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Non-malignant diseases associated with environmental arsenic exposure in Taiwan, Chile, and Bangladesh

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Abstract

Extensive epidemiological studies in Taiwan, Chile, and Bangladesh have shown that chronic arsenic exposure is associated with increased incidence and prevalence of skin lesions, cancers, as well as non-malignant disorders such as hypertension, diabetes mellitus, cardiovascular diseases, and respiratory diseases. However, the underlying mechanisms of how arsenic facilitates vascular disorders and diabetes remained unclear. To understand biochemical mechanisms related to arsenic-induced non-malignant diseases, we have examined the relationships between disease-related blood biomarkers and arsenic exposure levels in the arsenic-contaminated area in the western region of Bangladesh. In this review, we presented a summary of the findings of our studies in Bangladesh and discussed their significances in comparison with epidemiological observations in Taiwan and Chile. We have identified arsenic-induced changes in the biomarkers reflecting oxidative stress, inflammation, dyslipidemia, vasoconstriction, monocyte adhesion, and angiogenesis, all related to promoting atherosclerosis and hypertension. Determinations of glucose intolerance, serum insulin and creatinine, and lean body mass suggested a potential role of arsenic-induced skeletal muscle atrophy and its association with insulin resistance. Respiratory function tests and measurements of serum immunoglobulin E and cytokines showed that arsenic-induced T helper 2 (Th2)-dominant immunomodulation might predispose to developing Th2-high type asthma. Thus, the investigation of disease-related biomarkers allowed us to provide novel insight into biochemical mechanisms of arsenic-associated increases in non-malignant diseases.

Key words: arsenic, Bangladesh, atherosclerosis, diabetes mellitus, asthma, cardiovascular disease, biomarker

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Abbreviations:

BFD, black foot disease; BMI, body mass index; COPD, chronic obstructive pulmonary disease; CRP, C-reactive protein; CVD, cardiovascular disease; DBP, diastolic blood pressure; DM, diabetes mellitus; FBG, fasting blood glucose; FEV1, forced expiratory volume in one second; FEV6, forced expiratory volume in six second; FVC, forced vital capacity; GTT, glucose tolerance test; HDL, high-density lipoprotein; HEALS, the Health Effects of Arsenic Logitudinal Study; HOMA-IR, homeostasis model assessment-insulin resistance; ICAM-1, intercellular adhesion

molecule-1; IFN-γ, interferron-γ; IgE, immunoglobulin E; IGT, impaired glucose tolerance; IL, interleukin; LBM, lean body mass; LDL, low-density lipoprotein; LOX-1, lectin-like oxidized LDL receptor-1; MMP, matrix metalloprotease; NO, nitric oxide; NOX2, NADPH oxidase 2; OR, Odds ratio; oxLDL, oxidized low-density lipoprotein; RAO, reversible airway obstruction; senescence-associated secretory phenotypes, SASP; SBP, systolic blood pressure; SMR, standardized mortality ratios; sTM, soluble thrombomodulin; Th2, T helper 2; TNF-α, tumor necrosis factor-α; VCAM-1, vascular cell adhesion molecule-1; VEGF, vascular endothelial growth factor

1. Introduction

Since ancient times, arsenic has long been known as a poison and used for purposes such as assassination because of its colorless, tasteless, and odorless nature. Today, however, arsenic has become an environmental pollutant harming people globally. Arsenic pollution occurs mainly from groundwater naturally contaminated by the arsenic leached from the earth's crust, contrasting with other toxic metals, such as cadmium, mercury, and lead, originating mainly from mines and industrial products and wastes. Health hazards due to groundwater-derived arsenic have been reported in Taiwan, Bangladesh, India, China, and Mongolia in Asia and Chile, Argentina, and Mexico in Central and South America. It is estimated that more than 200 million people worldwide are exposed to arsenic from drinking water containing arsenic over 10 μg/L, the permissible limit set by WHO [1].

Health hazards caused by chronic arsenic exposure include skin lesions, such as hyperpigmentation, depigmentation and keratosis, and cancers of the skin, liver, lung, bladder, and kidneys [2,3]. Chronic arsenic exposure is also linked to non-malignant diseases such as cardiovascular diseases (CVDs) [4], diabetes mellitus (DM) [5], and respiratory diseases [6]. However, the causal relationships between arsenic exposure and these common diseases and the underlying mechanisms have yet to be clarified because many confounding factors are involved in these lifestyle-related diseases.

Although arsenic contamination is a global issue, Taiwan, Chile, and Bangladesh have suffered from particularly severe arsenic-related health problems, including non-malignant diseases, each having different modes of arsenic exposure and disease development. In the southwestern region in Taiwan, exposure to extremely high concentrations of arsenic from well-water for a long time caused a severe peripheral vascular disorder called black foot disease. In a city in Chile, tap water contamination by arsenic occurred from 1958 to 1970. More than 40 years' follow-up studies in Chile have demonstrated delayed health effects on CVDs, DM, and respiratory functions even after arsenic contamination had ceased. In Bangladesh, where more than eight million tube-wells have been installed throughout the country, an unprecedented scale exposure to arsenic has occurred and is continuing.

We have been investigating the health effects of chronic arsenic exposure among the residents in arsenic-contaminated areas in western Bangladesh, focusing on non-malignant diseases. This review will discuss possible mechanisms underlying arsenic-induced non-malignant diseases based on our human studies in Bangladesh. First, we briefly describe the findings of other epidemiological studies on the effects of chronic arsenic exposure on non-malignant diseases in Taiwan and Chile. It will clarify the similarities and differences among the three countries, including dose levels, exposure durations, and the effects of confounding factors such as obesity. Although arsenic exposure similarly elevated the risks of CVDs and DM in the three countries, detailed mechanistic approaches have not been fully achieved in Taiwan or Chile because arsenic exposure had occurred more than 50 years ago (**Fig. 1**). In Bangladesh, where exposure to arsenic is still ongoing, we were able to use blood biomarkers to examine the mechanisms by which arsenic increases the risks of non-malignant diseases.

