Published in final edited form as:

Toxicol Appl Pharmacol. 2017 September 15; 331: 62–68. doi:10.1016/j.taap.2017.05.014.

ARSENIC EXPOSURES ALTER CLINICAL INDICATORS OF ANEMIA IN A MALE POPULATION OF SMOKERS AND NON-SMOKERS IN BANGLADESH

Mohammed Parvez^a, Sebastian Medina^b, Regina M. Santella^a, Tariqul Islam^c, Fredine T. Lauer^b, Pam Factor-Litvak^a, Habibul Ahsan^d, Joseph H. Graziano^a, Ke Jian Liu^b, and Scott W. Burchiel^{b,*}

^aMailman School of Public Health, Department of Environmental Health, Columbia University, New York, NY 10032

^bThe University of New Mexico College of Pharmacy, Department of Pharmaceutical Sciences, Albuquerque, NM 87131

^cUniversity of Chicago Field Research Office, Dhaka-1230, Bangladesh

^dUniversity of Chicago, Division of Public Health, Chicago, IL 60637

Abstract

Drinking water arsenic (WAs) exposure has been linked to a number of detrimental health outcomes including anemia, primarily among pregnant women. Little is known about the effects of As on hematological disorders among men. We have examined the role of As exposure on hematological indicators of anemia in a group of men exposed to a wide range of As in their drinking water. We conducted a cross-sectional investigation among 119 healthy men in the Health Effects of As Longitudinal Study cohort, in rural Bangladesh. The participants are part of an ongoing study focused on evaluating the influence of As and smoking on immune function. Samples were collected at recruitment and analyzed for WAs, urinary As (UAs) and UAs metabolites to assess As exposure. Blood samples were also collected at recruitment and assayed immediately for hematological parameters. We found that increased WAs was associated with decreased red blood cell counts [$\beta = -0.13$, $p < 0.0001$] as well as hematocrit packed cell volume [$\beta = -0.68$, $p = 0.008$] following adjustment for age, smoking, body mass index and polycyclic aromatic hydrocarbon-DNA adducts. Other measures of As exposure (UAs and its metabolites) demonstrated similar associations. Slightly stronger effects were observed among smokers. We also observed an effect of As on hemoglobin among smokers in relation to urinary As [$\beta = -0.54$, $p < 0.05$]. Our analysis revealed effects of As exposure on hematological indicators of anemia in a group of healthy male smokers and non-smokers.

Keywords

Bangladesh; HEALS cohort; arsenic; drinking water; anemia; smokers

*To whom correspondence should be addressed. Fax: (505) 272-6749. sburchiel@salud.unm.edu.

INTRODUCTION

Arsenic (As) contamination in drinking water is a global health problem affecting millions of people worldwide. The major arsenicals found in drinking water are the pentavalent and trivalent inorganic forms arsenate (As^{+5}) and arsenite (As^{+3}), respectively (Styblo et al., 2000; Naujokas et al., 2013). As a result, exposure to these inorganic arsenicals is of particular concern from a public health standpoint (Styblo et al., 2000). High-level As exposure has been linked with internal and external cancers, lung disease, immunosuppression, and cognitive impairment in children (Naujokas et al., 2013; Hughes et al., 2002; Tyler and Allan 2014; Ferrario et al., 2016). A few studies have also indicated an association between chronic As exposure and hematological disorders such as anemia, particularly among women during pregnancy (Hopenhayn et al., 2006; Heck et al., 2008; Surdu et al., 2015; Kile et al., 2016).

Anemia is characterized by reduced red blood cell counts (RBC) and decreased hemoglobin (Hgb) levels in the blood. Anemia has been linked with a number of deleterious health effects, including fatigue, increased susceptibility to infection, cognitive and motor impairments, low birth weights, and risk of maternal and neonatal mortality (Balarajan et al., 2011; WHO 2011). A cross-sectional study of 217 Romanian women, found higher prevalence of anemia [prevalence proportion ratio (PPR) = 1.71, 95% (CI) 0.75–3.88], and particularly anemia during pregnancy [(PPR) = 2.87, 95% (CI) 0.62–13.26] associated with As exposure (Surdu et al., 2015). Similarly, a follow-up study of 810 pregnant women from two Chilean cities observed increased incidences of anemia related to As exposure. This study also revealed increasing prevalence of anemia with the progression of pregnancy among As exposed as compared to unexposed women (49% versus 17%) (Hopenhayn et al., 2006).

We have reported that higher urinary As ($>200 \mu\text{g/L}$) was linked to low Hgb ($<10 \text{ g/dL}$) among 1,954 men and women in the Health Effects of As Longitudinal Study (HEALS) cohort in Bangladesh (Heck et al., 2008). Two studies from Bangladesh also reported an association of Hgb on arsenical-induced skin lesions. In a group of 147 women, Kile et al., (2016) observed that low Hgb ($<12 \text{ g/dL}$) significantly increased risk [(OR) = 3.32, 95% (CI) 1.29, 8.52] of As-related skin lesions among women with normal Hgb levels after adjusting for As levels in drinking water and other covariates. Interestingly, the relationship of Hgb and arsenical skin lesions was found to be gender specific in a case-control study of 900 individuals from the same area of Bangladesh. Hgb was significantly associated with skin lesions only among males with a 40% reduction in the odds of skin lesions for every 1 g/dL increase in Hgb (OR = 0.60; 95% CI, 0.49–0.73). However, no associations ($n=184$) were observed between toenail As or UAs species and Hgb levels (Breton et al., 2006).

