



Association between chronic arsenic exposure and the characteristic features of asthma

Abu Ebrahim Siddique^{a,1}, Mizanur Rahman^{a,1}, Md. Imam Hossain^{b,1}, Yeasir Karim^a, M.M. Hasibuzzaman^a, Sheta Biswas^a, Md. Shofikul Islam^c, Atiqur Rahman^a, Faruk Hossen^a, Victor Mondal^a, Hasan Ul Banna^a, Nazmul Huda^d, Mostaque Hossain^e, Papia Sultana^f, Farjana Nikkon^a, Zahangir Alam Saud^a, Azizul Haque^g, Keiko Nohara^h, Lian Xinⁱ, Seiichiro Himenoⁱ, Khaled Hossain^{a,*}

^a Department of Biochemistry and Molecular Biology, University of Rajshahi, Rajshahi, 6205, Bangladesh

^b Department of Biotechnology and Genetic Engineering, Mawlana Bhashani Science & Technology University, Tangail, 1902, Bangladesh

^c Department of Applied Nutrition and Food Technology, Islamic University, Kushtia, 7003, Bangladesh

^d Department of Medicine, Rajshahi Medical College, Rajshahi, 6000, Bangladesh

^e Department of Medicine, Shaheed Taj Uddin Ahmad Medical College, Gazipur, Dhaka, Bangladesh

^f Department of Statistics, University of Rajshahi, Rajshahi, 6205, Bangladesh

^g Department of Microbiology and Immunology, Medical University of South Carolina, Charleston, SC, 29425, USA

^h Center for Health and Environmental Risk Research, National Institute for Environmental Studies, Tsukuba, 305-8506, Japan

ⁱ Laboratory of Molecular Nutrition and Toxicology, Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Tokushima, 770-8514, Japan

HIGHLIGHTS

- Chronic arsenic exposure was inversely associated with lung function.
- Arsenic was positively associated with the risk of reversible airway obstruction.
- Arsenic exposure was also associated with asthma-like symptoms and serum IgE levels.
- Serum IgE levels were higher in the subjects with reversible airway obstruction.
- Arsenic exposure-related asthma may be allergic in nature.

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ABSTRACT

Widespread contamination of arsenic (As) has become a global public health concern. Exposure to As causes respiratory complications. Asthma, a major respiratory complication, is increasing worldwide. However, the effect of chronic As exposure on the risk of asthma remains to be clarified. This study aims to examine the associations between As exposure (water, hair and nail As) and the risk of asthma among 842 individuals exposed to a wide range of As concentrations through drinking water in Bangladesh. Subjects' As exposure levels were measured with ICP-MS. Lung function was examined by a handheld spirometer. Characteristic features of asthma were evaluated by bronchodilator-mediated reversibility in airway obstruction and asthma-like symptoms through a structured questionnaire. Total serum immunoglobulin E (sIgE) levels were measured by immunoassay. As exposure metrics showed inverse associations with lung function measures (FEV1, FEV6, and FEV1/FEV6 ratio) and positive associations with the risks of airway obstruction (AO), reversible airway obstruction (RAO), and asthma-like symptoms. The majority of AO patients (70 of 97) were RAO with one or more characteristic symptoms of asthma. Intriguingly, subjects' As exposure levels showed positive associations with total sIgE levels. Total sIgE in RAO patients was significantly ($p < 0.001$) higher than that in the control group. Thus the results revealed that chronic As exposure was associated with the risk of the characteristic features of asthma.

* Corresponding author.

E-mail address: khossain@ru.ac.bd (K. Hossain).

¹ Contributed equally.

Additionally the association between As exposure and subjects' total IgE levels and an elevated level of total IgE in RAO group suggested that As exposure-related asthma might be allergic in nature.

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1. Introduction

Exposure to arsenic (As) via drinking water and food is a global public health problem. As contamination has been found in many countries in the world such as Bangladesh, India, Pakistan, China, Mongolia, Myanmar, Taiwan, Vietnam, Cambodia, Argentina, Chile, Mexico, USA and Hungary. Bangladesh is the most severely As affected country in the world where 61 out of 64 districts (administrative unit) have been contaminated by As affecting more than half of the total population of the country. Groundwater of many areas in Bangladesh contains abnormally high levels of As (Anwar et al., 2002; Rahman et al., 2009; Nordstrom, 2002; Kinniburgh and Smedley, 2001). As crisis has turned into a catastrophe in the country causing thousands of deaths (Chowdhury et al., 2000). As crisis in Bangladesh has been recognized as the "largest mass poisoning of a population in history" (WHO, 2000). Drinking of contaminated groundwater is the main cause of As poisoning in Bangladesh. However, alarmingly high concentrations of As have been found in rice, vegetables and other food items because of the extensive use of groundwater for irrigation purposes. Chronic As exposure is associated with cancer, cardiovascular diseases, and other illnesses (Kuo et al., 2017; Paul et al., 2019).

Several earlier studies showed associations between As exposure and respiratory complications including asthma-like symptoms using drinking water and urinary As concentrations as exposure metrics (Parvez et al., 2013, 2010; 2008; Das et al., 2014; Smith et al., 2006). All these studies reported associations between As exposure and respiratory complications by either determining lung function or evaluating respiratory symptoms, but none of them provided direct evidence of an association between As exposure and a specific pattern of respiratory complications such as asthma (Sanchez et al., 2018).

Asthma, a major form of non-neoplastic respiratory complication, is a significant health burden that increases morbidity and mortality all over the world (Braman, 2006). Asthma is manifested by reversible airway obstruction with symptoms of coughing, wheezing, chest tightness, and shortness of breath. Most of the asthma are allergic in nature and are mediated through IgE (Sunyer et al., 1996; Sears et al., 1991). An individual with an elevated level of total IgE is generally susceptible to developing asthma (Sunyer et al., 1996). Genetic and non-genetic factors such as environmental chemicals are associated with asthma (A joint projects of GINA and GOLD, 2015; Martinez, 1997; D'amato et al., 2005). To our knowledge, no systematic study has been conducted showing the association between environmental As exposure and the risk of asthma. Therefore, this study was designed to examine the association between As exposure and the risk of asthma among the individuals in low- and high-As-exposure areas in Bangladesh.