2. Black foot disease in Taiwan

Arsenic-induced black foot disease (BFD) occurred in the coastal area north of Tainan City in southwestern Taiwan (**Fig. 1**). BFD was found among the people using artesian wells to pump up the groundwater from 120-180 m depth, which contained high concentrations of arsenic [7]. They began to use the deep-well groundwater in the 1920s because the groundwater from shallow wells (12-18 m depth) was salty in this coastal area. The average arsenic concentration in the groundwater collected from the BFD-endemic area in 1959 was 780 μg/L, ranging from 350 to over 1050 μg/L [8]. The incidence of BFD peaked during 1956-1960, indicating that exposure to high-level arsenic for more than 30 years resulted in developing BFD [9].

BFD is a peripheral vascular disease with characteristic blackened skin of the lower limbs, which eventually progresses to ulceration and gangrene in the toes and feet, forcing many patients to have their toes and feet amputated. BFD patients also showed typical symptoms of arsenic poisoning, including skin pigmentation and keratosis. A 15-year follow-up study of 789 BFD

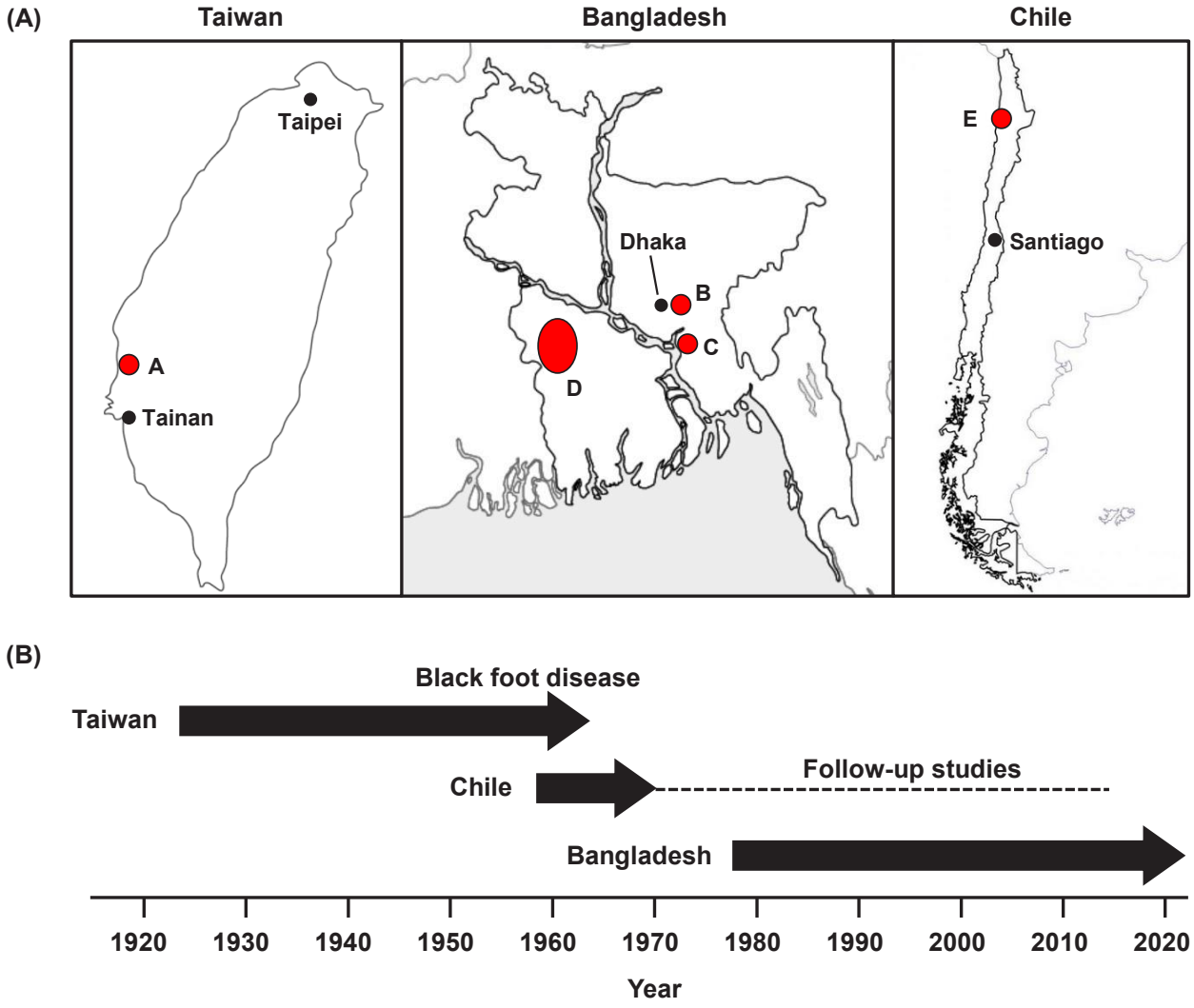

Fig. 1. **(A)** Maps of Taiwan, Bangladesh, and Chile. A, The black foot disease-endemic area in Taiwan; B, Araihazar, C, Matlab, and D, our research areas in Bangladesh; E, Antofagasta in Chile.
(B) Roughly estimated duration of arsenic contamination in Taiwan, Chile, and Bangladesh.

patients showed that the standardized mortality ratios (SMRs) for skin, lung, bladder, and liver cancers were 4.5, 2.8, 2.6, and 2.5, respectively, compared to the general population in Taiwan [10]. The SMRs of peripheral vascular disease and cardiovascular disease were 3.5 and 1.6, respectively. The prevalence of ischemic heart disease increased dose-dependently in the BFD-endemic areas [11]. The elevated prevalence of DM [12,13] and hypertension [14] was also found in the BFD-endemic area compared with the non-endemic area. Thus, many cancerous and non-cancerous diseases, currently recognized as arsenic-related diseases, had already been observed in the BFD-endemic area in Taiwan [7].

