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Higher Risk of Hyperglycemia with Greater Susceptibility in Females in Chronic Arsenic-Exposed Individuals in Bangladesh

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Abstract

Arsenic (As) toxicity and diabetes mellitus (DM) are emerging public health concerns worldwide. Although exposure to high levels of As has been associated with DM, whether there is also an association between low or moderate As exposure and DM remains unclear. We explored the dose-dependent association between As exposure levels and hyperglycemia, with special consideration of the impact of demographic variables, in 641 subjects from rural Bangladesh. The total study participants were divided into three groups depending on their levels of exposure to As in drinking water (low, moderate and high exposure groups). Prevalence of hyperglycemia, including impaired glucose tolerance (IGT) and DM was significantly associated with the subjects' drinking water arsenic levels. Almost all exposure metrics (As levels in the subjects' drinking water, hair and nails) showed dose-dependent associations with the risk of hyperglycemia, IGT and DM. Among

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the variables considered, sex, age, and BMI were found to be associated with higher risk of hyperglycemia, IGT and DM. In sex-stratified analyses, As exposure showed a clearer pattern of dose-dependent risk for hyperglycemia in females than males. Finally, drinking water containing low-to-moderate levels of As (50.01–150 µg/L) was found to confer a greater risk of hyperglycemia than safe drinking water (As < 10 µg/L). Thus the results suggested that As exposure was dose-dependently associated with hyperglycemia, especially in females.

Keywords

Arsenic; Hyperglycemia; Impaired glucose tolerance; Diabetes; Bangladesh

Introduction

Arsenic (As) poisoning is a serious threat to public health worldwide and Bangladesh is one of the most severely affected countries. Approximately 35–70 million people in Bangladesh have been exposed to As through drinking water (Kinniburgh and Smedley, 2001). The neoplastic effects of As have been well established. However, accumulating evidence suggests that chronic As exposure is also associated with several common non-malignant illnesses, such as cardiovascular diseases (CVDs) (Chen et al., 2011; Hasibuzzaman et al., 2017; Huda et al., 2014; Islam et al., 2015; Islam et al., 2011; Karim et al., 2013, 2010; Meliker et al., 2007; Navas-Acien et al., 2005). Exposure to high levels of arsenic has also been reported to be associated with diabetes (Lai et al., 1994; Rahman et al., 1999, 1998; Tseng et al., 2000). Individuals with diabetes mellitus (DM) are at particularly major risk of developing CVDs (Haffner et al., 1998; Huxley et al., 2006; Juutilainen et al., 2005).

DM is a chronic metabolic disorder characterized by hyperglycemia. Hyperglycemia can be differentiated into impaired glucose tolerance (IGT) and DM based on the glucose levels in blood. According to The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus (2009), patients with IGT are referred to as having “pre-diabetes,” indicating their greater susceptibility to diabetes (American Diabetes Association, 2009). DM is the most prevalent form of the disease worldwide. It is often asymptomatic in its early stages and can remain undiagnosed for many years. Due to its high prevalence and severe consequences, DM has become a major public health concern worldwide. Indeed, the incidence of DM is expected to nearly double by 2030 (Forbes and Cooper, 2013).

While the association between high levels of As exposure and DM has been well established, there are inconsistent findings in regard to the association between low-to-moderate levels of As and DM (Chen et al., 2007; Huang et al., 2011; Longnecker and Daniels, 2001; Maullet et al., 2012; Navas-Acien et al., 2006; Pan et al., 2013; Rahman et al., 1996; Tseng et al., 2002). An important limitation of most of the previous epidemiological studies was that they did not use biomarkers of chronic As exposure (Islam et al., 2012; Jovanovic et al., 2013; Lai et al., 1994; Lewis et al., 1999; Rahman et al., 1999, 1998; Tseng et al., 2000; Wang et al., 2003). A systematic review of studies on As exposure and diabetes showed that most of the studies used ecological measurements or calculated cumulative As dose rather than the actual biomarkers of chronic exposure, and did not consider the

confounders that might influence the association (Maull et al., 2012). Demographic variables, especially sex, are associated with higher prevalence of some chronic As exposure-related diseases. For example, women are found to be more vulnerable to As-related kidney, lung and bladder cancers than men (Chiu et al., 2004; Guo et al., 2004; Smith et al., 1998; Vahter et al., 2007; Wu et al., 1989). However, little is known about the effect of sex or other confounding variables on the prevalence of As-related hyperglycemia or DM. In order to better clarify the hyperglycemic effects of chronic exposure to As, more epidemiological studies will be needed, particularly studies using appropriate biomarkers of chronic exposure and a more thorough consideration of confounding variables.

In this cross-sectional study, we evaluated the dose-response relationship between hyperglycemia and both external and internal As exposure metrics in human subjects from rural areas in Bangladesh with low and high As exposure, with a focus on the impact of potentially confounding demographic variables. We hypothesized that there would be a concentration-dependent association between As exposure and risk of hyperglycemia, including both IGT and DM. We also hypothesized that demographic variables, especially sex, might correlate with the differences in hyperglycemic susceptibility.