Although a link between smoking and anemia has yet to be established, multiple diseases caused by smoking can result in anemia (Leifert 2008). Several studies have found that smoking results in red blood cell hemolysis, which may be a contributing factor to anemia development (Minamisawa et al., 1990; Masilamani et al., 2016). Conversely, smoking has also been reported to complicate the identification of anemia by increasing Hgb and hematocrit packed cell volume (HCT/PCV) (Sagone and Balcerzak 1975; Nordenberg et al.,

1990; Anandha, et al., 2014). These studies highlight the equivocal nature of the relationship between smoking and anemia and emphasize the need for further investigations.

There is strong evidence supporting an association between drinking water As exposure and anemia among women; however, very few studies have evaluated these relationships in men. To our knowledge, no studies have been conducted to determine the effects of smoking on anemia in As exposed individuals. Taking this into consideration, the present study assessed the relationships between chronic drinking water As exposure and hematological indicators of anemia among healthy male non-smokers and smokers in the HEALS cohort who live in rural Bangladesh.

METHODS

Study population

The study was implemented in the HEALS cohort, an ongoing population-based prospective cohort in Araihaazar, Bangladesh, a rural sub-district, near the capital Dhaka (Parvez et al., 2006). The HEALS cohort was established to investigate the health effects of inorganic As (InAs) exposure from drinking water (Ahsan et al., 2006; Parvez et al., 2006). Briefly, between October 2000 and May of 2002 a total of 11,746 married (to reduce loss to follow-up) men and women were recruited between the ages of 18 to 75 years from a well-defined 25-km² geographical area where they had been residing for at least 3 years. The cohort was expanded to include an additional 8,287 participants using the same methodology between 2006 and 2008. Most recently an additional 15,000 participants were recruited to the HEALS cohort totaling slightly over 35,000 adults. Participants in the HEALS cohort underwent demographic and lifestyle data collection using standardized questionnaires. Information on As exposure is assembled by collecting water and urine samples at multiple time points. A detailed description of the study cohort has been previously published and described (Ahsan et al., 2006).

Recruitment Procedure

We adopted a similar protocol for recruiting study participants based on our past procedures. The study protocol was approved by the Institutional Review Board of Columbia University and the Bangladesh Medical Research Council. Informed consent was obtained from all participants. The study team visited the potential study participants at their home, explained the study procedure, and ascertained their eligibility criteria using a structured questionnaire. A potential list of participants for this study was formed based on As exposure, age, and smoking status from the HEALS central database. Upon further examination of the potential participants list a number of participant were deceased, migrated out of the study area, or had been suffering from a serious illness. Some eligible participants could not participate because they had taken employment out of the study area and visit home infrequently only during weekends. A large number of participants were not eligible to participate because they stopped drinking water from wells with As concentration (>50 µg/L). Persons identified with illnesses related to immune function and/or taking any medication that might have an impact on immune function, including medications for cardiovascular disease or diabetes were not enrolled in the study. If the participant was eligible, willing, and consented, an

appointment was made for them at the field clinic. Our field team was able to identify 317 eligible participants to recruit for this study, out of which 267 participants visited our study clinic and completed the study procedure. We obtained and analyzed hematology and water arsenic (WAs) data from 103 individuals.

Questionnaire data, clinical examination and anthropometric measurement

Following consent to participate in the study, baseline in-person interviews were performed by trained personnel with detailed questionnaires on lifestyle characteristics. Participants were asked to provide information on demographics, medical co-morbidities, and cigarette and betel-nut usage. Questions were also answered on socioeconomic status defined by television ownership, land ownership, years of education, and occupation. Anthropometric measurements including height, weight, and blood pressure were performed using standard techniques.

Sample Collection, Processing, and Shipment

Information on drinking water was collected at the time of recruitment. If the study participants used the same water source since 2000 and it had been tested by Columbia University in the past, then the individuals were consented into the study. Following consent, trained physicians collected a 20 mL venous blood sample and 40 mL spot urine sample from each participant.

Blood samples were collected by venipuncture using a vein in the antecubital fossa into dipotassium EDTA or sodium heparin anticoagulant tubes. Blood samples in heparinized tubes were processed immediately to isolate peripheral blood mononuclear cells (PBMC) and blood collected in EDTA tubes was used for hematology. All hematological parameters were measured immediately at the Araihaaz Laboratory, Columbia University As and Health Research in Bangladesh. All the biological samples were labeled with machine-readable barcodes to maintain privacy of study participants.

Automated Complete Blood Count (CBC)

A CBC was performed using a MYTHIC 22, a fully automated (Microprocessor controlled) hematology analyzer used for the *in vitro* diagnostic testing of whole blood specimens. MYTHIC 22 is an optical measurement system for analyzing up to 22 hematological parameters.

Water sample collection and measurement of As

Procedures for field sample collection and laboratory analyses are described elsewhere in detail (Cheng et al., 2004; Van Geen et al., 2005; van Geen et al., 2007). Water samples were analyzed by high-resolution inductively coupled plasma mass spectrometry (HR ICP-MS) as previously described (van Geen et al., 2007). The analytical detection limit of the method is 0.1 µg/L; the standard deviation of a single measurement is conservatively estimated at 4 µg/L (van Geen et al. 2005).

Urine collection, and UAs and creatinine assays

Spot urine samples were collected in 50 mL acid-washed tubes and stored at -80°C until shipped to Columbia University on dry ice for analysis. UAs assays were performed with a graphite furnace atomic-absorption (GFAA) instrument using a Perkin-Elmer Analyst 600 graphite furnace system as described (Nixon et al., 1991). The detection limit for urinary As was $2\text{ }\mu\text{g/L}$. Urinary creatinine was analyzed by a colorimetric method based on the Jaffe reaction.