Pathological examination of the amputated parts of extremities from 51 BFD patients provided insight into the etiology of BFD [7,15]. Notably, histopathological manifestations of all BFD patients were similar to the well-known peripheral arterial diseases; 70% of the patients exhibited symptoms similar to arteriosclerosis obliterans, and 30% did thromboangiitis obliterans. Arteriosclerosis obliterans are characterized by blood vessel occlusion due to extensive accumulation of atherosclerotic plaques, causing limited blood flow to the lower extremities and, eventually, necrosis and gangrene of the toes and feet. Thromboangiitis obliterans is another type of peripheral arterial disease characterized by inflammation of the artery walls, causing the development of clots and eventually the occlusion of blood vessels in the toes and fingers. Although thromboangiitis obliterans is not directly related to atherosclerosis, examinations of the autopsy cases, who had thromboangiitis obliterans, showed extensive atherosclerosis in the arteries of the other organs [7]. Thus, both types of BFDs are occlusive vascular diseases with the extensive formation of

atherosclerosis-related clots, leading to necrosis and gangrene of the lower limbs.

Based on histopathological observations of BFD, arsenic is thought to cause vascular lesions such as atherosclerosis and inflammation of blood vessels. Diabetes and hypertension are also the risk factors driving arteriosclerosis obliterans; terminally ill diabetic patients may develop arteriosclerosis obliterans, resulting in amputating the lower limbs, even without arsenic contamination. Because the prevalence of DM and hypertension was high in the BFD-endemic area [12-14], the arsenic-induced peripheral arterial lesions might have been further accelerated by DM and hypertension.

Since the overt BFD has been rarely observed in the other arsenic-contaminated areas than Taiwan, it was initially speculated that additional unknown factors unique to this region of Taiwan might be involved in this rare disease. However, many human and experimental studies have provided evidence of atherosclerotic changes caused by arsenic exposure [16,17]. In Taiwan, the high concentrations (780 μg/L on average) of arsenic in water and the long duration of exposure (20-30 years) might have caused BFD, the severest case of atherosclerotic and inflammatory vascular lesions. However, the mechanisms of how arsenic caused atherosclerosis, hypertension, and DM in humans remained unclarified.

3. Long-term follow-up studies in Chile

In the South American countries, including Chile, arsenic leaching from volcanic rocks of the Andes Mountains has caused contamination of rivers, lakes, and groundwater. Particularly, in Antofagasta city in northern Chile (**Fig. 1**), significant health problems occurred due to the contamination of the municipal tap water that used arsenic-contaminated river water as a water source. Residents were exposed to about 860 μg/L arsenic via tap water for 12 years from 1958 to 1970. In 1971, a facility for removing arsenic was installed in the water supply system, and the exposure to high concentrations of arsenic ended [18,19]. Since there was no alternative water source, most residents in Antofagasta consumed the tap water containing approximately the same concentration of arsenic during the same period. Such a unique situation has offered a rare opportunity to conduct epidemiological studies with a certainty of information on arsenic exposure levels and durations.

The follow-up studies on the residents in Antofagasta, especially those born between 1958 and 1970, have demonstrated that arsenic exposure *in utero* and early childhood increased the incidences of cancers and non-malignant diseases 20-40 years later [18, 19]. The age-adjusted mortality rates from ischemic heart disease, especially acute myocardial infarction, were increased in males in the arsenic-contaminated regions more than 20 years after the cessation of high-level arsenic exposure [20]. SMRs for acute myocardial infarction among the people born in 1950-1957 were highest at the age of 40-49, and those born in 1958-1970 were highest at the age of 30-39. These results suggest that high-level arsenic exposure *in utero* or early childhood increased the risk of mortality from myocardial infarction even 20-30 years after the cessation of arsenic exposure.

A case-control study conducted in 2007-2010 showed that higher levels of arsenic exposure elevated the risk of hypertension [21]. The relationship between DM and arsenic exposure was also examined using the same data sources. The odds ratios (ORs) for DM after adjustment for sex, age, and BMI were higher in the highest arsenic exposure group than the low exposure group in tertiles [22]. However, the BMI-stratified analysis showed a significant increase in the OR of DM by arsenic exposure only in the group with BMI >30. These results suggest that arsenic exposure potentiated the risk of DM, which is primarily associated with obesity.

Early-life exposure to arsenic also caused increased mortality from bronchiectasis, the end-stage pulmonary disease with abnormal bronchial dilatation and epithelial inflammation. Mortality from bronchiectasis at the age of 30-49 years among the residents born in Antofagasta between 1958 and 1970 was markedly higher than the mortality in the general population in Chile [23]. The residents born in Antofagasta during 1958-1970 showed elevated prevalences of shortness of breath, persistent cough, and persistent sputum when examined during 2009-2011 [24]. The prevalence of asthma and chronic bronchitis slightly increased but was not statistically significant. The FVC (forced vital capacity) in non-smokers in the residents of Antofagasta was lower than that in the uncontaminated area [25]. However, the BMI-stratified analysis showed that only the non-smokers with BMI >30 showed significantly lowered FVC.

Thus, decades-long follow-up studies in Antofagasta, Chile, have demonstrated that early-life exposure to arsenic increased the risk of cancers, CVDs, DM, and respiratory diseases in later life. In addition, confounding effects of obesity on arsenic-associated diseases have been observed.