Methods

Study areas and study participants

The Institute of Biological Sciences, University of Rajshahi gave the ethical approval for this study (661/320/IAMEBBC/IBSc). Each participant provided written consent. The process used to select the study areas was described in our previous studies (Ali et al., 2010; Hossain et al., 2012). In brief, subjects were selected from the highly As-exposed villages in the northwestern region of Bangladesh, which included Marua in the Jessore District, Dutpatila, Jajri, Vultie and Kestopur in the Chuadanga District, Khemirdia (Bheramara) in the Kushtia District and Kazirpara in the Rajshahi District. First, we visited each of the As-endemic areas, and we found that many local residents had hyperkeratosis and melanosis, typical skin symptoms of chronic As exposure. The physicians involved in our study carefully examined the local residents for As-induced skin lesions. As an area of low As exposure, we selected Chowkoli, a village in the northern district of Naogaon that had no previous history of As poisoning. We did not find any residents with As exposure-related skin lesions in this low-exposure area. For the further confirmation of the non-endemic or low-exposure area, we randomly selected several tube wells and checked the As concentrations in their well water. In all these samples, the As concentrations were below the permissive limit ($<50 \mu\text{g/L}$) set by the Bangladesh Government. Our team visited the families in both As-endemic and non-endemic (low-exposed) villages and requested that all adult (18–60 years of age) family members convene at a specific location of each village for primary enrollment in our study. The individuals who responded to this call for voluntary participation and who had lived in their local area for at least five years were enrolled as study subjects irrespective of their skin symptoms. Pregnant and lactating mothers were excluded from this study, as were subjects with a history of drug addiction, hepatotoxic or antihypertensive drug use, malaria or leishmaniasis, or a history of hepatic, renal or severe cardiac diseases. The team members who measured the subjects' blood glucose levels were blind to their As levels. Appropriately

trained members of the research team conducted interviews of the study subjects using a standard questionnaire as described in our previous reports (Karim et al., 2013; Huda et al., 2014; Hasibuzzaman et al., 2017). The information obtained from the questionnaire included the sources of water for drinking and daily household uses, the history of drinking water consumption, socioeconomic status (occupation, education and income), food habits, alcohol intake, cigarette smoking, personal and family medical history, history of diabetes and other major diseases, previous physicians' reports and body mass index (BMI).

Outcome measurement

Subjects' fasting (10–12 hours) blood glucose (FBG) levels were determined using an Accu Chek Active (Roche, Mannheim, Germany) glucometer. After measuring FBG, the subjects were provided with 75g of glucose and asked to consume it with 250–300 ml of water within 5 minutes. After two hours, blood glucose levels were measured again with the same glucometer. Hyperglycemia, IGT or DM were defined according to WHO diagnostic criteria. Pre-diabetic and diabetic individuals were identified by two-hour blood glucose (2h-BG) levels 140–199 mg/dL and 200 mg/dL, respectively, as determined by OGTT (oral glucose tolerance test). Ten participants had been previously diagnosed as diabetic patients by their physicians (based on the physician reports). We measured the FBG levels of these 10 patients and all 10 were found to be diabetic (FBG level >126 mg/dL). These diabetic patients were excluded from the OGTT.

Exposure estimates at the individual level

We collected the water, hair and nail samples of the participants according to previously described methods (Ali et al., 2010; Hasibuzzaman et al., 2017; Hossain et al., 2017; Van geen et al., 2008). Hair and nail samples were washed and digested as described previously (Ali et al., 2010; Hasibuzzaman et al., 2017). The As concentrations in drinking water and digested hair and nails were analyzed by inductively coupled plasma mass spectroscopy (ICP-MS) (Agilent 7700x Japan). River water (NMIJ CRM 7202-a No.347; AIST, Japan) and human hair (NCS DC73347a; China National Analysis Center) were used as certified reference materials (CRM) to verify the accuracy of the measurement of drinking water, and hair and nail As levels, respectively. All samples and CRM were analyzed in duplicate and triplicate, respectively, and the average values of As were used for the analysis. The average value (mean $\pm$ SD) of As in the triplicate CRM samples in water was 1.161 ± 0.082 μ g/L (certified value, 1.18 μ g/L), and the corresponding value in hair was 0.278 ± 0.006 μ g/g (certified value, 0.28 μ g/g).

Statistical analyses

A frequency test was used to stratify participants into low, moderate and high groups based on their As exposure levels; each group contained an equal number of subjects. The same concentrations of As in water, hair, and nails were used to categorize low, moderate and high exposure groups for the sex-stratified analyses. An F-test (one-way ANOVA) was performed to compare the average age, BMI, As exposure levels, monthly income, FBG and 2h-BG, whereas a Chi-square test was performed for occupation, educational status, family history of DM, and prevalence of hyperglycemia, IGT and DM. We performed binary logistic regression analyses to examine the relative risk of As and demographic variables (age, sex,

BMI and smoking habit) on the prevalence of hyperglycemia in all and sex-stratified (males and females) subjects. Multinomial logistic regression analyses were performed to evaluate the relative risk of As and demographic variables on the prevalence of IGT and DM. Categorized age (<40 years and > 40 years) and BMI (normal = 18.5–24.9; low = < 18.5; high = > 24.9) were used in both binary and multinomial regression models where subjects under 40 years ages and subjects with normal BMI, respectively, were considered as the referent group. Male and non-smoker subjects were used as referent groups in their respective analyses. We further categorized the subjects into three groups (10 µg/L, 10.01–50 µg/L and > 50 µg/L) based on the maximum acceptable level of As in water set by WHO (10 µg/L) and the Bangladesh Government (50 µg/L). Finally, we split the > 50 µg/L group into a 50.01–150 µg/L group and > 150 µg/L group. Risks of hyperglycemia in these groups were examined through binary logistic regression analyses. A Spearman correlation test was used to analyze the association of As exposure with fasting and 2h-BG in males and females. All statistical analyses were performed using SPSS software version 21.0.