2. Materials and methods

2.1. Study areas and subjects

Ethics committees of Bangladesh Medical Research Council, Mohakhali, Dhaka-1212 (BMRC/ERC/2007-2010/558) and Institute of Biological Sciences, University of Rajshahi, Bangladesh (104/320/IAMEBBC/IBSc and 661/320/IAMEBBC/IBSc) approved the study

procedures. The study was conducted between February 2016 to March 2019. All study participants gave their written consent. We provided detailed description regarding the selection of study areas and study populations in our previous studies (Paul et al., 2019; Hossain et al., 2012; Karim et al., 2010; Karim et al., 2019). We recruited subjects from highly As-exposed (As-endemic) villages in the northwestern region of Bangladesh. The selected As-endemic villages were Dutpatila, Jajri, Vultie, and Kestopur in the Chaudhaga district, Khemirdia (Bheramara) in the Kushtia district, Marua in the Jessore district, and Kazirpara in the Rajshahi district. Large numbers of individuals with arsenical skin lesions (hyperkeratosis and melanosis) were found in these villages. These lesions were screened by the physicians involved in our research and were confirmed by a dermatologist. We also recruited subjects from a low-exposure (non-endemic) village (Chowkoli) in a northern district (Naogaon) as described elsewhere (Karim et al., 2019; Paul et al., 2019). We asked local residents to convene at a specific location irrespective of their skin symptoms. We primarily enrolled villagers who participated in this study spontaneously. Adults aged 18–60 years who had lived in the study areas continuously for at least the past 5 years were selected for this study. After the screening, a structured interview was conducted through the household visit of each study subjects as described (Karim et al., 2019). Excluded from the study were pregnant and lactating mothers and individuals with a history of drug addiction, recent major surgery, hepatitis B, severe hepatic or renal disease, any kind of malignancy, or severe cardiac disease. We primarily recruited 191 and 664 subjects from non-endemic and As-endemic areas, respectively. Among them, 2 (hepatic diseases = 1 and renal disease = 1) from low- and 11 (hepatic B positive = 3; hepatic diseases = 2; stroke = 1; recent surgical operation = 1; renal disease = 3; pregnancy = 1) from high-As-exposure areas were excluded. After exclusions, the total number of subjects was 842 (189 from non-endemic areas and 653 from As-endemic areas).

2.2. Exposure estimates

In our study, subjects' drinking water, hair and nail As concentrations were used as exposure markers. Subjects' drinking water As concentrations reflect the external exposure levels, while hair and nail As concentrations reflects the internal exposure levels. Hair samples reflect the past several months of As exposure, while nail samples reflects the exposure level from several months to years. Nails are relatively resistant to external contamination. Therefore, nail has been recognized as a reliable marker of prolonged exposure to As (Agahian et al., 1990; Karagas et al., 2000). People in the rural Bangladesh almost completely depend on groundwater (tube well water) for their drinking and household purposes. Each tube well identified by the study participant was pumped for 5 min and then water was collected in an acid-washed container. Each water sample was immediately acidified with nitric acid (final concentration of acid was approx. 2%). Hair and toe nail samples were collected from each study subject as described elsewhere (Rahman et al., 2015). All samples were shipped to the laboratory (Laboratory of Molecular Nutrition and Toxicology, Tokushima Bunri University, Japan) of our collaborator in Japan for the determination of total As concentrations. Hair and nails

samples were cleaned and allowed to dry as described previously (Ali et al., 2010; Chen et al., 1999). Cleaned and dried samples were digested with nitric acid and As concentrations in the water, hair and nails samples were measured with inductively coupled plasma mass spectrometer (ICP-MS) (Agilent 7700x, Japan) with the detection limit of 3.33×10^{-3} ppb. We used "River water" (National Metrology Institute of Japan [NMIJ] CRM 7202-a No. 347, National Institute of Advanced Industrial Science and Technology, Japan) as a certified reference material (CRM) to determine the accuracy of As concentrations in the water samples. The average value (mean $\pm$ SD) of As in the triplicate CRM samples was 1.161 ± 0.082 $\mu\text{g/L}$ (certified value: 1.18 $\mu\text{g/L}$). We used 'human hair' (NCS DC73347a, China National Analysis Center) as a CRM to verify the accuracy of the measurement of the hair and nail samples. The average value (mean $\pm$ SD) of As in the 'human hair' in the triplicate was 0.278 ± 0.006 $\mu\text{g/g}$ (certified value: 0.28 $\mu\text{g/g}$).

2.3. Questionnaire for asthma symptoms

Trained team members including a physician interviewed the study subjects with a questionnaire to assess asthma symptoms. A shortened version of the International Union Against Tuberculosis and Lung Disease Bronchial Symptoms Questionnaire for the determination of asthma symptoms (Ravault and Kauffmann, 2001) was used (Table S1). This questionnaire has good diagnostic rates in different languages (Burney et al., 1989). We translated the questionnaire from English into the local language (Bangla) and had this translation checked by independent translation back into English. Physicians included in the team examined chest sound by stethoscope. Interviewers were instructed to read the questions to the subjects without any more comment than appeared on the questionnaire. The reliability and reproducibility of the questionnaire were tested on 100 randomly selected subjects applying the test-retest method. The reproducibility of the questionnaire was verified by an independent group of individuals in our research team who were blind to the subjects' As levels, to the information obtained through the questionnaire, and to the spirometric data. We found 90% reproducibility of the questionnaire. We made minor modifications to the questionnaire to adapt it to the local context. For example, we used "hard work" instead of "exercise" according to the suggestion of a physician involved in the study. This was because most of the villagers do not exercise regularly. Wheezing, coughing, shortness of breath, and chest tightness have been recognized as the 4 main symptoms of asthma (Bateman et al., 2008). The first question refers to wheezing, the second to chest tightness, the third, fourth, and fifth to shortness of breath, and the sixth and seventh to coughing (Ravault and Kauffmann, 2001; Toren et al., 1993). We considered these 4 symptoms as asthma-like symptoms.