4. A large-scale arsenic poisoning in Bangladesh

Bangladesh has undergone an unprecedented scale of arsenic poisoning. Since the country's independence in 1971, a tremendous number (finally more than 8 million) of tube-wells have been installed with the support of UNICEF to provide pathogen-free water instead of surface water from ponds and rivers. Owing to these wells, the incidence of waterborne diseases had decreased. However, nationwide groundwater contamination with arsenic was recognized in the 1990s. Arsenic-contaminated groundwater over 50 μg/L arsenic (the limit set by the Bangladesh government) has been found in 61 out of 64 districts in Bangladesh [26], and the population exposed to arsenic was estimated to be more than 35 million [27]. Due to the difficulties in installing arsenic removal equipment in millions of wells and the low coverage of tap water, especially in rural areas, a large population is still obliged to use arsenic-contaminated well-water. The prevalence of lifestyle-related disorders such as hypertension and DM is high in the arsenic-contaminated areas in Bangladesh, where obesity is low, almost nobody drinks alcohol due to religious reasons, and women do not smoke.

Two large-scale prospective cohort studies have been carried out to examine the health effects of chronic arsenic exposure in Bangladesh. In Matlab, located 53 km southeast of Dhaka (**Fig. 1**), the International Centre for Diarrheal Disease Research, Bangladesh (icddr, b) has maintained a Health and Demographic Surveillance System covering 142 villages since 1966. Arsenic concentrations in half of the wells in Matlab were higher than 150 μg/L, and about 40% were in a range of 150-500 μg/L [27]. In Araihazar, located close to Dhaka (**Fig. 1**), approximately 12,000 residents have participated in the Health Effects of Arsenic Longitudinal Study (HEALS) since 2000 [28]. Arsenic concentrations in about 80% of the wells in Araihazar were less than 180 μg/L, representing low-to-moderate level arsenic exposure. Studies conducted in the two areas have provided extensive findings regarding the effects of chronic arsenic exposure on skin lesions, cancers, and non-malignant diseases [29,30].

Since 2009, we have started studying the health effects of arsenic exposure in the western region of Bangladesh, including Jessore, Chuadanga, and Kushtia districts (**Fig. 1**). Several hundred km from big cities like Dhaka, these rural areas were largely left out of groundwater mitigation, and most residents continue to use arsenic-contaminated groundwater with average concentrations of 170-190 μg/L ranging from low to high (up to about 1,000 μg/L) levels [16,31,32]. Naogaon district in the northwestern part was included as a non-endemic low-arsenic area. Because the villages to be surveyed are scattered over a large area, we took a cross-sectional approach. The total number of participants was small initially but increased gradually during the past ten years to more than 800 [33].

Unlike Taiwan and Chile, arsenic exposure is still ongoing, especially in rural areas like our study areas in Bangladesh. This situation has rendered us opportunities to examine the changes of disease-related biomarkers induced by the recent exposure to arsenic. In Bangladesh, groundwater is also used for rice-field irrigation, and the per capita rice consumption is very high. Hence, determining water arsenic concentrations in each well is insufficient to assess the individual arsenic exposure levels. To overcome this problem, we measured arsenic concentrations in the residents' hair and nails, which reflect relatively long-term arsenic intake [34,35], in addition to their drinking water. We have attempted to examine dose-response relationships between these individual-level arsenic exposure indicators and the changes in biomarkers for various diseases to clarify the mechanisms underlying arsenic-induced disease development in humans. The results of our studies thus far published [16,31-33,36-46] are summarized in **Table 1**.

5. Changes in biomarkers related to CVDs by arsenic exposure

Prospective cohort studies conducted in Matlab in Bangladesh have reported that arsenic exposure increased mortality from CVDs [47,48]. Another cohort study among the participants in HEALS has also shown that arsenic exposure increased mortality from ischemic heart disease and other forms of heart diseases [49]. Prevalence of hypertension was increased dose-dependently by arsenic exposure in Matlab [50]. A seven-year follow-up study among the participants in HEALS showed that age-dependent increases in blood pressure were accelerated by arsenic exposure [51]. Our studies have also shown that the average levels of SBP and DBP and the prevalence of hypertension among the subjects in the arsenic-contaminated areas were significantly higher than those in the non-contaminated area [16,31,32,39,42].

As a blood biomarker for hypertension, we focused on endothelin-1, a vasoconstrictor secreted from vascular endothelial cells. Since the half-life of endothelin-1 in the blood is very short, we measured the plasma levels of its precursor, Big-endothelin-1, which has a longer half-life in the blood. We found that plasma levels of Big-endothelin-1 were higher among the subjects in the

arsenic-contaminated areas than those in the non-contaminated area and significantly higher in hypertensive than in normotensive individuals [31]. Arsenic concentrations in drinking water, hair, and nails all showed significant positive correlations (r >0.4) with plasma Big-endothelin-1 concentrations. It is known that blood endothelin-1 levels reflect abnormalities in vascular endothelial cells and are elevated in patients with CVDs and DM [52,53]. Our results suggest that arsenic exposure enhanced secretion of endothelin-1 from the damaged vascular endothelial cells, contributing to the elevation of blood pressure.

We next investigated the relationships between arsenic exposure indicators and the concentrations of blood lipids, inflammation markers, and soluble forms of adhesion molecules related to atherosclerosis [16]. We found that plasma levels of triglycerides and total cholesterol were not increased by arsenic exposure. However, oxidized LDL (OxLDL) levels were increased, HDL levels were decreased, and the ratios of OxLDL/HDL were increased in an arsenic concentration-dependent manner, suggesting the involvement of reactive oxygen species (ROS) induced by arsenic. We also found that plasma levels of C-reactive protein (CRP), an indicator of inflammation, and circulating adhesion molecules, such as soluble VCAM-1 (sVCAM-1) and sICAM-1, were elevated dose-dependently by arsenic exposure. The reduced levels of HDL, which has antioxidant properties, may fail to suppress LDL oxidation and OxLDL accumulation in macrophages [54]. These results suggest that arsenic accelerates the initiation process of atherosclerosis via enhancing oxidative stress and inflammation and activating circulating monocytes' adhesion to endothelial cells and transmigration to the intima (**Fig. 2**).