Results

Characteristics of the study participants

Table 1 shows the basic characteristics of the study participants. A frequency test was used to group all participants into low, moderate and high exposure groups based on the As levels in their drinking water. The average (mean ± SD) age, BMI and the parameters (occupation, education and monthly income) related to socioeconomic condition were similar in the low, moderate and high exposure groups. Most of the female subjects were housewives and most of the males were farmers. Approximately half of the total subjects had no formal education and the rate was similar in each group. We did not find any female smokers. The As levels in the drinking water ($p<0.001$), hair ($p<0.001$) and nail ($p<0.001$) samples were significantly increased in the higher exposure groups.

Comparisons of hyperglycemia (IGT and DM) and other related parameters in the low, moderate and high exposure groups

Table 2 shows the comparisons of hyperglycemia and other related parameters in the low, moderate and high exposure groups. A majority of the study subjects (79.3%) had no family history of DM, whereas 18.7% of the subjects could not provide information about the family history of DM. Only 2% of the subjects' had a family history of diabetes. Of the total subjects, 1.6% were known cases of DM. FBG and 2h-BG levels were significantly ($p<0.01$ for FBG and $p<0.001$ for 2h-BG) increased with the increasing levels of As in drinking water. We found a higher prevalence of hyperglycemia (30%) in the high exposure group than the moderate (21.5%) and low (12.7%) exposure groups. Increased concentrations of As in drinking water were significantly ($p<0.01$) associated with IGT and DM. The prevalences of IGT in the low, moderate and high As-exposure groups were 8%, 8.9% and 16.4%, respectively, and those of DM were 4.7%, 12.6% and 13.6%, respectively.

Effects of As exposure and other relevant variables on the risk for hyperglycemia (IGT and DM)

Table 3 shows the relative effects of As exposure and other relevant variables on hyperglycemia. Age, sex, BMI and smoking habits were considered as variables. In binary logistic regression, the adjusted odds ratios (ORs) for hyperglycemia in the moderate and high exposure groups were approximately 2-fold ($OR = 1.9$, $p < 0.05$) and 3-fold ($OR = 2.96$, $p < 0.001$) higher, respectively, than in the group with low concentration of As in water. Adjusted ORs in the moderate and high groups were approximately 2-fold higher ($OR = 1.84$, $p < 0.05$ for moderate and $OR = 1.94$, $p < 0.05$ for high concentration of As in hair, and $OR = 1.95$, $p < 0.05$ for moderate and $OR = 1.99$, $p < 0.01$ for high concentration of As in nails) than the groups with low concentrations of As in hair and nails. Interestingly, females showed approximately 2-fold higher OR ($OR = 1.95$, and $p < 0.01$ for As concentrations in water, $OR = 2$, and $p < 0.05$, and $OR = 1.96$, and $p < 0.01$ for As concentrations in nails) for hyperglycemia than their male counterparts. Adjusted ORs were 2-fold higher in the > 40 years of age group compared to the < 40 years group across all the exposure metrics. The high BMI group showed approximately 2-fold higher ORs compared to the normal BMI group.

Next, we performed multinomial logistic regression analyses to separate the hyperglycemic subjects into IGT and DM groups. The adjusted OR for IGT in the group with exposure to a high concentration of As in water was approximately 2.5-fold higher ($OR = 2.56$, $p < 0.01$) than that in the low exposure group. We also found that the ORs for IGT in the groups with exposure to high concentrations of As in hair and nails were approximately 2-fold higher ($OR = 1.83$, $p = 0.064$ for As in hair and $OR = 1.83$, $p = 0.057$ for As in nails) than those in the corresponding low exposure groups, and these differences were border line significant. On the other hand, ORs for DM in the moderate and high exposure groups were significantly higher than those in the low exposure groups for each exposure metric. ORs for IGT and DM in subjects older than 40 years of age and in the high BMI group were similar, as observed in the ORs of hyperglycemia across all the exposure metrics.

As exposure and risk of hyperglycemia in males and females

We next performed sex-specific analyses splitting the subjects into three exposure groups based on the concentrations of As in water, hair and nails (Figure 1). In females, the ORs for the groups with moderate and high concentrations of As in drinking water after adjustment for BMI and age were approximately 2-fold ($OR = 2.11$, $p < 0.05$) and 4-fold ($OR = 3.8$, $p < 0.001$) higher, respectively, than those of the corresponding low concentration group. On the other hand, the adjusted ORs for the groups with moderate and high concentrations of As in hair were approximately 2-fold ($OR = 2.14$, $p < 0.05$) and 3-fold ($OR = 2.97$, $p < 0.01$) higher, respectively, than those of the low concentration group. The ORs were approximately 3-fold higher in both the group with moderate ($OR = 2.81$, $p < 0.01$) and the group with high ($OR = 2.62$, $p < 0.01$) concentration of As in nails compared to the low concentration group. In the case of males, the adjusted ORs were higher in the groups with moderate and high As concentrations in water and nails than in their low-concentration counterparts, but these differences were not significant.

Comparisons of hyperglycemia and other parameters between males and females

The finding that the risk of hyperglycemia was greater in females than males (Figure 1) was intriguing, and led us to perform sex-specific analyses to compare hyperglycemia, IGT, DM and other parameters between the two groups (Table 4). We found that the prevalence of hyperglycemic patients (26.9%) in females was significantly ($p < 0.01$) higher than that (15.8%) in males. The percentages of DM and IGT patients were also significantly ($p < 0.01$ for both) higher in females than males. As expected, the average (mean $\pm$ SD) levels of FBG and 2h-BG were significantly ($p < 0.01$) higher in females than males. The average (mean $\pm$ SD) As levels were also higher in females than males, but this difference was only significant ($p < 0.01$) for the nail samples. Moreover, FBG and 2h-BG levels in females but not in males showed weak but significant associations with arsenic exposure metrics (Figure S1 and Figure S2).