Urinary As metabolites assay

UAs metabolites were assayed by inductively coupled plasma-mass spectrometry (ICP-MS-DRC) coupled to high performance liquid chromatography (HPLC). ICP-MS-DRC was used as a detector for As metabolites chromatographically separated on an Anion Exchange Column (Hamilton PRP-X100) with 10 mM ammonium nitrate/ammonium phosphate, pH 9.1, as the mobile phase (van Geen et al., 2002). The excellent separation power of HPLC coupled with the very low detection limits of ICP-MS-DRC allowed us to detect InAs (i.e. As^{+3} and As^{+5}), total monomethylarsinic acid (MMA), and total dimethylarsinic acid (DMA).

PBMC isolation from peripheral blood

Blood from heparinized tubes was transferred into two 15 mL centrifuge tubes and was mixed with approximately 14 ml sterile phosphate buffered saline (PBS); 7 mL of the mixture was layered on top of 4 mL Ficoll-Paque Plus (at room temperature; RT), a ratio of approximately 2:1 (v/v) and samples were centrifuged at 2700 rpm at RT for 25 min. Following centrifugation PBMC were collected from the interface of the ficoll. Cells were diluted with sterile PBS and centrifuged this was repeated two more times. PBMC were cryopreserved and held in liquid nitrogen until shipped on dry ice to the U.S. for analysis of PAH-DNA adducts.

Polycyclic Aromatic Hydrocarbon-DNA (PAH-DNA) Adducts

PAH diol epoxide-DNA adducts were analyzed by competitive ELISA, using methods described previously (Divi et al., 2002). Briefly, 96 microwell plates coated with 2 ng of benzo(a)pyrene diol epoxide (BPDE)-I-DNA ($5\text{ adducts}/10^3\text{ nucleotides}$) and rabbit antiserum #29 (Poirier 1980) was used with BPDE-DNA as a standard. DNA was isolated from frozen PBMC samples according to standard procedure using phenol/chloroform/isoamyl alcohol. DNA was assayed for PAH-DNA adducts after sonication and denaturation by laboratory technicians blinded to exposure status. For analytical purposes, those samples with $<15\%$ inhibition are considered non-detectable and assigned a value of $1\text{ adduct}/10^8\text{ nucleotides}$, an amount midway between the lowest positive value and zero. A 5% blinded duplication was carried out using those subjects with the most DNA available. As an additional quality control, a DNA sample from an animal treated with BP was also assayed was run with the sample batch.

Statistical analysis

Summary statistics were calculated to describe the distribution of all variables for outliers. Similarly, we used frequency tables, crosstabs, box plots, and scatter plots to better understand the distribution of the data. T-tests and ANOVA were used to detect differences in categorical and continuous variables. Water and UAs as well as PAH-DNA adduct values were log- transformed to normalize their distributions to meet assumptions of ANOVA and reduce the impact of extreme values in linear regression analysis. Spearman correlation coefficients were used to evaluate bivariate associations among hematological parameters and the exposure variables. We estimated associations between As exposure (water, urine and UAs metabolites) and different hematological parameters in separate linear regression models, with and without adjusting for socio-demographic characteristics, and other potential confounders (age, body mass index, smoking, length of education and PBMC PAH-DNA adduct). We repeated the same analysis among smokers and non-smokers in separate linear regression models adjusting for the same variables mentioned previously. We also examined interactions between smoking and PAH-DNA adducts for each measure of hematological parameters by including multiplicative interaction terms in the models. All statistical analyses were conducted using Statistical Analysis Software (SAS 9.4) (SAS Institute Inc.).

RESULTS

Characteristics of the study population

The characteristics of the study population are described in Table 1. A majority of the participants were more than 50 years old (60%) and had a BMI less than 25 (75%). The majority of participants had at least one or more years of schooling (70%). About half of the study participants were active smokers (46%) and more than 60% were exposed to WAs >50 µg/L, a drinking water standard for As in Bangladesh.

Relationships among measures of exposure

The different measures of As exposure (WAs, UAs, and InAs, MMA and DMA) were moderately correlated with each other (r-values between 0.36 and 0.52 and $p < 0.0001$, data not shown). However, the correlations among As exposures measures were relatively stronger among never smokers (r-values between 0.51 and 0.62 and $p < 0.0001$, data not shown) as compared to smokers. Statistically significant correlations were observed only between WAs and UAs among smokers ($r = 0.40$, $p = 0.007$). Similarly, PAH-DNA adducts were correlated only with WAs ($r = 0.40$, $p < 0.001$) which did not differ appreciably due to smoking status (data not shown).

Relationships of As exposure and hematological parameters

In unadjusted models, significant inverse correlations were found between WAs and RBC and HCT/PCV (Fig. 1A and Fig. 1B). Furthermore, a dose dependent decrease in RBC and HCT/PCV was also observed with increasing WAs levels among all participants (Fig. 2A and Fig. 2B). Inverse relationships were also found between WAs and UAs and MCV ($\beta = 0.38$, 95% CI: $-0.05, 1.47$; $p = 0.06$) and Hgb ($\beta = 0.03$, 95% CI: $-0.10, 0.16$; $p = 0.66$, data

not shown). Associations between UAs and RBC and HCT/PCV as well as other parameters were similar to those observed in relation to WAs (data not shown). However, a significant inverse correlation was observed between UAs and Hgb ($\beta = -0.38$, 95% CI: $-0.65, -0.12$; $p = 0.005$, data not shown).