2.4. Spirometric measurement

The subjects' lung function was measured by a portable spirometer (Hi-Checker, Vitalograph, Ireland). Our team members were trained by a physician who was well experienced in spirometric measurement. Forced expiratory volume in 1 s (FEV1)/forced vital capacity (FVC) was used in a clinical setting to define AO. However, the ratio of FEV1/forced expiratory volume in 6 s (FEV6) determined by a portable spirometer can be used as a valid alternative to FEV1/FVC in the diagnosis of airway obstruction, particularly for screening purposes (Vandevorde et al., 2006). The subjects were asked to take a deep breath and then blow into the mouthpiece of the spirometer vigorously and continuously for 6 s. AO was defined as a FEV1/FEV6 ratio <0.73 according to the guidelines of the American Thoracic Society (ATS) and the European

Respiratory Society (ERS) Task Force (Vandevorde et al., 2006; Laszlo, 2006; Bhatt et al., 2014). Ten minutes after the initial spirometry, the subject was offered a bronchodilator reversibility test after the administration of an adequate dose (400 μg) of the bronchodilator salbutamol (Sultolin, Square Pharmaceutical, Bangladesh) by means of an inhaler to which a spacer was attached (Buffels et al., 2004). Subjects with $>12\%$ increases in FEV1 in response to the treatment have been primarily recognized as asthmatic (A joint projects of GINA and GOLD, 2015; Buffels et al., 2004). Spirometry-based reversibility of airway obstruction ($>12\%$ increase in FEV1 after treatment) can't completely confirm a diagnosis of asthma until other criteria are met, such as medical history, symptoms, and exclusion of alternative diagnoses that mimic asthma (Eder et al., 2006). Therefore, we used the term "reversible airway obstruction" (RAO) in the case of asthma primarily defined by spirometry. We found 2 self-reported asthma cases. These subjects were also included in the spirometric measurement; questionnaire and blood analysis.

2.5. Blood collection and measurement of total sIgE

Fasting blood was collected from each study participant and serum was separated as described previously (Karim et al., 2019). Total sIgE levels were determined using a commercially available immunoglobulin E (IgE) ELISA kit (DRG, USA) through a microplate reader (Mikura Ltd., UK). Each standard and serum sample was analyzed in duplicate. Finally average values of the duplicate standard and serum samples were considered for analysis. The intra- and inter-assay coefficients of variation (CVs) were $\leq 10\%$. Out of 842 subjects who participated in lung function testing, 95 were unavailable or declined to provide blood. Furthermore, we could not analyze 43 samples because of the limited number of total sIgE kits and the use of serum samples for other experiments. Two samples showed values below the detectable range of total sIgE and were excluded from the analysis. Finally, the total number of samples for the total sIgE assay was 702.

2.6. Statistical analyses

We used Statistical Packages for Social Sciences (SPSS version 21.0, SPSS, USA) software for data analysis. Age, BMI, As exposure levels, income, spirometry measures and total sIgE levels were compared between As endemic and non-endemic areas by the independent sample *t*-test, whereas occupation, education, skin symptoms, smoking, AO, RAO and asthma-like symptoms were compared by the chi-squared test. Through frequency test, we categorized the total subjects into three groups (low, medium, and high) with equal population size based on their As exposure (water, hair and nail As) levels. We conducted bivariate analyses to estimate the crude effects of primary exposure variables and potential confounding variables (age, sex, BMI, smoking habits, education, occupation and income) on the outcomes adjusting for the effect of village level clustering. We then conducted multivariable analyses that included the primary exposure, primary outcomes and potential confounders in the respective models. All of the potential variables associated (*p*-value <0.1) with the exposure and outcomes in the bivariate analysis were considered for final multivariable models.

Associations between As exposure and spirometric measures were examined by multiple linear regression analysis. Effects of As exposure on AO, RAO and asthma-like symptoms were assessed by Binary logistic regression analyses. The chi-squared test was performed for the comparison of asthma-like symptoms between RAO and control groups. Relationships of As exposure metrics with total sIgE levels were examined through multiple linear regression

analyses. Difference in total sIgE levels between the RAO and control groups were assessed by the independent sample *t*-test.

3. Results

3.1. Characteristics of the study subjects recruited from As-endemic and non-endemic areas

Table 1 shows the characteristics of the study subjects between As-endemic and non-endemic areas. Of the 842 study subjects, 189 were from non-endemic and 653 were from As-endemic areas. Subjects' age, BMI, smoking habits, and socioeconomic criteria (education, income and occupation) were similar in the As-endemic and non-endemic areas. Most of the male subjects were farmers, and the vast majority of the female subjects were housewives. A majority (53.0%) of the study subjects had no formal education. The ranges of subjects' drinking water, hair and nail As concentrations were 0.03–1800 µg/L, 0.03–62 µg/g, and 0.03–47.8 µg/g, respectively. Endemic population had significantly ($p < 0.001$) higher As exposure levels (average concentrations of drinking water, hair and nail As) compared to the non-endemic population.

3.2. Comparison of outcome variables between As-endemic and non-endemic populations

Outcome variables (spirometric measures, AO, RAO, asthma like

symptoms and total sIgE levels) of the As-endemic population were significantly higher than those of the non-endemic population (Table S2).

3.3. Associations of As exposure metrics with spirometric measures

We categorized all the subjects into low (water As range = 0.03–5.30 µg/L; hair As range = 0.03–0.80 µg/g; and nail As range = 0.03–2.02 µg/g), medium (water As range = 5.32–134 µg/L; hair As range = 0.81–2.86 µg/g; and nail As range = 2.03–6.43 µg/g) and high (water As range = 135–1800 µg/L; hair As range = 2.88–62.0 µg/g; and nail As range = 6.44–47.8 µg/g) exposure groups based on the subjects' drinking water, hair and nail As concentrations. Table 2 shows the adjusted associations between As exposure and spirometric measurements. Compared to the groups with low concentrations of As in water and nails, medium and high groups showed significant associations with FEV1 and FEV6. Only group with high concentration of hair As shows significant association with FEV1 compared to the low group of hair As. All exposure groups with high concentration of As showed significant associations with FEV1/FEV6 compared to the groups with low concentrations of As. Among other variables, age ($p = 0.002$ in water, $p = 0.003$ in hair and $p = 0.001$ in nail As for FEV1; and $p < 0.001$ for FEV1/FEV6 across all exposure metrics) and BMI ($p = 0.014$ in water, $p = 0.039$ in hair and $p = 0.048$ in nail for FEV1/FEV6) showed significant associations with the spirometric measurements.

