesions and should not be dismissed.

The method used for determining anemia, Sahli's colorimetric method, was chosen primarily for its ability to provide immediate results to participants. It is highly sensitive but not very specific compared with more sophisticated methods for determining anemia such as HemoCue (24). Thus, whereas our estimate of anemia prevalence may be overestimated due to a high number of false positives, it is not likely to be exposure dependent. Furthermore, our estimate falls well below the Bangladesh national prevalence estimates. In our main analyses, however, we used Hb values on a continuous scale. Whereas the field team could not be completely blinded to case or exposure status, we believe it unlikely that any error associated with the measurement method would be differential particularly because Hb measurements were not the primary objective of the original study.

In conclusion, we report a novel observation of a protective effect of increasing Hb levels on the presence of skin lesions among men but not among women and have presented several possible explanations. However, we have no explanation for why this protective effect was observed only among men, although it is possible that the gender interaction is a marker for some unmeasured exposure that occurs more frequently in men than in women. For example, some recent animal studies have shown an interaction between arsenic and UV in the development of skin lesions (46, 47).

Additional research into the mechanism by which Hb may confer protection against skin lesions is warranted. We found no evidence to suggest arsenic directly influences Hb levels or that Hb directly alters metabolism of arsenic to its urinary metabolites; however, our primary study aims were not designed to detect this. Nevertheless, other hematologic variables may play a role in chronic inorganic arsenic toxicity and more research is needed to fully understand the biological mechanisms contributing to the development of arsenic-induced skin lesions. Moreover, in a country in which anemia is so prevalent, low Hb levels only serve to exacerbate the detrimental health effects of chronic arsenic poisoning. Whereas providing clean water remains the optimal solution to Bangladesh's problem of arsenic poisoning, improving nutrition and reducing iron-deficiency anemia may ameliorate some of the negative health effects of chronic arsenic toxicity such as skin lesions.

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