Based on our results and recent evidence on the processes of atherosclerosis supporting our results [55-66], we propose the mechanisms of arsenic-induced vascular damages as illustrated in **Fig. 2**. Arsenic was shown to activates NADPH oxidase 2 (NOX2) in vascular endothelial cells, promoting ROS formation [55,56]. ROS-induced OxLDL activates endothelial cells via binding to LOX-1, the receptor for OxLDL expressed in endothelial cells [57,58] in addition to the engulfment by intimal macrophages. The internalization of OxLDL via LOX-1 plays a crucial role in initiating atherosclerosis by activating various downstream genes in endothelial cells, including VCAM-1, ICAM-1, endothelin-1, and CRP, with concomitant production of ROS [59-62]. The secreted endothelin-1 and CRP, in turn, enhance the expression of LOX-1 and increases OxLDL uptake

Table 1. | Lists of biomarkers and their changing tendencies dependent on arsenic exposure in our studies in western Bangladesh

Blood biomarkers	Changes caused by arsenic exposure	Ref.
Choline esterase	Choline esterase ↓	36
Lactate dehydrogenase (LDH)	LDH ↑	37
Alanine succinate transferase (AST), Alanine lactate transferase (ALT)	AST ↑, ALT ↑	38
Big endothelin-1 (precursor of endothelin-1)	Big endothelin-1 ↑	31
Triglyceride, Total cholesterol, LDL, HDL, Oxidized LDL (OxLDL), C-reactive protein (CRP), sVCAM-1, sICAM-1	OxLDL ↑, HDL ↓, OxLDL/HDL ratio ↑, CRP ↑, sVCAM-1 ↑, sICAM-1 ↑	16
Uric acid	Uric acid ↑	39
Vascular endothelial growth factor (VEGF)	VEGF ↑	40
Matrix metalloproteases (MMP)	MMP-2 ↑, MMP-9 ↑	41
Long interspersed nuclear element-1 (LINE-1)	Methylation of LINE-1 ↓	42
sThrombomodulin (sTM)	sTM ↑	32
Fasting blood glucose (FBG), 2h-blood glucose at glucose tolerance test	FBG ↑, 2h-blood glucose ↑ Odds ratio of hyperglycemia ↑ in females	43
Brain-derived neurotrophic factor (BDNF)	Cognitive function test ↓, BDNF ↓	44
Total IgE	FEV1 ↓, FEV1/FEV6 ↓, Reversible airway obstruction ↑, Asthma-like symptoms ↑, Total IgE ↑	33
FBG, Insulin, Creatinine	FBG ↑, Insulin ↑, HOMA-IR ↑, Creatinine ↓, Lean body mass ↓	45
IL-4, IL-5, IL-13, IFN-γ, TNF-α, Eotaxin	IL-4 ↑, IL-5 ↑, IL-13 ↑, Eotaxin ↑	46

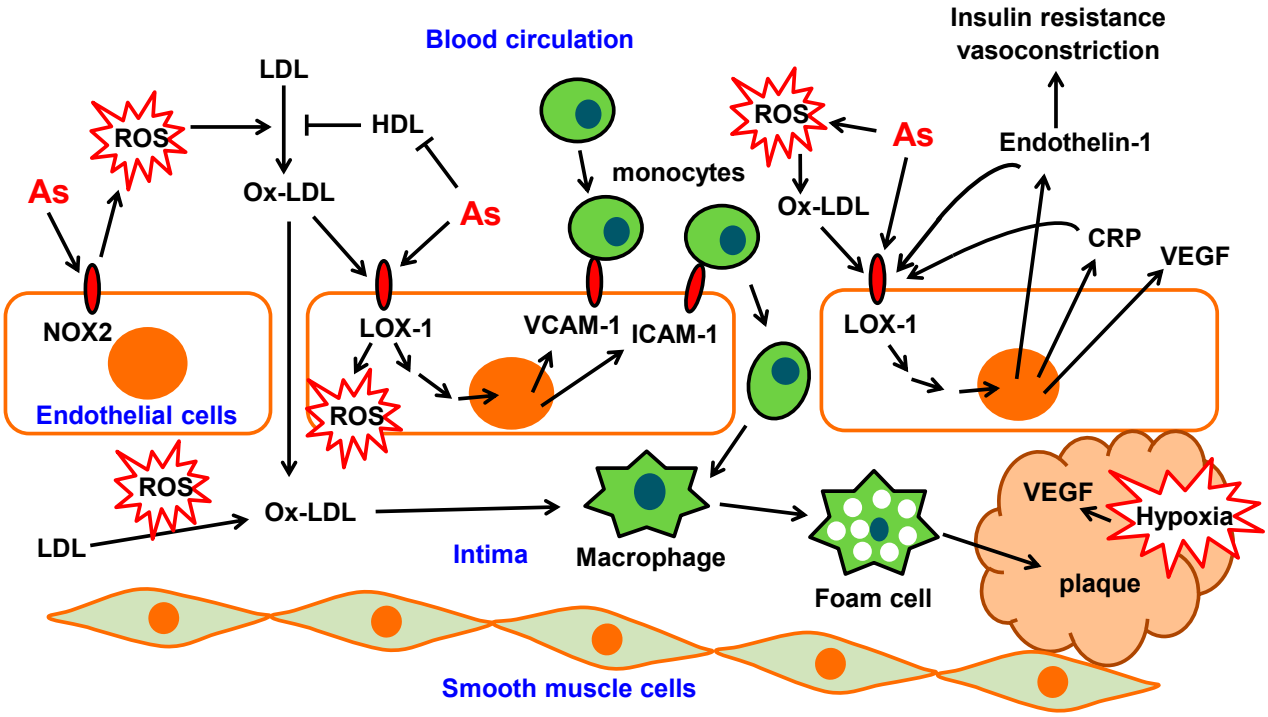

Fig. 2. Vascular events involved in arsenic-induced endothelial damages and their relations to recently found mechanisms of atherosclerosis development. Details are explained in the text.

and downstream gene expression, forming a reinforcing loop [61,63,64]. Notably, treatment of cultured endothelial cells with arsenic was shown to increase the expression of LOX-1, thereby accelerating the binding of OxLDL to LOX-1 and downstream pathways [65]. Another experimental study showed that the administration of arsenic to mice enhanced ROS-mediated expression of VCAM-1 in endothelial cells [66]. We have determined plasma levels of Big-endothelin-1, the precursor to endothelin-1, as a vasoconstrictor in our previous study [31]. However, recent evidence has shown that endothelin-1 plays a broader role in developing both atherosclerosis and hypertension.