Table 1
Characteristics of the study subjects recruited from As-endemic and non-endemic areas.

Parameters	All	Non-endemic	Endemic	<i>p</i> -value
Subjects (n)	842	189	653	
Sex [n (%)]				
Male	430 (51.1)	85 (45.0)	345 (52.8)	
Female	412 (48.9)	104 (55.0)	308 (47.2)	
Age, (min - max) yrs ^a	36.3 ± 11.5 (18–60)	35.4 ± 11.6 (18–60)	36.5 ± 11.5 (18–60)	0.255*
BMI, kg/m ^{2a}	21.6 ± 3.53	21.3 ± 3.15	21.6 ± 3.63	0.228*
Occupation [n (%)]				
Males:				
Farmers	319 (74.2)	66 (77.6)	253 (73.3)	0.290 [†]
Students	45 (10.5)	12 (14.1)	33 (9.60)	
Workers	30 (7.00)	1 (1.20)	29 (8.40)	
Business	10 (2.30)	1 (1.20)	9 (2.60)	
*Others	26 (6.00)	5 (5.90)	21 (6.10)	
Females:				
Housewives	392 (95.1)	99 (95.2)	293 (95.1)	0.707 [†]
Students	10 (2.40)	2 (1.90)	8 (2.60)	
Workers	6 (1.50)	3 (2.90)	3 (1.00)	
[†] Others	4 (1.00)	0 (0)	4 (1.30)	
Education [n, (%)]				
No formal education	446 (53.0)	103 (54.5)	343 (52.5)	0.306 [†]
Primary	253 (30.0)	60 (31.7)	193 (29.6)	
Secondary	94 (11.2)	17 (9.0)	77 (11.8)	
Higher	49 (5.80)	9 (4.80)	40 (6.10)	
Income/month, USD\$ ^a	25.8 ± 9.38	25.7 ± 8.08	25.9 ± 9.73	0.850*
Smoking in males [n, (%)]				
Yes	158 (36.7)	29 (34.1)	129 (37.4)	0.576 [†]
No	272 (63.3)	56 (65.9)	216 (62.6)	
Skin symptoms [n, (%)]				
Yes	332 (39.4)	0 (0)	332 (50.8)	<0.001 [†]
No	510 (60.6)	189 (100)	321 (49.2)	
Drinking water As, µg/L ^a	128 ± 191	2.58 ± 3.77	165 ± 203	<0.001*
Range (min - max)	(0.03–1800)	(0.03–25.4)	(0.08–1800)	
Hair As, µg/g ^a	3.35 ± 5.21	0.36 ± 0.30	4.22 ± 5.63	<0.001*
Range (min - max)	(0.03–62.0)	(0.03–1.87)	(0.05–62.0)	
Nail As, µg/g ^a	6.48 ± 7.27	1.33 ± 1.34	7.97 ± 7.60	<0.001*
Range (min - max)	(0.03–47.8)	(0.03–8.13)	(0.11–47.8)	

As: Arsenic, BMI: body mass index. ^aPresented as mean ± SD. BMI was calculated as body weight (kg) divided by body height squared (m²). **p*- and [†]*p*-values were from the independent sample *t*-test and chi-squared test, respectively. *Others were teacher, village doctor, rickshaw puller, driver, day-laborer and banker, and [†]others were teacher, farm worker and village doctor.

Table 2
Associations of As exposure with spirometric measures.

Exposure metrics	Dependent variables					
	FEV1†		FEV6†		FEV1/FEV6†	
	β ^a (95% CI)	p-value	β ^a (95% CI)	p-value	β ^a (95% CI)	p-value
Water As (μg/L)						
Low (0.03–5.30)	1 (referent)	—	1 (referent)	—	1 (referent)	—
Medium (5.32–134)	−0.123 (−0.219, −0.028)	0.011	−0.122 (−0.211, −0.033)	0.007	−0.018 (−0.040, 0.005)	0.123
High (135–1800)	−0.203 (−0.334, −0.071)	0.003	−0.169 (−0.309, −0.028)	0.019	−0.055 (−0.082, −0.028)	<0.001
Hair As (μg/g)						
Low (0.03–0.80)	1 (referent)	—	1 (referent)	—	1 (referent)	—
Medium (0.81–2.86)	−0.111 (−0.240, 0.019)	0.093	−0.081 (−0.179, 0.018)	0.110	−0.023 (−0.047, −0.000)	0.049
High (2.88–62.0)	−0.185 (−0.314, −0.057)	0.005	−0.133 (−0.271, 0.005)	0.058	−0.055 (−0.087, −0.024)	0.001
Nail As (μg/g)						
Low (0.03–2.02)	1 (referent)	—	1 (referent)	—	1 (referent)	—
Medium (2.03–6.43)	−0.109 (−0.181, −0.036)	0.004	−0.142 (−0.249, −0.034)	0.010	−0.009 (−0.026, 0.009)	0.334
High (6.44–47.8)	−0.185 (−0.247, −0.124)	<0.001	−0.199 (−0.312, −0.086)	0.001	−0.038 (−0.076, −0.000)	0.049

Results were derived from multivariate linear regression analyses. Abbreviations: FEV1, forced expiratory volume in 1 s; FEV6, forced expiratory volume in 6 s. Low group was used as a referent. Sample number (n) in water As groups: Low, n = 279; Medium, n = 281 and High, n = 282. Sample number (n) in hair As groups: Low, n = 279; Medium, n = 279 and High, n = 284. Sample number (n) in nails As groups: Low, n = 278; Medium, n = 278 and High, n = 286. ^a Adjusted for age, sex, BMI, smoking habits, income, education and occupation. †Adjusting for clustering village.