Association between the regulatory upper limit of water As and risk of hyperglycemia

Finally, the subjects were split into three groups ($< 10 \mu\text{g/L}$, $10.01\text{--}50 \mu\text{g/L}$ and $> 50 \mu\text{g/L}$) based on the maximum permissible limit of As in water set by WHO and the Bangladesh Government to examine the dose-response relationship of hyperglycemia in these groups (Table S1). We found that the risk of hyperglycemia was increased with the increasing concentration of As in water. However, only the subjects in the $> 50 \mu\text{g/L}$ group had significantly ($\text{OR} = 2.46$ and $p < 0.001$) higher risk of hyperglycemia compared to the subjects in the $< 10 \mu\text{g/L}$ group. To determine the critical dose of As in water that might be responsible for the development of a hyperglycemic condition, we further divided the $> 50 \mu\text{g/L}$ group into a $50.01\text{--}150 \mu\text{g/L}$ group and a $> 150 \mu\text{g/L}$ group. Interestingly, the risks of hyperglycemia were significantly ($\text{OR} = 2.3$, and $p < 0.01$ for the $50.01\text{--}150 \mu\text{g/L}$ group and $\text{OR} = 2.71$, and $p < 0.001$ for the $> 150 \mu\text{g/L}$ group) higher in the population groups exposed to $50.01\text{--}150 \mu\text{g/L}$ and $> 150 \mu\text{g/L}$ As in water than in the group exposed to $< 10 \mu\text{g/L}$ As in water, suggesting that both low-to-moderate and high levels of drinking water As might be risks for hyperglycemia.

Discussion

In this study, we found that As concentrations in drinking water were positively associated with the prevalence of hyperglycemia, including both IGT and DM (Table 2). The risks of hyperglycemia were significantly higher in the moderate and high As-exposure groups than the low exposure groups across all three exposure metrics (Table 3). We also found that sex, age and BMI had significant associations with the risk of IGT and DM (Table 3). The subjects with low-to-moderate ($50.01\text{--}150 \mu\text{g/L}$) and high concentration ($> 150 \mu\text{g/L}$) of As in water had significantly higher risk of hyperglycemia than those with a low concentration ($< 10 \mu\text{g/L}$) of As in water (Table S1).

The association between high concentration of As and DM is generally well accepted, but there is disagreement in regard to the association between DM and low-to-moderate concentrations ($< 150 \mu\text{g/L}$) of As in water (Islam et al., 2012; Lai et al., 1994; Maull et al., 2012; Rahman et al., 1999, 1998, 1996; Tseng et al., 2000; Wang et al., 2003). An important caveat in regard to the previous epidemiological studies, which we have addressed herein, is

that most of them lacked a biomarker for chronic As exposure. Rather, the previous studies used external or ecological concentrations, such as drinking water As, as an exposure metric (Islam et al., 2012; Lai et al., 1994; Lewis et al., 1999; Rahman et al., 1999, 1998; Tseng et al., 2000; Wang et al., 2003). DM or hyperglycemia is a chronic disease. To establish the role of an environmental pollutant on the development of any chronic disease, a biomarker of prolonged exposure is more reliable than an ecological or immediate exposure marker. Ecological exposure metrics do not represent the actual exposure levels. Chen et al. (2010) used As concentrations in drinking water and urine as exposure markers and found no link between As exposure and DM in a cohort in Bangladesh. Urinary As does not represent chronic exposure; it is a biomarker of short term exposure with a half-life of only about 72 hours. As such, an investigation using chronic exposure biomarkers was warranted.

In the present study, we used biomarkers of chronic As exposure, and our findings corresponded well to those of Pan et al. (2013), which showed a positive link between As exposure and DM. As levels in hair and nails are effective markers of prolonged or chronic exposure (Garland et al., 1993; Michaud et al., 2004). As far as we are aware, no previous study has shown a dose-dependent association between As exposure and hyperglycemia using three kinds of exposure metrics. More importantly, we found that the As concentrations in subjects' hair and nails corresponded well with the concentrations of As in drinking water (data not shown). These interrelationships among exposure metrics and the dose-response relationship of the three exposure metrics with hyperglycemia were the important strengths of our study. The subjects of this study had a wide variation of As concentrations. Moreover, individuals who had been living in the study areas for at least 5 years were recruited for this study. Our use of subjects with prolonged residency in the study areas and of multiple biomarkers of prolonged exposure provided evidence that the As exposures observed in our study were chronic in nature. Therefore, the individuals enrolled in this study were appropriate subjects for exploring the association between As exposure and chronic diseases such as hyperglycemia.