We also examined other biomarkers related to vascular disorders and CVDs, including vascular endothelial growth factor (VEGF), matrix metalloprotease-2 (MMP-2), MMP-9, uric acid, and soluble thrombomodulin (sTM). It is known that VEGF and MMPs play critical roles in cancer cell infiltration and invasion, and angiogenesis in cancer tissue [67,68]. VEGF has also been shown to promote angiogenesis within atherogenic plaques and cause plaque instability in advanced atherosclerosis [69,70]. Intraplaque VEGF expression is induced by hypoxia in the advanced plaques [69], but the OxLDL-LOX-1 interaction also upregulates VEGF expression in endothelial cells [71] (**Fig. 2**). MMP-2 and MMP-9 contribute to the degradation of the extracellular matrix, leading to remodeling of the vasculature and instability of plaques [72]. We found that arsenic exposure dose-dependently increased serum VEGF, MMP-2, and MMP-9 levels among our study subjects in the arsenic-contaminated areas [40,41]. These results suggest that either arsenic directly upregulated the expression and production of these molecules, contributing to advancing atherosclerosis, or that the elevations of these molecules in serum merely reflected the consequences of arsenic-induced advancement of atherosclerosis. Furthermore, the elevated serum VEGF, MMP-2, and MMP-9 levels may partly reflect arsenic-induced cancer cell infiltration, if any.

Because blood levels of uric acid and sTM are known to be elevated in patients with vascular disorders [73-75], we also determined the concentrations of these circulating molecules. Under physiological conditions, uric acid plays the role of antioxidant, but higher levels of uric acid promote ROS formation in endothelial cells and induce endothelin-1 expression [75]. We found plasma uric acid levels among our study subjects in the arsenic-contaminated areas were higher than those in the non-contaminated area [39]. Hypertensive individuals showed higher levels of plasma uric acid than normotensive individuals in the

arsenic-contaminated areas, suggesting the association of uric acid with vascular dysfunctions. Thrombomodulin at the surface membrane of endothelial cells plays an antithrombotic role, but the serum levels of sTM reflect the proteolytic degradation of thrombomodulin due to endothelial damages [76]. The serum levels of sTM were shown to be elevated in patients with peripheral vascular diseases [73,74]. We found that serum levels of sTM were elevated among our study subjects in the arsenic-contaminated areas with significant correlations with blood pressure and serum levels of sVCAM-1 and sICAM-1 [32].

Thus, examining the vascular lesion-related blood biomarkers allowed us to find dose-dependent changes in these biomarkers by arsenic exposure. All these data suggest that arsenic is a vascular toxicant affecting the expression of various target molecules related to hypertension and atherosclerosis in endothelial cells.

6. Arsenic-induced DM and insulin resistance

A link between arsenic exposure and DM has been observed in Taiwan, Chile, Mexico, India, and Bangladesh. Previous studies in Bangladesh have shown that fasting blood glucose (FBG) or HbA1c levels were elevated by arsenic exposure [77-79]. A study conducted among the participants of HEALS failed to show significant associations of arsenic exposure with HbA1c levels, possibly due to the low-to-moderate arsenic concentrations in the well-water (most of them <300 μg/L) in this area [80].

In our study, a glucose tolerance test (GTT) was carried out in addition to FBG determination [43]. We divided the subjects into tertile of low (<10.6 μg/L), moderate (10.6 – 168 μg/L), and high (168 – 1000 μg/L) exposure groups based on water arsenic concentration. Both FBG and the blood glucose levels 2 hr after the loading of 75 g glucose increased significantly as the arsenic exposure levels increased, suggesting an impaired glucose tolerance (IGT). DM was diagnosed based on GTT results according to the WHO's criteria. The ORs of DM were 3.00 (1.39-6.45) in the moderate and 3.63 (1.7-7.76) in the high arsenic exposure group after adjustment for sex, age, BMI, and smoking status. Similar associations were observed with hair and nail arsenic concentrations. Sex showed significant confounding effects on the associations, and the prevalences of hyperglycemia and IGT were higher in females than in males.

IGT is caused by either reduced insulin secretion or elevated insulin resistance; the latter generally develops at the initiation phase among most patients with type 2 DM. However, the mechanisms underlying arsenic-related DM remain elusive. Both possibilities that arsenic causes pancreatic damage, leading to reduced insulin secretion, and that arsenic disturbs insulin signaling in glucose-uptaking tissues, causing insulin resistance, have been proposed and argued [5,81]. We, therefore, determined serum insulin concentrations among the subjects who participated in the previous DM study [43]. Based on serum insulin concentrations, HOMA-IR, an indicator of insulin resistance, and HOMA-β, an indicator of decreased pancreatic function, were calculated. We found that serum insulin and HOMA-IR levels were significantly higher in the high arsenic exposure group than in the low exposure group [45]. Even the moderate exposure group showed significant increases in serum insulin and HOMA-IR levels in females. For the first time, these results provided evidence indicating that arsenic-induced hyperglycemia and DM are primarily related to elevated insulin resistance among the populations exposed to moderate-to-high levels of arsenic.