3.4. Associations of As exposure and the risks of AO and RAO

Table 3 shows the adjusted associations of As exposure with the risks of AO and RAO. The adjusted ORs of AO in the medium and high exposure groups were approximately 2-fold ($p = 0.042$) and 4-fold ($p < 0.001$), respectively, that of the low-exposure water As group. Adjusted ORs of AO in the medium and high exposure groups of hair As were approximately 2 fold ($p = 0.033$) and 4-fold ($p = 0.002$), while adjusted ORs in the high exposure groups of nail As were approximately 2 fold ($p = 0.016$), respectively, that of the low-exposure hair and nail As groups. The adjusted ORs of RAO in the medium and high groups were approximately 2-fold ($p = 0.046$) and 4-fold ($p < 0.001$), respectively, that of the low-exposure water As group. The adjusted ORs of RAO were approximately 3-fold ($p = 0.001$) in the medium and 7-fold ($p < 0.001$) in the high group that of the low group of hair As exposure. The adjusted ORs were approximately 2-fold ($p < 0.001$) and 4-fold ($p = 0.001$) in the medium and high groups, respectively, that of the low group in nail As concentrations. Among the variables considered, age showed significant ($p < 0.001$ across all exposure metrics) confounding effect on AO. Age also showed confounding effect on RAO with boarder line significant values ($p = 0.048$ for water, $p = 0.068$ for hair and $p = 0.058$ for nail As). Additionally, BMI

showed significant ($p = 0.024$ for water, $p = 0.018$ for hair and $p = 0.022$ for nail As) confounding effect on RAO.

3.5. As exposure and risk of asthma-like symptoms

Table 4 shows the adjusted associations between As exposure and the risk of asthma-like symptoms. The adjusted ORs for 1 through 4 asthma-like symptoms in the groups with exposure to high concentrations of As were significantly higher than in the groups with low concentrations of As across all the exposure metrics. Among the other variables, only age showed significant ($p < 0.001$) ORs for the symptoms across all the exposure metrics. Additionally, the prevalences of 1 (100%), 2 (97.1%), 3 (87.1%), and 4 (77.1%) asthma-like symptoms were much higher in RAO patients than in the control group (Table S3).

3.6. Associations of As exposure with total sIgE levels

Table 5 shows the adjusted relationship of As exposure metrics with total sIgE levels. The subjects with medium and high concentrations of As showed positive associations with total sIgE levels. Among the other variables, income and occupation showed significant effects on total sIgE levels.

Table 3
As exposure and the risks of AO and RAO.

Exposure metrics	AO†			RAO†		
	No. of cases	OR ^a (95% CI)	p-value	No. of cases	OR ^a (95% CI)	p-value
Water As (μg/L)						
Low (0.03–5.30)	14	1 (referent)	—	10	1 (referent)	—
Medium (5.32–134)	29	1.94 (1.03, 3.65)	0.042	19	1.76 (1.01, 3.06)	0.046
High (135–1800)	54	3.65 (1.84, 7.24)	<0.001	41	3.81 (1.85, 7.85)	<0.001
Hair As (μg/g)						
Low (0.03–0.80)	12	1 (referent)	—	6	1 (referent)	—
Medium (0.81–2.86)	32	2.23 (1.07, 4.68)	0.033	22	3.40 (1.67, 6.74)	0.001
High (2.88–62.0)	53	3.69 (1.60, 8.51)	0.002	42	6.87 (2.78, 17.0)	<0.001
Nail As (μg/g)						
Low (0.03–2.02)	17	1 (referent)	—	9	1 (referent)	—
Medium (2.03–6.43)	27	1.27 (0.81, 1.97)	0.295	19	1.86 (1.36, 2.54)	<0.001
High (6.44–47.8)	53	2.36 (1.17, 4.73)	0.016	42	3.85 (1.72, 8.65)	0.001

Results were derived from binary logistic regression analysis. OR, odds ratio; CI, confidence interval. Low group was used as a referent. Number of case indicates the number of subjects with AO or RAO. Sample number (n) in water As groups: Low, n = 279; Medium, n = 281 and High, n = 282. Sample number (n) in hair As groups: Low, n = 279; Medium, n = 279 and High, n = 284. Sample number (n) in nails As groups: Low, n = 278; Medium, n = 278 and High, n = 286. ^a Adjusted by age, sex, BMI, smoking habits, income, education and occupation. †Adjusting for clustering village.

Table 4
As exposure and the risk of asthma-like symptoms.

Parameters	Presence of 1 symptom†			Presence of 2 symptoms†			Presence of 3 symptoms†			Presence of 4 symptoms†		
	No. of cases	OR ^a (95% CI)	p-value	No. of cases	OR ^a (95% CI)	p-value	No. of cases	OR ^a (95% CI)	p-value	No. of cases	OR ^a (95% CI)	p-value
Water As (µg/L)												
Low (0.03–5.30)	118	1 (referent)	—	66	1 (referent)	—	27	1 (referent)	—	12	1 (referent)	—
Medium (5.32–134)	141	1.20 (0.78, 1.85)	0.401	87	1.34 (1.04, 1.72)	0.025	45	1.10 (0.74, 1.64)	0.629	26	2.04 (1.06, 3.92)	0.033
High (135–1800)	198	2.90 (1.90, 4.43)	<0.001	128	2.45 (1.94, 3.10)	<0.001	73	1.69 (1.42, 2.03)	<0.001	49	3.69 (2.23, 6.11)	<0.001
Hair As (µg/g)												
Low (0.03–0.80)	116	1 (referent)	—	66	1 (referent)	—	20	1 (referent)	—	10	1 (referent)	—
Medium (0.81–2.86)	156	1.45 (0.90, 2.34)	0.125	97	1.37 (0.83, 2.25)	0.215	57	1.89 (0.86, 4.13)	0.113	29	2.50 (1.07, 5.87)	0.035
High (2.88–62.0)	185	2.03 (1.32, 3.11)	0.001	118	1.78 (1.35, 2.35)	<0.001	68	2.05 (1.41, 2.96)	<0.001	48	4.12 (2.24, 7.60)	<0.001
Nail As (µg/g)												
Low (0.03–2.02)	120	1 (referent)	—	64	1 (referent)	—	29	1 (referent)	—	14	1 (referent)	—
Medium (2.03–6.43)	154	1.23 (0.73, 2.05)	0.441	102	1.59 (0.99, 2.56)	0.056	52	1.10 (0.71, 1.70)	0.666	26	1.40 (0.83, 2.38)	0.209
High (6.44–47.8)	183	1.94 (1.53, 2.44)	<0.001	115	2.14 (1.70, 2.71)	<0.001	64	1.52 (1.10, 2.12)	0.012	47	2.64 (1.81, 3.83)	<0.001