Table 2 shows associations of As exposure with hematological parameters in adjusted models. With adjustment for the covariates including age, BMI, educational attainment, smoking, and PAH-DNA adducts, significant inverse associations were observed between WAs and RBC ($\beta = -0.13$, 95% CI: $-0.19, -0.07$; $p < 0.0001$) and HCT/PCV ($\beta = -0.68$, 95% CI: $-1.17, -0.19$; $p = 0.008$). The relationship between RBC and HCT/PCV with UAs was found to be slightly stronger (Table 2). Similarly, a strong positive correlation was observed for total white blood cell counts (WBC) ($\beta = 516.11$, 95% CI: $70.16, 962.06$; $p = 0.002$) in relation to UAs.

Relationships of urinary As metabolites and hematological parameters

We further examined the role of urinary As metabolites on hematological parameters in adjusted models (Table 3). Associations between urinary As metabolites and hematological parameters were consistent with those observed for water and urinary As. As stated, we observed a significant inverse relationship between RBC and InAs ($\beta = -0.16$, 95% CI: $-0.26, -0.23$; $p = 0.004$), MMA ($\beta = -0.12$, 95% CI: $-0.21, -0.02$; $p = 0.01$), and DMA ($\beta = -0.11$, 95% CI: $-0.22, 0.11$; $p = 0.05$). A significant inverse association of InAs ($\beta = -0.12$, 95% CI: $-0.21, -0.02$; $p = 0.01$) with HCT/PCV was also found. We observed a positive association of DMA with HCT/PCV ($\beta = 2.71$, 95% CI: $0.62, 4.81$; $p = 0.01$). There was a marginally significant inverse relationship between InAs and Hgb ($\beta = -0.18$, 95% CI: $-0.39, 0.03$; $p = 0.09$) and this relationship was stronger among smokers ($\beta = -0.27$, 95% CI: $-0.50, -0.04$; $p = 0.02$, data not shown). Significant negative correlations were also found between percent lymphocytes and InAs ($\beta = -1.87$, 95% CI: $-3.22, -0.53$; $p = 0.007$), MMA ($\beta = -1.37$, 95% CI: $-2.57, -0.16$; $p = 0.02$), and DMA ($\beta = -1.45$, 95% CI: $-2.92, 0.02$; $p = 0.05$). Similarly, InAs was negatively correlated with percent neutrophils ($\beta = 1.60$, 95% CI: $0.09, 3.11$; $p = 0.04$).

Relationships of As exposure and hematological parameters by smoking status

Table 4 shows correlations between As exposure and hematological parameters among smokers and non-smokers. Among non-smokers, the negative correlation with RBC remained significant in relation to WAs. Interestingly, the magnitude of the associations of As exposure, particularly with UAs on RBC ($\beta = -0.4$, 95% CI: $-0.66, -0.14$; $p = 0.005$) as well as HCT/PCV ($\beta = -1.98$, 95% CI: $-4.19, 0.23$; $p = 0.08$) became stronger among smokers. Additionally, there was also a strong effect of UAs on Hgb among smokers in relation to urinary As ($\beta = -0.54$, 95% CI: $-1.09, 0.00$; $p = 0.05$). In contrast, a significant association was observed for WBC with WAs as well as UAs, which was non-significant among smokers. We have found a marginally significant interaction with water As and smoking ($p = 0.09$) on RBC.

DISCUSSION

The goal of this study was to evaluate the effects of chronic drinking water As exposure on clinical indicators of anemia among healthy male smokers and non-smokers in the HEALS cohort living in rural Bangladesh. To our knowledge, the present study is the first to investigate associations between As exposure (as indicated by WAs, UAs, and UAs metabolites) and clinical indicators of anemia among smokers. A dose-dependent reduction in RBC and HCT/PCV was found with increasing WAs levels. In models adjusted based on age, BMI, educational attainment, smoking, and PAH-DNA adducts, inverse relationships were also found between measures of As exposure and RBC and HCT/PCV. The correlations between As exposure with RBC and HCT/PCV became stronger among smokers, suggesting potential interactive effects between As and smoking on clinical indicators of anemia. Additionally, associations between As exposure and WBC and white blood cell subsets were also identified.

A high prevalence of anemia exists among the general population in rural Bangladesh (Ahmed 2000; Jamil et al., 2008). Anemia in this region is often associated with nutritional deficiencies of iron, folate, and vitamins B₆ and B₁₂ (Ahmed 2000), but a number of studies have also reported As exposure to be a contributing risk factor (Hopenhayn et al., 2006; Heck et al., 2008; Surdu et al., 2015; Kile et al., 2016). It is important to note that nearly all of these referenced studies only included women, so there is a lack of information concerning the relationships between As exposure and anemia among men. A previous study by Heck et al. (2008), found that men exposed to elevated As were susceptible to anemia, whereas only women with predispositions (i.e., Hgb <10 g/dL) showed a relationship between As exposure and anemia. These findings emphasize the need for gaining a deeper understanding of the influences of As exposure and anemia among men.

As exposure has been reported to contribute to anemia in humans and rodents by depressing the function of the bone marrow (Szymanska-Chabowska et al., 2002). A number of studies have indicated As-induced toxicity to red blood cells (Winski et al., 1997; Winski and Carter 1998; Zhang et al., 2000; Biswas et al., 2008; Mahmud et al., 2009). As has been shown to cause oxidative stress, deplete intracellular ATP, and to alter the morphological characteristics of red blood cells resulting in compromised membrane integrity, eryptosis, and intra-and-extravascular hemolysis (Winski and Carter 1998; Biswas et al., 2008; Mahmud et al., 2009). There is also a possibility that the inverse relationship between RBC and As exposure is related to reduced erythropoietic capacity of the bone marrow. In fact, we recently reported that drinking water exposure of adult male mice to low levels of As⁺³ (500 µg/L) disrupts erythropoiesis in the bone marrow by inhibiting the colony-forming ability of early erythroid progenitors and suppressing the differentiation of later stage erythroblasts (Medina et al., submitted).