We found that low-to-moderate (50.01–150 µg/L) and moderate-to-high doses (> 150 µg/L) of As in drinking water were associated with approximately 2-fold and 3-fold higher risk of hyperglycemia, respectively, than the low dose (< 10 µg/L) of As in drinking water (Table S1). Our results were in line with the findings of Grau-Perez et al. (2017) and Pan et al. (2013), which differed from those of Chen et al. (2010). Chen's study did not find a link between low-to-moderate dose of As exposure and DM. Temporality of subject's drinking water might have been one of the major reasons for these inconsistent results. Several other factors (i.e., age, sex, BMI, lifestyle, food habits, smoking, duration of As exposure, family history of DM, co-exposure to other metals, etc.) might also be responsible for inconsistencies in the association of As exposure with DM, as suggested by previous studies (Maull et al., 2012; Pan et al., 2013; Tseng et al., 2002). In our study, we found that sex, age and BMI had confounding effects on hyperglycemia (Table 3). It is now well established that high BMI, an important indicator of obesity, is a risk factor for DM. Older age has been almost unanimously found to be a risk factor for the majority of chronic diseases including DM (Kirkman et al., 2012; Niccoli and Partridge, 2012). The prevalences of IGT and DM observed in our study were approximately 11% and 10%, and these values appeared to be close to those of other studies conducted on the general population in Bangladesh (Sayeed et

al., 2003, 2007). However, the population of our study was younger than the populations recruited for the other studies (Table 1). A large portion of our population (approximately 69%) was under 40 years of age. If we compare the subjects of similar age, the prevalence of IGT or DM was higher in our study, indicating that chronic As exposure increases the risks of IGT and DM in younger populations.

The most intriguing finding of our study was the pronounced effect of sex on the ORs. Gender has been reported to be associated with higher prevalence of some chronic As exposure-related diseases (Chiu et al., 2004; Guo et al., 2004; Smith et al., 1998; Vahter et al., 2007; Watanabe et al., 2001; Wu et al., 1989). In sex-specific analyses, As exposure levels in females showed a clear pattern of dose-response relationship with the risk of hyperglycemia; however, exposure levels in males were weakly and non-significantly associated with the risk of hyperglycemia (Figure 1). In the comparison between males and females, we found a significantly higher prevalence of hyperglycemia (IGT and DM) in females (Table 4). Females also showed stronger associations between As exposure metrics and FBG (Figure S1) or 2h-BG (Figure S2) levels than males. Hence these results clearly indicated that females had greater susceptibility to As-related hyperglycemia. This finding was in line with previous studies (Chiu et al., 2006; Wang et al., 2003). Chiu's study showed that diabetic-related mortality was more common in females than males after withdrawal of As-contaminated water in an endemic area of black foot disease in Taiwan. Wang's group found that an endemic area-related risk of diabetes was consistently greater in females than in males (Wang et al., 2003). Neither of these studies used As-exposure metrics to examine the association between As exposure and prevalence of DM. To the best of our knowledge, our study is thus the first to provide direct evidence in support of the greater susceptibility of females to As-related DM than males. While we could not clarify the causes of the female group's greater susceptibility to hyperglycemia, one reason may have been the difference in As exposure levels between the male and female groups. The average concentration of As in the nail samples was significantly higher in females than males (Table 4). Although the differences were not significant, females also had higher levels of As in drinking water and hair (Table 4). The demographic characteristics of the male and female participants of this study suggested that the water sources of males were more variable than those of females (Table 1). That is, in our study, most of the females were housewives, whereas most of the males were farmers or farm workers (Table 1). Farmers and farm workers in Bangladesh usually work in agricultural fields far from their homes, which leads to variations in their drinking water sources. Most of our female subjects, and especially the housewives, remained near their homes for a longer period than males. Thus the reduced variability in the drinking sources of the female subjects may have increased the females' susceptibility to As-related hyperglycemia and DM. Female hormones may be another factor in the greater susceptibility of females to As-related hyperglycemia. Recently, Huang et al. (2015) found that As-treated oophorectomized mice were susceptible to hyperglycemia, which was reversed by 17 β -estradiol supplementation. The results of their study suggested that estrogen deficiency causes an enhanced susceptibility to As-related hyperglycemia in females (Huang et al., 2015). However, more studies are required to explain the greater susceptibility of females to developing hyperglycemia than males.

IGT has also been associated with the underlying mechanism of DM. Glucose uptake and utilization can be impaired because of the development of insulin resistance. During the developmental stage of IGT, beta cells increase production and secretion of insulin to overcome insulin resistance. When beta cells of islets fail to produce insulin excessively to overcome insulin resistance, hyperglycemia develops, leading to IGT and DM. Dyslipidemia is a major cause of insulin resistance. Previously we reported that chronic As exposure was associated with dyslipidemia through an increase in the oxidized low density lipoprotein (x-LDL) levels and concomitant reduction of high density lipoprotein cholesterol (HDL-C) levels (Karim et al., 2013). These increased oxidized LDL and decreased HDL levels have been recognized as risk factor for the development of insulin resistance (Linna et al., 2015; Vergès, 2009). The results of our previous study provided strong evidence in support of the notion that chronic As exposure induces hyperglycemia through an insulin-resistant mechanism. This idea was further supported by the recent results of Park et al. (2016), who found that As exposure is associated with diminished insulin sensitivity but not the function of beta cells in islets.

In spite of its several noted strengths, this study also had some limitations that should be pointed out. Although the glucometer we used for this study meets analytical and clinical quality requirements (Dhatt et al., 2011), we did not check its reliability before using it for our field study. Laboratory facilities are not readily available and often poorly equipped in rural Bangladesh. A portable glucometer can be used for screening rather than diagnosis of diabetes (Agarwal et al. 2008). The team members who measured the blood glucose levels were blind to the subjects' As exposure levels. Therefore, it is unlikely that misclassification due to inaccuracies in the measurement of blood glucose would have disrupted the associations observed in our study. We considered that age, sex, BMI and smoking habit were possible confounders for the observed associations of As exposure with hyperglycemia. However, other variables and/or the presence of other metals could affect the observed associations. If other confounders did affect the observed associations, they would have followed the same concentration gradients in parallel with the As concentrations. Although unexpected, such effects cannot be completely ruled out, and more studies are warranted to better control for them. Food habits and various lifestyle components may influence DM. In our study, the socioeconomic conditions (occupation, education and monthly income) of the low, moderate and high As-exposure groups were similar (Table 1). Hence it is unlikely that the food habits or lifestyles of the three groups were substantially different.