Results were derived from binary logistic regression analysis. OR, odds ratio; CI, confidence interval. Low group was used as a referent. Number of case indicates the number of subjects with asthma-like symptom(s). Sample number (n) in water As groups: Low, n = 279; Medium, n = 281 and High, n = 282. Sample number (n) in hair As groups: Low, n = 279; Medium, n = 279 and High, n = 284. Sample number (n) in nails As groups: Low, n = 278; Medium, n = 278 and High, n = 286. ^a Adjusted by age, sex, BMI, smoking habits, income, education and occupation. †Adjusting for clustering village. Wheezing, coughs, shortness of breath and chest tightness were considered the major asthma-like symptoms. One symptom = presence of any 1 asthma-like symptom; Two symptoms = presence of any 2 asthma-like symptoms; Three symptoms = presence of any 3 asthma-like symptoms; Four symptoms = presence of all asthma-like symptoms.

Table 5
Associations of As exposure with total sIgE levels.

Independent variables	sIgE†	
	β (95% CI)	p-value
Water As		
Low (0.03–5.30)	1 (referent)	—
Medium (5.32–134)	0.127 (0.023, 0.231)	0.016
High (135–1800)	0.237 (0.109, 0.364)	<0.001
Hair As		
Low (0.03–0.80)	1 (referent)	—
Medium (0.81–2.86)	0.234 (0.156, 0.312)	<0.001
High (2.88–62.0)	0.247 (0.151, 0.343)	<0.001
Nail As		
Low (0.03–2.02)	1 (referent)	—
Medium (2.03–6.43)	0.137 (–0.014, 0.288)	0.060
High (6.44–47.8)	0.215 (0.081, 0.350)	0.002

Results were derived from univariate linear regression analyses. sIgE, serum immunoglobulin E. Log₁₀-transformed values of total sIgE and As concentrations were used. Low group was used as a referent. Adjusted by age, sex, BMI, smoking habits, income, education, occupation and skin symptoms. †Adjusting for clustering village.

3.7. Comparisons of total sIgE levels between RAO and control groups

Fig. 1 compares the total sIgE levels (geometrical mean) between the RAO and control (without RAO) groups. The RAO group showed significantly ($p < 0.001$) higher levels of total sIgE than the control group.

4. Discussion

This study shows for the first time that chronic As exposure is positively associated with characteristic features of asthma, including reversibility in airway obstruction, asthma-like symptoms, and total sIgE levels among human subjects exposed to a wide range of As concentrations through drinking water.

Our previous studies revealed that the study subjects' external

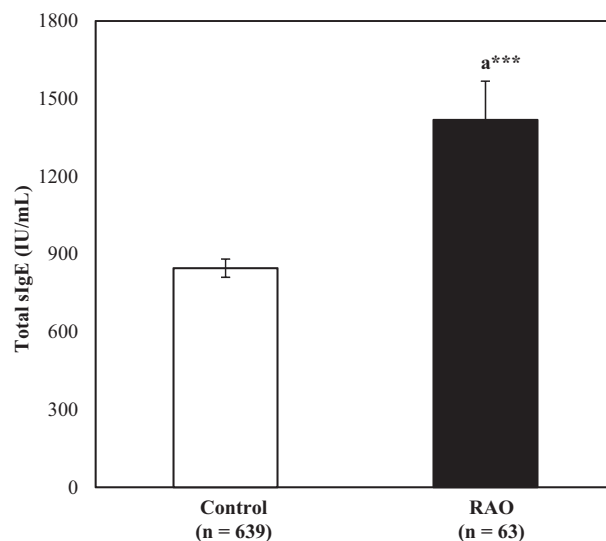


Fig. 1. Comparison of total sIgE levels between control (without RAO) and RAO groups. Data are presented as mean $\pm$ SE. Control group indicates all the study subjects from both As-endemic and non-endemic areas except the study subjects with reversible airway obstruction (RAO). 'a' significant difference from control group. *** $p < 0.001$. p -values were from the independent sample t -test.

exposure marker (drinking water As) was significantly correlated with internal exposure markers (hair and nail As) (Hossain et al., 2019; Islam et al., 2011; Karim et al., 2010). Results of the present study also showed that drinking water As concentrations were significantly correlated with subjects' hair and nail As concentrations (data not shown) suggesting that drinking water is a major source of human exposure to As in Bangladesh. The average levels of drinking water and nail As concentrations of our subjects in As-endemic areas were 165 µg/L and 7.97 µg/g, respectively (Table 1). These results were similar with some other studies conducted in Bangladesh (Pan et al., 2013; Parvez et al., 2008; Islam et al., 2012).

However, drinking water As levels and human exposure to As through drinking water vary from one location to another location or from one country to another country. Groundwater of many areas in Bangladesh contains abnormally high levels of As (Anawar et al., 2002; Rahman et al., 2009; Nordstrom, 2002; Kinniburgh and Smedley, 2001). In agreement with these studies, we also found that some tube well water in our endemic areas contained abnormally high concentrations of As (approx. 1% and 4% study subjects' drinking water As level were >1000 µg/L and >500 µg/L, respectively).

We found that As exposure metrics were inversely and dose-dependently associated with FEV1, FEV6, and the FEV1/FEV6 ratio (Table 2). These results agree with those of the previous studies (Parvez et al., 2013, 2008 and 2010) suggesting that a positive association exists between As exposure and impaired lung function. We also found that As exposure was dose-dependently associated with the risk of AO (Table 3). Based on the levels of reversibility in airway obstruction (>12% increase in FEV1) in response to the use of a bronchodilator, we found that a large portion of the AO (70 of 97) patients were RAO (Table 3). Intriguingly, subjects' As exposure levels conferred the risk of RAO dose-dependently (Table 3).