The major form of As found in drinking water in Bangladesh is As⁺³ (Zheng et al., 2004), which is the most toxic inorganic arsenical (Styblo et al., 2000). As⁺³ is metabolized in the liver, kidneys, and lungs through a series of methylation reactions catalyzed by the As3MT enzyme to yield MMA and DMA (Vahter 2002). Although some interindividual variation is expected, DMA and MMA are the forms of arsenic most readily excreted in urine (Vahter

2002). The relative amounts of excreted As metabolites are used as indicators to determine the efficiency of InAs metabolism (Gamble et al., 2005).

The positive relationship between DMA and HCT/PCV found in our studies is not surprising, as high levels of DMA in urine are indicative of more efficient As metabolism and are often associated with decreased toxicity (Vahter and Concha 2001; Vahter 2002). We found a positive relationship between DMA and HCT/PCV, which likely reflects a greater As metabolic capacity among some participants. Multiple As forms exist simultaneously in the body (Vahter 2002), so it is possible that the discrepancy between the direction of relationship with DMA and RBC was influenced by toxic effects caused by other As metabolites.

Cigarette smoke has been shown to cause red blood cell hemolysis in animal models and humans (Minamisawa et al., 1990; Masilamani et al., 2016). In a group of 62 men and women, Masilamani et al., (2016) recently found that smoking causes morphological abnormalities on the surface of red blood cells that result in hemolysis. The authors also indicate that such cell surface damage facilitates the entry of toxins into red blood cells (Masilamani et al., 2016). The stronger relationships identified between RBC and HCT/PCV in smokers may therefore be attributed to potential interactive effects on red blood cell hemolysis induced by combined exposure to As and cigarette smoke. However, this hypothesis requires further investigation. The inverse relationship between UAs and Hgb is consistent with previous findings that As binds to heme and reduces heme metabolism resulting in decreased Hgb levels in red blood cells (Delnomdedieu et al., 1994; Hernández-Zavala et al., 1999; Lu et al., 2004). It is interesting to note that this association was detected only among smokers. Previous studies have indicated elevated RBC and HCT/PCV among smokers (Sagone and Balcerzak 1975; Nordenberg et al., 1990; Anandha, et al., 2014), but these studies did not consider the effects of combined exposures with other pollutants. This suggests that smoking may have differential effects on red blood cells when combined with other toxicants, such as As.

Interestingly, As exposure and smoking were correlated with hematological indicators of immune status (i.e., WBC and white blood cell subsets). The loss of positive associations between As exposure and WBC among smokers as well as the inverse relationships between all UAs metabolites and lymphocyte percentages suggest potential immune alterations among study participants and will be the topic of our future investigations.

In summary, results from this study provide evidence that chronic drinking water exposure to As results in hematological alterations that are consistent with anemia in a male cohort of smokers and non-smokers in rural Bangladesh. In addition, we also found that smoking may exacerbate As-induced effects on clinical indicators of anemia. Collectively, these findings show that men are sensitive to As-induced anemia and emphasize the necessity for further research concerning the mechanistic basis for the relationship between chronic As exposure and anemia.

Acknowledgments

FUNDING

This work was supported by the National Institutes of Environmental Health Sciences (NIEHS) ViCTER Program R01 ES019968S1 and by the NIEHS Superfund Basic Research Program P42 and ES009089.