DM is one of the major causes of death worldwide. On the other hand, As is an emerging public health problem in many countries. Even small effects of As on the development of DM could produce thousands of additional DM patients. In our study, the hyperglycemic patients included both cases of IGT and DM. IGT patients have 3–9% likelihood per year of developing diabetes (Edelstein et al., 1997). IGT is an asymptomatic form of hyperglycemia that is not treated with pharmaceuticals. However, individuals with IGT may delay the onset of diabetes if they change their lifestyles. The higher percentage of IGT patients (Table 2) in our high As-exposure group indicates that lifestyle change and immediate withdrawal of As-contaminated drinking water or food may help delay DM development. IGT and DM are

well known risks for developing CVDs. IGT and DM are also reported to be associated with poor pregnancy outcomes (Negrato et al., 2012; Yang et al., 2002).

Conclusions

Using three different exposure metrics, this study showed a dose-dependent relationship between chronic As exposure and the risk of hyperglycemia. BMI, age and sex were associated with the risk of hyperglycemia. Females had greater susceptibility to As exposure-related hyperglycemia, than males. Finally, the higher prevalence of IGT in the high As-exposure group, and the confounding effects of demographic variables particularly BMI on IGT indicate that withdrawal of As-contaminated water and food, and lifestyle changes may delay the onset of DM.

Supplementary Material

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Highlights

- Arsenic exposure is dose-dependently associated with the risk of hyperglycemia.
- Females are found to be more susceptible to arsenic-related hyperglycemia.
- Low to moderate doses of water arsenic is related to the risk of hyperglycemia.

Fig. 1.

As exposure and relative risk of hyperglycemia in females (A) and males (B). Results were derived from binary logistic regression analysis. Odds ratios (ORs) were adjusted by age (<40 years used as referent) and BMI (18.5–24.9 used as referent) in females and by age (<40 years used as referent), BMI (18.5–24.9 used as referent) and smoking habit (non-smokers were used as referent) in males. Arsenic levels in water: low (0.03–10.59 $\mu\text{g/L}$, number of cases = 15 for females and 12 for males), medium (12.5–168 $\mu\text{g/L}$, number of cases = 26 for females and 20 for males) and high (168.01–1006.7 $\mu\text{g/L}$, number of cases = 46 for females and 18 for males). Arsenic levels in hair: low (0.01–0.9 $\mu\text{g/L}$; number of cases = 17 for females and 14 for males), medium (0.93–2.95 $\mu\text{g/L}$, number of cases = 30 for females and 23 for males) and high (3–62.02 $\mu\text{g/L}$; number of cases = 40 for females and 13 for males). Arsenic levels in nails: low (0.05–2.16 $\mu\text{g/L}$; number of cases = 17 for females and 15 for males), medium (2.17–6.88 $\mu\text{g/L}$, number of cases = 30 for females and 21 for males) and high (6.89–47.83 $\mu\text{g/L}$; number of cases = 40 for females and 14 for males).

Table 1.

Basic characteristics of the study population based on As levels in their drinking water.

Parameters	All (0.03 – 1006.7 µg/L)	Low (0.03 – 10.59 µg/L)	Moderate (12.5 – 168 µg/L)	High (168.01 – 1006.7 µg/L)	p-value
Study subjects (n)	641	213	214	214	
Sex (n)					
Male	317	108	115	94	
Female	324	105	99	120	
Age (years)^a	36.50 ± 11.36	36.40 ± 11.57	36.96 ± 11.45	36.15 ± 11.1	0.753*
BMI (kg/m²)^a	22.00 ± 3.66	21.65 ± 3.3	22.17 ± 3.74	22.18 ± 3.9	0.233*
Occupation [n, (%)]					
Male					
Farmers	229 (72.2)	86 (79.6)	73 (63.5)	70 (74.5)	
Business	11 (3.5)	4 (3.7)	5 (4.3)	2 (2.1)	
Students	29 (9.1)	10 (9.3)	12 (10.4)	7 (7.4)	0.131 [†]
Workers	27 (8.5)	3 (2.8)	16 (13.9)	8 (8.5)	
‡Others	21 (6.6)	5 (4.6)	9 (7.8)	7 (7.4)	
Female					
Housewives	313(96.6)	103 (98.1)	96 (97)	114 (95)	
Students	5(1.5)	1(1)	1 (1)	3 (2.5)	0.872 [†]
Workers	2 (0.6)	0 (0)	1 (1)	1 (0.8)	
‡Others	4(1.2)	1 (1)	1 (1)	2 (1.7)	
Education [n, (%)]					
No formal education	338 (52.7)	122 (57.3)	99 (46.3)	117 (54.7)	
Primary	188 (29.3)	59 (27.7)	69 (32.2)	60 (28)	0.121*
Secondary	73 (11.4)	18 (8.5)	34 (15.9)	21 (9.8)	
Higher	42 (6.6)	14 (6.6)	12 (5.6)	16 (7.5)	
Income/month (US\$)^a	25.13 ± 10.59	24.16 ± 8.46	25.16 ± 12.15	26.07 ± 10.78	0.174 [†]
Smoking in male [n, (%)]					
Yes	136 (42.9)	40 (37)	58 (50.4)	38 (40.4)	
No	181 (57.1)	68 (63)	57 (49.6)	56 (59.6)	0.110 [†]
Drinking water As (µg/L)^a	134.89 ± 169.98	1.89 ± 2.45	74.87 ± 44.53	327.29 ± 162.28	< 0.001*
Hair As (µg/g)^a	3.7 ± 5.86	0.89 ± 2.51	3.20 ± 4.49	7.01 ± 7.6	< 0.001*
Nail As (µg/g)^{a∞}	7 ± 7.96	1.94 ± 3.2	6.66 ± 6.08	12.43 ± 9.38	< 0.001*