There is no gold standard for accurately identifying asthma (A joint projects of GINA and GOLD, 2015). Clinically, asthma can be diagnosed through several approaches, including reversibility of airway obstruction, symptoms, physical examination, and exclusion of alternative diagnoses that mimic asthma (Eder et al., 2006). To confirm the spirometry-based identification of RAO patients as asthmatic in nature, we assessed asthma-like symptoms among all of the study subjects through a questionnaire (Table S1). The four most common symptoms of asthma are thought to be wheezing, coughing, chest tightness, and shortness of breath (Bateman et al., 2008). All three As exposure metrics showed dose-dependent associations with asthma-like symptoms (Table 4). Additionally, the prevalence of asthma-like symptoms was much higher in RAO patients than in the control (without RAO) group (Table S3).

IgE is a key element in the pathogenesis of allergic asthma. A number of studies have shown an association between the prevalence of asthma and elevated total sIgE (Sunyer et al., 1996; Sears et al., 1991; Platts-Mills et al., 2016). Elevated levels of total sIgE predict the development of asthma (Sunyer et al., 1996). We have found that all three As exposure metrics were positively correlated with total sIgE levels in a dose-dependent fashion (Table 5). We also found that RAO patients had significantly ($p < 0.001$) higher levels of total sIgE than the control group (Fig. 1). Our findings suggested that IgE-sensitive pathways might be implicated in the development of As-related characteristic features of asthma. In Fig. 1, the control group included many subjects from As-endemic areas who were exposed to high levels of As. This is the reason for relatively higher level of total sIgE in this group compared to the normal value (maximum 150 IU/mL). In fact, the control group included all the subjects ($n = 639$) except the subjects who had RAO ($n = 63$). Moreover, the average level of total sIgE (344 IU/mL) in non-endemic population in our study was also higher than the normal value (Table S2). This value was close to the previous study that was conducted on the population in India (Papiha et al., 1980). In this paper, the authors stated that "IgE level lying between 400 and 500 IU/mL should be taken as a normal standard for Indian populations". In another study (Sharma et al., 2006) conducted also on Indian population, the range of total sIgE levels in healthy individuals was 10–725 IU/mL. Bangladesh shares common border and almost same socio-cultural background with India. Recently Rahman et al. (2016) conducted a study in Bangladesh and the range of total sIgE of the healthy control was 1.2–1045 IU/mL (average value: 224 IU/mL). All these papers justify the high levels of total sIgE in non-endemic population observed in our study.

The results of the present study did not clarify how As exposure increases the risk of asthma. It has been reported that increased vascular permeability due to changes in the airway vascular architecture by environmental agents is associated with the risk of asthma (D'amato et al., 2005). Therefore, one possible mechanism could be that a high concentration (>50 µg/L) of As changes the airway microvasculature, resulting in increased vascular permeability for environmental allergens. Our previous studies on the associations of As exposure with blood biomarkers of vascular dysfunction, such as VEGF, ICAM-1, VCAM-1, MMP-2 and -9, soluble thrombomodulin, and big endothelin-1, provided strong evidence that changes in the microvascular architecture are caused by chronic exposure to As (Hossain et al., 2012; Hasibuzzaman et al., 2017; Rahman et al., 2015; Karim et al., 2013; Islam et al., 2015).

We showed associations using three exposure metrics (drinking water, hair, and nail As concentrations), and all these metrics were strongly correlated with each other, as described previously (Islam et al., 2011; Ali et al., 2010). Strong interrelationships between an ecological marker (water As) and individual exposure markers (hair and nail As) and similar associations of asthma features across these three exposure metrics suggest that possibilities of exposure-based misclassification are minimal. An understanding of the dose-response relationship of any toxic chemical is important to accurately predict the health risk of exposure. Therefore, one of the major strengths of this study was the dose-dependent relationship between As exposure and the risk of RAO, asthma-like symptoms and total sIgE levels in a good number of subjects with wide variations in As exposure levels.

We used a handheld spirometer (Hi-Checker) to identify airway obstruction. A handheld spirometer is less expensive and easily portable. It is especially useful for screening airway obstructions in epidemiological settings where diagnostic facilities are limited or unavailable. Hi-Checker is a good alternative to conventional spirometry for identifying airway obstruction (Yoshimoto et al., 2014; Nishimura et al., 2011) although the measurement may not be completely identical to that of conventional spirometry because of random measurement errors.

Environmental, occupational, and many other factors are associated with asthma. We selected subjects from rural Bangladesh who were almost entirely homogeneous. Demographic and socio-economic characteristics of the subjects between As-endemic and non-endemic areas were similar (Table 1). Based on the bivariate analyses, we considered age, sex, BMI, smoking habit, education, occupations and monthly income as confounding variables in our all regression models that were adjusted for clustering of villages. Age was found to be positively associated with the risk of spirometric measures, AO, RAO and asthma symptoms. It has been reported that factors prevalent in childhood may predict asthma in adulthood (Rasmussen et al., 2002). The association of age with the risk of RAO and asthma-like symptoms observed in our study argues that, as a prevailing factor, prolonged exposure to As may lead to future development of asthma. In contrast to age, BMI showed a significant negative interaction with RAO. It has been reported that underweight and overweight people are prone to develop asthma (Von Mutius et al., 2001; Celedón et al., 2001). The average BMI of our study subjects was 21.6 ± 3.53 (Table 1); approximately 22% of total subjects and 30% of RAO patients were below (<18.5) the normal range of BMI (data not shown). Such a large number of subjects with low BMI might be the cause of the significant negative association with the risk of RAO.

Most of our subjects were in a lower income group, their average BMI was close to the lower end of the normal range, and approximately 51% of endemic population had skin symptoms (Table 1). All these features may limit the generalizability of the

present findings. Because of the cross-sectional nature of the study design, the associations observed in our study may not exactly explain the cause-effect relationship. A longitudinal study is required in future to validate the results observed in our study. The prevalence of asthma is increasing globally. It decreases quality of life and creates both health and economic burdens on the patient, and can even cause death. The risk factors of asthma have not yet been completely understood. As exposure is also a major threat to public health worldwide. Therefore, the findings of our study have important implications for public health.