References

- Ahmed F. Anaemia in Bangladesh: a review of prevalence and aetiology. *Public Health Nutr.* 2000; 3(4):385–393. [PubMed: 11135792]
- Ahsan H, Chen Y, Parvez F, Argos M, Hussain AI, Momotaj H, Levy D, Van Geen A, Howe G, Graziano J. Health effects of arsenic longitudinal study (HEALS): description of a multidisciplinary epidemiologic investigation. *J. Expo. Sci. Environ. Epidemiol.* 2006; 16:191–205. [PubMed: 16160703]
- Anandha L, Anandhi L, Ganesh KP, Saravanan A. Effect of intensity of cigarette smoking on haematological and lipid parameters. *J. Clin. Diagn. Res.* 2014; 8(7):BC11–BC13.
- Balarajan Y, Ramakrishnan U, Özaltın E, Shankar AH, Subramanian SV. Anaemia in low-income and middle-income countries. *Lancet.* 2011; 378:2123–2135. [PubMed: 21813172]
- Biswas D, Banerjee M, Sen G, Das JK, Banerjee A, Sau TJ, Pandit S, Giri AK, Biswas T. Mechanism of erythrocyte death in human population exposed to arsenic through drinking water. *Toxicol. Appl. Pharmacol.* 2008; 230:57–66.
- Breton CV, Houseman EA, Kile ML, Quamruzzaman Q, Rahman M, Mahiuddin G, Christiani DC. Gender-specific protective effect of hemoglobin on arsenic-induced skin lesions. *Cancer Epidemiol. Biomarkers Prev.* 2006; 15(5):902–907. [PubMed: 16702368]
- 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; 379:512–518. [PubMed: 15098084]
- Delnomdedieu M, Basti MM, Styblo M, Otvos JD, Thomas DJ. Complexation of Arsenic Species in Rabbit Erythrocytes. *Chem. Res. Toxicol.* 1994; 7:621–627. [PubMed: 7841340]
- Divi RL, Beland FA, Fu PP, Von Tungeln LS, Schoket B, Camara JE, Ghei M, Rothman N, Rashmi Sinha R, Poirier MC. Highly sensitive chemiluminescence immunoassay for benzo[a]pyrene-DNA adducts: validation by comparison with other methods, and use in human biomonitoring. *Carcinogenesis.* 2002; 23(12):2043–2049. [PubMed: 12507927]
- Ferrario D, Gribaldo L, Hartung T. Arsenic exposure and immunotoxicity: a review including the possible influence of age and sex. *Curr. Environ. Health Rep.* 2016; 3(1):1–12. [PubMed: 26875182]
- Gamble MV, Liu X, Ahsan H, Pilsner JR, Ilievski V, Slavkovich V, Parvez F, Levy D, Factor-Litvak P, Graziano JH. Folate, homocysteine, and arsenic metabolism in arsenic-exposed individuals in Bangladesh. *Environ. Health Perspect.* 2005; 113(12):1683–1688. [PubMed: 16330347]
- Heck JE, Chen Y, Grann VR, Slavkovich V, Parvez F, Ahsan H. Arsenic exposure and anemia in Bangladesh: a population-based study. *J. Occup. Environ. Med.* 2008; 50(1):80–87. [PubMed: 18188085]
- Hernández-Zavala A, Del Razo LM, García-Vargas GG, Aguilar C, Borja VH, Albores A, Cebrián ME. Altered activity of heme biosynthesis pathway enzymes in individuals chronically exposed to arsenic in Mexico. *Arch. Toxicol.* 1999; 73:90–95. [PubMed: 10350189]
- Hopenhayn C, Bush HM, Bingcan A, Hertz-Picciotto I. Association between arsenic exposure from drinking water and anemia during pregnancy. *J. Occup. Environ. Med.* 2006; 48(6):635–643. [PubMed: 16766928]
- Hughes MF. Arsenic toxicity and potential mechanisms of action. *Toxicol. Lett.* 2002; 133:1–16. [PubMed: 12076506]
- Jamil KM, Rahman AS, Bardhan PK, Khan AI, Chowdhury F, Sarker SA, Khan AM, Ahmed T. Micronutrients and anaemia. *J. Health Popul. Nutr.* 2008; 26(3):340–355. [PubMed: 18831229]
- Kile ML, Faraj JM, Ronnenberg AG, Quamruzzaman Q, Rahman M, Mostofa G, Afroz S, Christiani DC. A cross sectional study of anemia and iron deficiency as risk factors for arsenic-induced skin lesions in Bangladeshi women. *BMC Public Health.* 2016; 16(158):1–10. [PubMed: 26728978]
- Leifert JA. Anaemia and cigarette smoking. *Int. J. Lab. Hematol.* 2008; 30:177–184. [PubMed: 18479294]

- Lu M, Wang H, Li X, Lu X, Cullen WR, Arnold LL, Cohen SM, Le XC. Evidence of hemoglobin binding to arsenic as a basis for the accumulation of arsenic in rat blood. *Chem. Res. Toxicol.* 2004; 17:1733–1742. [PubMed: 15606151]
- Mahmud H, Föller M, Lang F. Arsenic-induced suicidal erythrocyte death. *Arch. Toxicol.* 2009; 83:107–113. [PubMed: 18636241]
- Masilamani V, AlZahrani K, Devanesan S, AlQahtani H, AlSalhi MS. Smoking induced hemolysis: spectral and microscopic investigations. *Sci. Rep.* 2016; 6:1–9. [PubMed: 28442746]
- Minamisawa S, Komuro E, Niki E. Hemolysis of rabbit erythrocytes induced by cigarette smoke. *Life Sci.* 1990; 47(24):2207–2215. [PubMed: 2266788]
- Naujokas MF, Anderson B, Ahsan H, Aposhian HV, Graziano JH, Thompson C, Suk WA. The broad scope of health effects from chronic arsenic exposure: update on a worldwide public health problem. *Environ. Health Perspect.* 2013; 121(3):295–302. [PubMed: 23458756]
- 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(9):1575–1579. [PubMed: 1893592]
- Nordenberg D, Yip R, Binkin NJ. The effect of cigarette smoking on hemoglobin levels and anemia screening. *JAMA.* 1990; 264(12):1556–1559. [PubMed: 2395196]
- Parvez F, Chen Y, Argos M, Hussain AZMI, Momotaj H, Dhar R, van Geen A, Graziano JH, Ahsan H. 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(3):355–359. [PubMed: 16507457]
- Poirier MC, Santella R, Weinstein B, Grunberger D, Yuspa SH. Quantitation of benzo(a)pyrene-deoxyguanosine adducts by radio immunoassay. *Cancer Res.* 1980; 40(2):412–416. [PubMed: 7356524]
- Sagone AL, Balcerzak SP. Smoking as a cause of erythrocytosis. *Ann. Intern. Med.* 1975; 82(4):512–515. [PubMed: 235231]
- Styblo M, Del Razo LM, Vega L, Germolec DR, LeCluyse EL, Hamilton GA, Reed W, Wang C, Cullen WR, Thomas DJ. Comparative toxicity of trivalent and pentavalent inorganic and methylated arsenicals in rat and human cells. *Arch. Toxicol.* 2000; 74(6):289–99. [PubMed: 11005674]
- Surdu S, Bloom MS, Neamtii IA, Pop C, Anastasiu D, Fitzgerald EF, Gurzau ES. Consumption of arsenic-contaminated drinking water and anemia among pregnant and non-pregnant women in northwestern Romania. *Environ. Res.* 2015; 140:657–660. [PubMed: 26073204]
- Szyma ska-Chabowska A, Antonowicz-Juchniewicz J, Andrzejak R. Some aspects of arsenic toxicity and carcinogenicity in living organism with special regard to its influence on cardiovascular system, blood and bone marrow. *Int. J. Occup. Environ. Med.* 2002; 15(2):101–116.
- Tyler CR, Allan AM. The effects of arsenic exposure on neurological and cognitive dysfunction in human and rodent studies: a review. *Curr. Environ. Health Rep.* 2014; 1:132–137. [PubMed: 24860722]
- Van Geen A, Ahsan H, Horneman AH, Dhar RK, Zheng Y, Hussain I, Ahmed KM, Gelman A, Stute M, Simpson HJ, Wallace S, Small C, Parvez F, Slavkovich V, Loiacono NJ, Becker M, Cheng Z, Momotaj H, Shahnewaz MM, Seddique AA, Graziano JH. Promotion of well-switching to mitigate the current arsenic crisis in Bangladesh. *Bull. World Health Organ.* 2002; 80(9):732–737. [PubMed: 12378292]
- Van Geen A, Cheng Z, Qing J, Seddique AA, Rahman MW, Rahman MM, Ahmed KM. Monitoring 51 community wells in Araihaazar, Bangladesh, for up to 5 years: implications for arsenic mitigation. *J. Environ. Sci. Health. Part A.* 2007; 42:1729–1740.
- Van Geen A, Cheng Z, Seddique AA, Hoque MA, Gelman A, Graziano JH, Ahsan H, Parvez F, Ahmed KM. Reliability of a commercial kit to test groundwater for arsenic in Bangladesh. *Environ. Sci. Technol.* 2005; 39:299–303. [PubMed: 15667109]
- Vahter M. Mechanisms of arsenic biotransformation. *Toxicology.* 2002; 181–182:211–217.
- Vahter M, Concha G. Role of metabolism in arsenic toxicity. *Pharmacol. Toxicol.* 2001; 89(1):1–5.