Data were presented as ^aMean ± SD. Abbreviations: As, arsenic; BMI, body mass index. BMI was calculated as body weight (kg) divided by body height squared (m²). **p*- and [†]*p*-values were determined by one-way ANOVA (F-test) and Chi-square test, respectively. ⁺Other occupations included village doctor, teacher, rickshaw puller, driver, banker, and carpenter. [‡]Others included farm worker and laborer. [∞]As concentrations in nail samples (n = 4) were not available.

Table 2.

Comparisons of hyperglycemia (IGT and DM) and other related parameters in the low, moderate and high exposure groups.

Parameters	All (0.03 – 1006.7 μg/L)	Low (0.03 – 10.59 μg/L)	Moderate (12.5 – 168 μg/L)	High (168.01 – 1006.7 μg/L)	<i>p</i> -value
Family history of DM [n, (%)]					
Unknown	120 (18.7)	46 (21.6)	37 (17.3)	37 (17.3)	0.696 [‡]
Yes	13 (2)	3 (1.4)	5 (2.3)	5 (2.3)	
No	508 (79.3)	164 (77)	172 (80.4)	172 (80.4)	
Previously diagnosed DM [n, (%)] [§]					
Yes	10 (1.6)	2 (0.9)	3 (1.4)	5 (2.3)	0.494 [‡]
No	631 (98.4)	211 (99.1)	211 (98.6)	209 (97.7)	
FBG (mg/dL) ^a	95.29 ± 25.84	90.25 ± 16.68	96.72 ± 28.03	98.88 ± 30.04	< 0.01*
2h-BG (mg/dL) ^{a§}	114.11 ± 48.48	103.54 ± 33.87	116.05 ± 52.22	122.81 ± 54.9	< 0.001*
Prevalence of hyperglycemia [%, (n)]	21.4 (137)	12.7 (27)	21.5 (46)	30 (64)	< 0.001 [‡]
Prevalence of IGT [%, (n)]	11.1 (71)	8 (17)	8.9 (19)	16.4 (35)	< 0.01 [‡]
Prevalence of DM [%, (n)]	10.3 (66)	4.7 (10)	12.6 (27)	13.6 (29)	

Data were presented as ^aMean ± SD. Abbreviations: DM, diabetes mellitus, FBG, fasting blood glucose; 2h-BG, 2 hours after blood glucose; IGT, impaired glucose tolerance. **p*- and [†]*p*-values were determined by one-way ANOVA (F-test) and Chi-square test, respectively. [§]Diabetes was diagnosed by physicians (based on physician reports) and the 2h-BG data were missing (n = 10) for these patients.

Table 3.
Effects of As exposure and other relevant variables on the risk for hyperglycemia (IGT and DM)

Variables groups	Hyperglycemia *		IGT #		DM #	
	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
Water As (µg/L)						
Low (0.03 – 10.59)	1 (referent)	-	1 (referent)	-	1 (referent)	-
Moderate (12.5 – 168)						
Before	1.89 (1.12 – 3.17)	< 0.05	1.24 (0.62 – 2.46)	0.543	2.99 (1.41 – 6.36)	< 0.01
After ^a	1.9 (1.11 – 3.24)	< 0.05	1.24 (0.62 – 2.51)	0.545	3 (1.39 – 6.45)	< 0.01
High (168.01 – 1006.7)						
Before	2.94 (1.79 – 4.84)	< 0.001	2.55 (1.38 – 4.74)	< 0.01	3.6 (1.7 – 7.62)	< 0.01
After ^a	2.96 (1.77 – 4.94)	< 0.001	2.56 (1.36 – 4.82)	< 0.01	3.63 (1.7 – 7.76)	< 0.01
Sex ^b	1.95 (1.18 – 3.21)	< 0.01	2.14 (1.1 – 4.17)	< 0.05	1.76 (0.91 – 3.42)	0.093
Age ^c	2.04 (1.35 – 3.1)	< 0.01	2.02 (1.18 – 3.46)	< 0.05	2.06 (1.19 – 3.57)	< 0.05
BMI						
Normal (18.5 – 24.9)	1 (referent)		1 (referent)		1 (referent)	
Low (< 18.5)	0.46 (0.24 – 0.9)	< 0.05	0.38 (0.14 – 1.01)	0.052	0.56 (0.23 – 1.28)	0.163
High (> 24.9)	1.93 (1.22 – 3.04)	< 0.01	2.04 (1.15 – 3.61)	< 0.05	1.82 (0.99 – 3.32)	0.053
Smoking ^d	1.26 (0.67 – 2.36)	0.476	1.3 (0.55 – 3.06)	0.553	1.22 (0.53 – 2.79)	0.643
Hair As (µg/g)						
Low (0.01 – 0.9)	1 (referent)	-	1 (referent)	-	1 (referent)	-
Moderate (0.93 – 2.95)						
Before	1.84 (1.11 – 3.05)	< 0.05	1.49 (0.78 – 2.85)	0.227	2.49 (1.25 – 4.96)	< 0.01
After ^a	1.84 (1.11 – 3.22)	< 0.05	1.43 (0.74 – 2.78)	0.288	2.39 (1.19 – 4.8)	< 0.05
High (3 – 62.02)						
Before	1.94 (1.17 – 3.22)	< 0.05	1.78 (0.95 – 3.32)	0.071	2.04 (1.01 – 4.14)	< 0.05
After ^a	1.94 (1.17 – 3.22)	< 0.05	1.83 (0.97 – 3.48)	0.064	2.09 (1.02 – 4.28)	< 0.05
Sex ^b	2 (1.22 – 3.28)	< 0.05	2.2 (1.13 – 4.26)	< 0.05	1.81 (0.94 – 3.5)	0.078
Age ^c	1.97 (1.3 – 2.98)	< 0.01	1.96 (1.15 – 3.35)	< 0.05	1.97 (1.14 – 3.4)	< 0.05