Further analyses of serum biomarkers enabled us to find the involvement of skeletal muscle atrophy as a possible cause for developing insulin resistance by arsenic exposure. Initially, we measured serum creatinine levels to see if DM caused kidney damages. However, almost none of the subjects showed an increase in serum creatinine concentration. Instead, serum creatinine concentrations decreased dose-dependently by arsenic exposure [45]. Consistent with our results, another study in Bangladesh also reported that serum creatinine levels among the residents in the arsenic-contaminated area were lower than those in the non-contaminated area [82]. Under conditions with no renal dysfunction, serum creatinine levels generally reflect skeletal muscle mass [83]. We also found that the levels of lean body mass (LBM), another surrogate marker for skeletal muscle mass, were significantly lower in the high exposure group than the low exposure group. The dose-dependent decreases in serum creatinine and LBM levels suggest that chronic arsenic exposure might have caused skeletal muscle atrophy.

We also found an inverse association of skeletal muscle mass with insulin resistance. When the subjects were divided into tertiles based on their serum creatinine or LBM levels, FBG and HOMA-IR levels increased with decreased serum creatinine and LBM levels [45]. These results suggest that arsenic-induced skeletal muscle atrophy is a likely cause of inducing insulin resistance. Although there were no sex differences in arsenic-induced reduction in skeletal muscle mass, the contributions of the muscle mass loss to the increases in FBG and HOMA-IR levels were more evident in females than males [45]. The higher risk of hyperglycemia in females observed in our previous study [43] may be explained, at least partly, by the higher sensitivity

of females to the effects of skeletal muscle atrophy on insulin sensitivity.

Skeletal muscle, the largest tissue in the body, plays a vital role in absorbing postprandially increased blood glucose. The interaction of DM and sarcopenia, the conditions of skeletal muscle loss and weakness with advanced aging, is currently attracting global attention [84]. Many clinical studies have been concerned about the worsening of diabetic symptoms by aging-associated sarcopenia. On the other hand, several prospective cohort studies have reported that the individuals with low serum creatinine levels at the beginning or those whose serum creatinine levels declined during the study period showed an increased risk of developing DM later, suggesting the role of skeletal muscle reduction in initiating DM [83,85-89]. Because our study on the association of muscle mass and insulin resistance is cross-sectional, we could not conclude that skeletal muscle mass atrophy is a preceding event causing insulin resistance. However, the average ages of our study subjects were below 40 [43,45], suggesting a lower possibility of aging-related sarcopenia. Furthermore, experimental studies have shown that arsenic affected the regeneration of the muscle [90] and mitochondrial morphology and functions of the muscle in mice [91], supporting our human studies. Our study highlighted the novel role of muscle as a target organ of arsenic toxicity and its relation to insulin resistance.

Skeletal muscle is also a target of endothelin-1. Endothelin-1 suppresses glucose uptake in skeletal muscle [92] and induces insulin resistance in skeletal muscle [93,94]. Since the high-level glucose is known to cause elevated production of endothelin-1 in endothelial cells [95], hyperglycemia may further facilitate endothelin-1-induced insulin resistance in skeletal muscle. Thus, the elevation of blood endothelin-1 levels among the arsenic-exposed individuals, which we found in our previous study [31], may be associated with multiple events, including hypertension, atherosclerosis, and skeletal muscle-related insulin resistance (**Fig. 2**).

7. Arsenic-induced respiratory dysfunctions involving immunomodulation

In Bangladesh, the incidence of respiratory symptoms, such as repeated cough, breathing problems, or blood in their sputum, increased in the arsenic-exposed subjects who participated in HEALS [96]. The respiratory functions determined by spirometric measures such as FEV1 (forced expiratory volume in one second) and FVC were also decreased by arsenic exposure among the participants in HEALS exposed to low-to-moderate level arsenic [97].

Since many people in our study areas also showed respiration difficulties, we conducted respiratory function tests using a portable spirometer (Hi-checker) and interviews regarding respiratory symptoms [33]. FEV6 was used as a surrogate for FVC. Reduction in FEV1/FEV6 suggests obstructive lung diseases, such as chronic obstructive pulmonary disease (COPD) and asthma. Although discriminating COPD and asthma has many difficulties in clinical settings, the recovery of FEV1 more than 12% after dosing a bronchodilator is internationally used as the indicator of reversible airway obstruction (RAO), a characteristic feature of asthma. We, therefore, examined the frequencies of airway obstruction (FEV1/FEV6 <0.73), RAO (FEV1/FEV6 <0.73 plus FEV1 recovery >12% by bronchodilator), and the presence of all four asthma-like symptoms (repeated cough, wheezing, shortness of breath, and chest tightness). Of 842 subjects participating in our study, 97, 70, and 87 showed airway obstruction, RAO, and all four asthma-like symptoms, respectively. The ORs of the airway obstruction, RAO, and asthma-like symptoms in moderate and high arsenic exposure groups were 1.94 and 3.65, 1.76 and 3.81, and 2.04 and 3.69, respectively, compared to the low exposure group (referent). The dose-dependent increases in the ORs of the RAO and asthma-like symptoms suggest that arsenic exposure is likely to enhance the risk of airway obstruction, especially asthma [33].

Asthma is not a single entity disease but includes multiple phenotypes and mechanistic pathways (endotypes). Our study showed that the average concentration of serum IgE in the subjects showing RAO was about two times higher than the rest of the study participants, suggesting a possibility of allergic (atopic) asthma [33]. Allergic asthma overlaps with the Th2-high endotype, the major type of asthma that involves the activation of Th2 cytokines and Th2-related inflammatory cascades in the airway [98,99]. When allergens are recognized by dendric cells in the lung, naïve CD4+ T cells differentiate to the Th2 phenotype and secrete Th2 cytokines such as IL-4, IL-5, and IL-13 [100]. IL-4 and IL-13 activate the switching of B cells to plasma cells producing IgE, and IgE activates mast cells, leading to enhanced histamine secretion. IL-5 stimulates the maturation of eosinophils in the bone marrow and facilitates the recruitment of eosinophils to the airway, resulting in elevated inflammation in the lung [101]. Eotaxin (CCL11), a chemokine, also promotes recruiting eosinophils and Th2 cells to the site of inflammation in the airway [102].