- Winski SL, Barber DS, Rael LT, Carter DE. Sequence of toxic events in arsine-induced hemolysis in vitro: implications for the mechanism of toxicity in human erythrocytes. *Fundam. Appl. Toxicol.* 1997; 38(2):123–128. [PubMed: 9299185]
- Winski SL, Carter DE. Arsenate toxicity in human erythrocytes: characterization of morphologic changes and determination of the mechanism of damage. *J. Toxicol. Environ. Health A.* 1998; 53(5):345–355. [PubMed: 9515938]
- World Health Organization (WHO). Arsenic in drinking-water. Geneva: WHO Press; 2011. Available: http://www.who.int/water_sanitation_health/dwq/chemicals/arsenic.pdf
- Zhang T, Gao Y, Lu J, Wang K. Arsenite, and arsenate and vanadate affect human erythrocyte membrane. *J. Inorg. Biochem.* 2000; 79:195–203. [PubMed: 10830866]
- Zheng Y, Stute M, van Geen A, Gavrieli I, Dhar R, Simpson HJ, Schlosser P, Ahmed KM. Redox control of arsenic mobilization in Bangladesh groundwater. *Appl. Geochem.* 2004; 19(2):201–214.

Figure 1. Correlations between red blood cell counts (RBC) and hematocrit packed cell volume (HCT/PCV) with water arsenic concentration in male participants (n=103). A. Relationship between RBC and water arsenic. B. Association between HCT/PCV and water arsenic. The red lines indicates 95% confidence intervals. Significance level is p 0.05.

Figure 2.

Changes in red blood cell counts (RBC) and hematocrit packed cell volume (HCT/PCV) as a function of increasing water arsenic levels in male participants. A. RBC and water arsenic. B. HCT/PCV and water arsenic. The red line indicates the mean and black line indicates the median (n=103). *Statistically significant difference compared to 0–10 $\mu\text{g/L}$ ($p < 0.05$). # Statistically significant difference compared to 10–100 $\mu\text{g/L}$ ($p < 0.05$).

Table 1Sociodemographic, exposure and hematology parameters of the study participants.^a

Sociodemographic	Mean (range)
Age	52 (36–65)
Body Mass Index (BMI)	22.26 (14.50–30.00)
Education (years)	4.50 (0–12)
Exposure history	
Water As (µg/L)	103.50 (0.10–363.00)
Urinary As (µg/g)	1730.39 (100–11160)
Years of well use	15.12 (3–40)
Smoking in years	22.00 (5–30)
Cigarettes/per day	18.65 (10–30)
Packs of cigarettes/per year	188.62 (0–900)
PAH-DNA adduct (ng/mL)	1.99 (0.90–8.00)
Hematology parameters	
RBC ($\times 10^6/\mu\text{L}$)	4.98 (3.72–8.98)
HCT/PCV (%)	42.32 (31.80–70.50)
MCV (fL)	85.47 (56.50–116.60)
Hgb (g/dL)	14.03 (10.20–18.20)
WBC (cell/cumm)	9,015.79 (4,200–14,700)
Lymphocytes (%)	34.59 (17.00–57.00)
Neutrophils (%)	56.18 (31.00–77.00)
Monocytes (%)	3.78 (1.00–10.00)
Eosinophils (%)	5.43 (1.00–13.00)

^aRed blood cells counts (RBC), hematocrit packed cell volume (HCT/PCV), mean corpuscular volume (MCV), hemoglobin (Hgb), and total white blood cell counts (WBC).