Variables groups	Hyperglycemia*		IGT [#]		DM [#]	
	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
BMI						
Normal (18.5 – 24.9)	1 (referent)		1 (referent)		1 (referent)	
Low (< 18.5)	0.48 (0.25 – 0.943)	< 0.05	0.39 (0.15 – 1.03)	0.059	0.58 (0.25 – 1.35)	0.203
High (> 24.9)	1.98 (1.26 – 3.11)	< 0.01	2.07 (1.17 – 3.66)	< 0.05	1.87 (1.03 – 3.42)	< 0.05
Smoking ^d	1.22 (0.66 – 2.29)	0.526	1.24 (0.53 – 2.93)	0.617	1.2 (0.53 – 2.75)	0.661
Nail As (µg/g)[∞]						
Low (0.05 – 2.16)	1 (referent)	-	1 (referent)	-	1 (referent)	-
Moderate (2.17 – 6.88)						
Before	1.78 (1.09 – 2.91)	< 0.05	1.12 (0.58 – 2.15)	0.739	2.89 (1.44 – 5.81)	< 0.01
After ^a	1.95 (1.17 – 3.23)	< 0.05	1.23 (0.63 – 2.4)	0.549	3.12 (1.53 – 6.36)	< 0.01
High (6.89 – 47.83)						
Before	1.91 (1.17 – 3.11)	< 0.01	1.76 (0.96 – 3.2)	0.067	2.17 (1.05 – 4.5)	< 0.05
After ^a	1.99 (1.20 – 3.3)	< 0.01	1.83 (0.98 – 3.4)	0.057	2.26 (1.08 – 4.76)	< 0.05
Sex ^b	1.96 (1.19 – 3.22)	< 0.01	2.05 (1.06 – 3.99)	< 0.05	1.87 (0.96 – 3.64)	0.067
Age ^c	2.08 (1.37 – 3.14)	< 0.01	2.05 (1.20 – 3.5)	< 0.01	2.1 (1.21 – 3.63)	< 0.01
BMI						
Normal (18.5–24.9)	1 (referent)	-	1 (referent)		1 (referent)	
Low (< 18.5)	0.48 (0.25 – 0.93)	< 0.05	0.39 (0.15 – 1.03)	0.057	0.57 (0.24 – 1.34)	0.571
High (> 24.9)	2 (1.27 – 3.14)	< 0.01	2.07 (1.17 – 3.66)	< 0.05	1.92 (1.05 – 3.51)	< 0.05
Smoking ^d	1.21 (0.65 – 2.25)	0.556	1.21 (0.51 – 2.84)	0.665	1.21 (0.53 – 2.77)	0.654

^{*}, [#] Results were derived from binary logistic regression analysis and multinomial logistic regression analysis, respectively.

^a Adjusted by age, sex, BMI, and smoking status of the subjects.

^b Males,

^c < 40 years of age,

^d non-smokers were used as reference categories.

[∞] As concentrations in nail samples (n = 4) were not available.

Table 4

Comparisons of the different parameters between males and females.

Parameters	Males	Females	<i>p</i> -value
Prevalence of hyperglycemia [% , (n)]	15.8 (50)	26.9 (87)	< 0.01 [†]
Prevalence of IGT [% , (n)]	7.6 (24)	14.5 (47)	< 0.01 [†]
Prevalence of DM [% , (n)]	8.2 (26)	12.3 (40)	
FBG (mg/dl) ^a	92.39 ± 23.39	98.13 ± 27.77	< 0.01*
2h-BG(mg/dl) ^{a§}	108.39 ± 40.94	119.7 ± 54.35	< 0.01*
Water As (µg/L) ^a	125.93 ± 169.46	143.66 ± 170.3	0.187*
Hair As (µg/g) ^a	3.49 ± 5.13	3.91 ± 6.5	0.362*
Nail As (µg/g) ^{a∞}	5.92 ± 6.47	8.05 ± 9.06	< 0.01*

Data were presented as ^a Mean ± SD. Abbreviation: DM, diabetes mellitus; FBG, fasting blood glucose; 2h-BG, 2 hours after blood glucose; IGT, impaired glucose tolerance. [§]Diabetes was diagnosed by physicians (based on physician reports) and data on 2h-BG were missing (n = 10) for these patients. **p*- and [†]*p*-values were determined by independent sample t-test and chi-square test, respectively. [∞]As concentrations in nail samples (n = 4) were not available.