Hence, we determined serum levels of Th1 cytokines, IFN-γ and TNF-α, and Th2 cytokines, IL-4, IL-5, and IL-13 among the subjects in the previous asthma study [33]. We also determined serum levels of eotaxin. Results showed that serum levels of IL-4, IL-5, IL-13, and eotaxin among the residents in the arsenic-contaminated areas were significantly higher than those in

the non-contaminated area [46]. In contrast, serum levels of IFN-γ and TNF-α were similar between arsenic-exposed and non-exposed subjects. When the subjects with RAO and without RAO were compared, the subjects with RAO had higher serum levels of IL-4, IL-5, IL-13, and eotaxin. These results suggest that arsenic exposure altered the Th1/Th2 balance toward Th2 dominance, predisposing the arsenic-exposed subjects to develop Th2-high type asthma.

8. Conclusions and future perspectives

Extensive epidemiological studies in Taiwan and Chile have demonstrated the associations between arsenic exposure and the increased risk of non-malignant diseases such as DM, CVDs, and respiratory diseases. However, the mechanisms underlying arsenic-induced increases in the risks of these diseases have remained unclear. We have explored biochemical mechanisms by examining the dose-response relationships between disease-related biomarkers and individuals' arsenic exposure indicators. Our studies have provided novel insights into biochemical mechanisms of arsenic-related development of hypertension, atherosclerosis, insulin resistance, DM, and respiratory diseases.

The inter-disease and inter-organ communications in the pathogenesis of DM, hypertension, and CVDs have recently been studied extensively. DM promotes atherosclerosis [103], insulin resistance exacerbates CVDs [104], and muscle fat infiltration develops insulin resistance and CVDs [105,106]. The vasculature is the central place for these interactions mediated by OxLDL, LOX-1, endothelin-1, VEGF, MMPs, and adhesion and inflammatory molecules. Our findings suggest that arsenic plays multiple roles in accelerating the interactions of these complex networks, facilitating atherosclerosis, hypertension, insulin resistance, and DM. However, many other pieces of this puzzle have yet to be investigated. For example, vascular constriction and relaxation depend on the balance of endothelin-1 and vasodilator, NO [107], but NO's contribution has not been elucidated in our study area. We found that skeletal muscle is a novel target of arsenic toxicity [45], but more detailed mechanisms causing insulin resistance via arsenic-related muscle atrophy should be elucidated. Effects of arsenic on muscle quality, such as ectopic lipid accumulation by fat infiltration [105,106], in addition to muscle quantity, need to be investigated. The Th2-dominant immunomodulation by arsenic exposure, which we found as a possible predisposing factor for developing asthma [46], may also be involved in other arsenic-associated diseases such as skin lesions and even cancers.

CVDs are considered a disease of premature aging of the vasculature [108, 109], and muscular atrophy is a clear reflection of aging [110]. In our studies, the increased blood pressure and atherosclerosis-related biomarkers and the reduced skeletal muscle mass were found in the participants whose average age was below 40 years [16,31,45]. In another study, we conducted cognitive function tests, initially developed for the elderly, among the arsenic-exposed adults with an average age below 40 years and found an arsenic concentration-dependent decrease in cognitive function [44]. Thus, there is a possibility that accelerated aging by arsenic exposure is the fundamental underlying mechanism of arsenic-induced increases in CVDs, DM, and muscle-related dysfunctions. Future studies may be necessary to measure the biomarkers for premature aging, such as senescence-associated secretory phenotypes (SASP) factors [111].

It should be argued here about the relationship between obesity and arsenic-induced DM or hypertension. Indeed, obesity is the primary risk factor for hypertension, atherosclerosis, DM, and CVDs. However, the average percentage of obesity (BMI >30) in Bangladesh in 2016 was 3.6%, while those in Chile, Mexico, and the USA are 28.0, 28.9, and 36.2, respectively [112], where the associations of arsenic exposure with DM and CVDs have been extensively investigated [20-22,113,114]. In Chile, the risk of DM was increased by early-life arsenic exposure only in the obese (BMI >30) people [22], suggesting that arsenic is a potentiating factor for the primarily obesity-associated DM. Even in Bangladesh, obesity has increased (1.0%, 1.9%, and 3.6% in 1996, 2006, and 2016, respectively) [112]. However, the participants of our studies in rural areas had an average BMI of 20-21 constantly during the past ten years [36,45], and only 1.6% of them showed BMI >30 [115]. The OR of hyperglycemia was increased even after adjusting BMI [43]. Thus, the confounding effects of obesity on analyzing the association of arsenic exposure with DM and hypertension may be minimal in Bangladesh, particularly the rural areas like our study area. Nevertheless, ectopic fat accumulation in the muscle or abdominal adipose tissues needs to be explored even among the Bangladesh populations with low BMI. Recent studies have implied that skeletal muscle atrophy is linked to intramuscular fat infiltration, which may be the ultimate cause for promoting insulin resistance [105,106].

The studies in Chile have demonstrated that arsenic exposure *in utero* and early childhood is crucial for developing cancers and non-cancer diseases in adulthood [18,19]. In Bangladesh, health hazards due to arsenic contamination of groundwater were

detected in the 1990s. However, long-term monitoring of arsenic concentrations in well-water at Matlab showed that arsenic concentrations began to increase in the 1970s [27]. It is necessary to identify the participants in our studies subjected to early-life exposure to arsenic and examine their disease development.

Our studies have limitations caused by the cross-sectional approach itself. We have shown the associations of arsenic exposure levels with the changes in biomarkers and pathological conditions, but these results are insufficient to prove cause-effect relationships. We have proposed several new hypotheses from our cross-sectional studies, such as that arsenic decreases muscle mass and increases insulin resistance or that arsenic causes a Th2 shift and promotes the development of asthma. These hypotheses require further scientific validation by future epidemiological and experimental studies.

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