Table 2Hematology parameters in arsenic-exposed men.^{a,b}

Hematology Parameters	Water Arsenic (µg/L) β (95% CI), p-value	Urinary As (µg/gm) β (95% CI), p-value
RBC (×10 ⁶ /µL)	−0.13 (−0.19, −0.07), <0.0001 *	−0.23 (−0.37, −0.09), 0.001 *
HCT/PCV (%)	−0.68 (−1.17, −0.19), 0.008 *	−1.08 (−2.2, 0.03), 0.06
MCV (fL)	0.67 (−0.16, 1.51), 0.11	1.43 (−0.41, 3.28), 0.13
Hgb (g/dL)	−0.06 (−0.18, 0.07), 0.33	−0.06 (−0.18, 0.07), 0.39
WBC (cell/cumm)	60.95 (−146.00, 267.9), 0.56	516.11 (70.16, 962.06), 0.02 *

^aRed blood cells counts (RBC), hematocrit packed cell volume (HCT/PCV), mean corpuscular volume (MCV), hemoglobin (Hgb), and total white blood cell counts (WBC).

^bParameter estimates are adjusted for age, BMI, smoking status, PAH-DNA adduct and level of education

* Significance level is p 0.05.

Table 3Hematology parameters and urinary arsenic metabolites of the study participants.^{a,b}

Hematology Parameters	In As (As ³⁺ As ⁵⁺) (µg/L)	MMA (µg/L)	DMA (µg/L)
	β (95% CI), p-value	β (95% CI), p-value	β (95% CI), p-value
RBC (×10 ⁶ /µL)	−0.16 (−0.26, −0.05), 0.004 *	−0.12 (−0.21, −0.02), 0.01 *	−0.11 (−0.23, 0.11), 0.05 *
HCT/PCV (%)	−0.77 (−1.6, 0.06), 0.07	−0.54 (−1.27, 0.19), 0.15	2.71 (0.62, 4.81), 0.01 *
MCV (fL)	0.8 (−0.57, 2.18), 0.25	0.58 (−0.63, 1.79), 0.34	0.51 (−0.95, 1.97), 0.49
Hgb(g/dL)	−0.18 (−0.39, 0.03), 0.09	−0.06 (−0.25, 0.12), 0.48	−0.05 (−0.27, 0.17), 0.65
WBC (cells/cumm)	224.14 (−113.31, 561.58), 0.19	154.22 (−141.58, 450.02), 0.30	181.07 (−177.05, 539.18), 0.32
Lymphocytes (%)	−1.87 (−3.22, −0.53), 0.007 *	−1.37 (−2.57, −0.16), 0.02 *	−1.45 (−2.92, 0.02), 0.05 *
Neutrophils (%)	1.6 (0.09, 3.11), 0.04 *	1.13 (−0.2, 2.46), 0.09	1.29 (−0.32, 2.91), 0.11
Monocytes (%)	−0.18 (−0.46, 0.09), 0.19	−0.12 (−0.37, 0.12), 0.32	−0.22 (−0.52, 0.07), 0.13
Eosinophils (%)	0.43 (−0.02, 0.88), 0.06	0.36 (−0.04, 0.75), 0.08	0.38 (−0.1, 0.86), 0.12

^a Red blood cells counts (RBC), hematocrit packed cell volume (HCT/PCV), mean corpuscular volume (MCV), hemoglobin (Hgb), and total white blood cell counts (WBC).

^b Parameter estimates are adjusted for age, BMI, smoking status, PAH-DNA adduct and level of education.

* Significance level is p 0.05.

Table 4Hematology parameters in arsenic-exposed men by smoking status.^{a,b}

Hematology Parameters	Never Smokers		Smokers	
	Water Arsenic (µg/L) β (95% CI), p-value	Urinary Arsenic (µg/gm) β (95% CI), p-value	Water Arsenic (µg/L) β (95% CI), p-value	Urinary Arsenic (µg/gm) β (95% CI), p-value
RBC (×10 ⁶ /µL)	−0.09 (−0.15, −0.03), 0.003 [*]	−0.1 (−0.24, 0.04), 0.17	−0.17 (−0.3, −0.04), 0.01 [*]	−0.4 (−0.66, −0.14), 0.005 [*]
HCT/PCV (%)	−0.38 (−0.9, 0.14), 0.15	−0.2 (−1.39, 0.99), 0.74	−0.87 (−1.96, 0.21), 0.12	−1.98 (−4.19, 0.23), 0.08
MCV (fL)	0.64 (−0.46, 1.73), 0.26	1.16 (−1.29, 3.61), 0.35	0.87 (−0.65, 2.4), 0.27	2.52 (−0.57, 5.61), 0.11
Hgb (g/dL)	−0.02 (−0.17, 0.13), 0.8	−0.02 (−0.34, 0.31), 0.92	−0.07 (−0.35, 0.21), 0.63	−0.54 (−1.09, 0.00), 0.05 [*]
WBC (cells/cumm)	239.36 (−2.07, 480.79), 0.05 [*]	776.17 (261.38, 1290.96), 0.004 [*]	−279.95 (−677.24, 117.33), 0.17	337.84 (−495.65, 1171.32), 0.43

^aRed blood cells counts (RBC), hematocrit packed cell volume (HCT/PCV), mean corpuscular volume (MCV), hemoglobin (Hgb), and total white blood cell counts (WBC).

^bParameter estimates are adjusted for age, BMI, smoking status, PAH-DNA adduct and level of education.

*Significance level is p<0.05.