5. Conclusions

In this study, As exposure was dose-dependently associated with the risk of RAO and characteristic symptoms of asthma. Additionally, As exposure-related total IgE levels were higher in RAO patients, suggesting that IgE-sensitive pathways might be implicated in As exposure-related asthma. Thus, the findings of our study reveal that chronic As exposure is linked to the risk of asthma, warranting immediate attention from policymakers and health professionals in the countries where As poisoning has become a serious concern to public health.

Authors' contribution

Khaled Hossain, Seiichiro Himeno, and Keiko Nohara planned the study and were involved in funding acquisition. Khaled Hossain oversaw the research, including study design, implementation, data collection and interpretation and drafting the manuscript. Abu Ebrahim Siddique, Mizanur Rahman, Md. Imam Hossain, Yeasir Karim, M. M. Hasibuzzaman, Lian Xin and Sheta Biswas carried out the experiments. Nazmul Huda, Mostaque Hossain, Farjana Nikkon and Zahangir Alam Saud planned the experiments and coordinated sample and data collections. Atiqur Rahman, Md. Shofikul Islam, Faruk Hossen, Victor Mondal and Hasan Ul Banna recruited the participants and prepared the samples. Abu Ebrahim Siddique, Mizanur Rahman, Md. Imam Hossain prepared manuscript. Azizul Haque was involved in the critical review of the manuscript and funding acquisition. Papia Sultana performed statistical analysis.

Declaration of competing interest

The authors declare that there is no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.chemosphere.2019.125790>.

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Table S1 Questions About Asthma and Asthma-like Symptoms in the Questionnaire from the International Union Against Tuberculosis and Lung Disease, the IUATLD (1986) Bronchial Symptoms Questionnaire

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1. Have you had wheezing or whistling in your chest, at any time in the last 12 months?
 2. Have you woken up with a feeling of tightness in your chest first thing in the morning at any time in the last 12 months?
 3. Have you at any time in the last 12 months had an attack of shortness of breath that came on during the day when you were not doing anything strenuous?
 4. Have you had an attack of shortness of breath that came on after you stopped hard work at any time in the last 12 months?
 5. Have you at any time in the last 12 months been woken at night by an attack of shortness of breath?
 6. Have you at any time in the last 12 months been woken at night by an attack of coughing?
 7. Do you usually cough first thing in the morning?
 8. Do you usually bring up phlegm from your chest first thing in the morning?
 9. Have you brought up phlegm from your chest like this most mornings for at least 3 months each year?
 10. Which of the following statements best describes your breathing?
 - a. I never or only rarely get trouble with my breathing
 - b. I get regular trouble with my breathing, but it always gets completely better
 - c. My breathing is never quite right
 11. When you are in a dusty part of the house or with animals (for instance dogs, cats, or horses) or near feathers (including pillows, quilts and eiderdown) do you ever:
 - a. Get a feeling of tightness in your chest?
 - b. Start to feel short of breath?
 12. Have you ever had asthma?
 13. Have you had an attack of asthma at any time in the last 12 months?
 14. Are you currently taking any medicines, (including inhalers, aerosols or tablets) for asthma?
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Table S2 Comparison of outcome variables between the subjects in arsenic-endemic and non-endemic areas

Outcome variables	Non-endemic	Endemic	<i>p</i> -value
FEV1, (L) ^a	1.64 ± 0.58	1.42 ± 0.58	<0.001*
FEV6, (L) ^a	1.68 ± 0.62	1.54 ± 0.64	0.005*
FEV1/FEV6 ^a	0.98 ± 0.06	0.93 ± 0.15	<0.001*
AO [n (%)]	5 (2.60)	92 (14.1)	<0.001 [†]
RAO [n (%)]	3 (1.60)	67 (10.3)	<0.01 [†]
Presence of 1 symptom [n (%)]	72 (38.1)	385 (59.0)	<0.001 [†]
Presence of 2 symptoms [n (%)]	37 (19.6)	244 (37.4)	<0.001 [†]
Presence of 3 symptoms [n (%)]	6 (3.20)	138 (21.2)	<0.001 [†]
Presence of 4 symptoms [n (%)]	4 (2.10)	83 (12.7)	<0.001 [†]
sIgE levels, (IU/mL) ^b	344 (3.30)	569 (3.11)	<0.001*

^aData are presented as mean ± SD. ^bData are presented as geometric mean (GSD). Abbreviations: FEV1, forced expiratory volume in one second; FEV6, forced expiratory volume in 6 seconds; RAO, reversible airway obstruction; AO, airway obstruction. Wheezing, coughs, shortness of breath and chest tightness were considered the major asthma-like symptoms. One symptom = presence of any 1 asthma-like symptom; Two symptoms = presence of any 2 asthma-like symptoms; Three symptoms = presence of any 3 asthma-like symptoms; Four symptoms = presence of all asthma-like symptoms. **p*- and [†]*p*-values were from the independent sample t-test and chi-squared test, respectively.

Table S3 Comparison of asthma-like symptoms between RAO and control groups

Asthma-like symptoms	Control [n (%)]	RAO [n (%)]	<i>p</i> -value
Presence of 1 symptom			
Yes	387 (50.10)	70 (100)	<0.001 [†]
No	385 (49.0)	0 (0)	
Presence of 2 symptoms			
Yes	213 (27.6)	68 (97.1)	<0.001 [†]
No	559 (72.4)	2 (2.9)	
Presence of 3 symptoms			
Yes	84 (10.9)	61 (87.1)	<0.001 [†]
No	688 (89.1)	9 (12.9)	
Presence of 4 symptoms			
Yes	33 (4.30)	54 (77.1)	<0.001 [†]
No	739 (95.7)	16 (22.9)	

[†] *p*-values were from the chi-squared test. Control group indicates all the study subjects from both arsenic-endemic and non-endemic areas except the study subjects with reversible airway obstruction (RAO). Wheezing, coughs, shortness of breath, and chest tightness were considered major asthma-like symptoms. One symptom = presence of any 1 asthma-like symptoms; Two symptoms = presence of any 2 asthma-like symptoms; Three symptoms = presence of any 3 asthma-like symptoms; Four symptoms = presence of all asthma-like symptoms.