

Arsenic Exposure and Subclinical Endpoints of Cardiovascular Disease

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Abstract Mechanistic evidence suggests that arsenic exposure from drinking water increases the production of reactive oxygen species and influences inflammatory responses and endothelial nitric oxide homeostasis. These arsenic-induced events may lead to endothelial dysfunction that increases the risk of atherosclerosis and cardiovascular disease. We reviewed accumulating epidemiologic evidence that evaluated the association between arsenic exposure and intermediate markers and subclinical measures that predict future cardiovascular risk. Cross-sectional studies have indicated positive associations between high or low-to-moderate levels of arsenic exposure with indices of subclinical atherosclerosis, QT interval prolongation, and circulating markers of endothelial dysfunction. The evidence is limited for other intermediate endpoints such as markers of oxidative stress and inflammation, QT dispersion, and lipid profiles. Prospective studies are needed to enhance the causal inferences of the effects of arsenic on subclinical endpoints of cardiovascular disease, especially at lower arsenic exposure levels.

Keywords Arsenic exposure · Cardiovascular disease · Subclinical atherosclerosis · QTc prolongation · Oxidative stress · Inflammation · Endothelial dysfunction · Susceptibility

Introduction

Arsenic is an abundant element of the Earth's crust and can enter drinking water supplies from natural deposits. Areas affected by arsenic exposure via contaminated drinking water span the globe, and significant exposures have been identified in Bangladesh, India, Taiwan, China, México, Argentina, Chile, and the US. Recent data also raise concerns for arsenic exposure via foods including appreciable levels of inorganic arsenic (InAs) in rice and rice products, fruit juices, and chicken [1–5]. These data suggest that arsenic exposure may be more widespread than previously considered and be of massive public health significance. As a group 1 human carcinogen, arsenic exposure from drinking water causes cancers of the skin, lung, and bladder [6–8]. In addition, arsenic exposure has been related to elevated risks of non-cancerous outcomes such as skin lesions [9, 10], lung disease [11, 12], and cardiovascular disease (CVD) [13].

High levels of arsenic exposure (>500 µg/L) have been related to an array of CVD in southwestern Taiwan, Bangladesh, and Chile [14–18]. Cross-sectional studies from north-eastern Taiwan and prospective studies from Bangladesh reported that moderate-to-high levels of arsenic exposure (<300 µg/L) were associated with CVD prevalence, incidence, and mortality [19–22]. Positive associations of arsenic exposure at low levels (<100 µg/L) have also been found with CVD mortality in a series of ecological studies from the US and Spain [23–28], and recently, with the risk of overall and subtypes of CVD in a prospective cohort study in the US [29]. Despite the clear epidemiologic links between arsenic

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exposure and CVD risk, the underlying mechanisms have not been well established.

Experimental studies have suggested that arsenic can increase the production of reactive oxygen species [30, 31], interfere with the activity of key antioxidant enzymes [32–34], and induce lipid peroxidation [33, 34]. The induction of oxidative stress may in turn mediate abnormal gene expression, inflammatory responses, or impaired nitric oxide homeostasis [35–37]. Arsenic up-regulates the expression of various inflammatory molecules [36, 38–40] and inhibits key regulators of lipid homeostasis [41], two key components in the initiation of atherosclerosis. These events may ultimately lead to endothelial dysfunction, which disrupts the balance in vasomotor tone between relaxation and contraction and increases CVD risk [42]. Arsenic may also enhance platelet aggregation and reduce fibrinolysis leading to arterial thrombosis and atherosclerosis [43]. Many mechanistic studies have suggested the possible pathways underlying the cardiovascular effects of arsenic exposure. In addition, epidemiologic studies that evaluate the associations between arsenic exposure and intermediate phenotypes along these pathways are accumulating. Reviews of epidemiologic studies on arsenic exposure and CVD [13, 44] as well as on the effects of arsenic on CVD risk factors such as diabetes mellitus and hypertension [45, 46] have been published. Here, we review the epidemiologic evidence on arsenic exposure and subclinical endpoints of CVD, including subclinical atherosclerosis (Table 1), electrocardiographic abnormalities (Table 2), markers of oxidative stress, inflammation, and endothelial dysfunction, as well as lipid profiles (Table 3). We also discuss studies on the individual susceptibility to some of these subclinical endpoints due to arsenic methylation capacity and genetic polymorphisms. The schematic depiction of epidemiologic evidence on possible mechanisms underlying the cardiovascular effects of arsenic exposure is presented in Fig. 1.

Subclinical Atherosclerosis

Carotid artery intima-media thickness (cIMT) and plaque, which can be detected non-invasively with reasonable precision using high-resolution ultrasound techniques, reflect a thickening of the carotid artery wall or the presence of large irregular arterial wall deposits, respectively. cIMT and plaque are established measures of subclinical atherosclerosis. Multiple independent studies have established consistent association of carotid phenotypes with coronary events and stroke in prospective studies of young, middle-aged, and older adults [47, 48], and recent consensus prevention guidelines cite cIMT as a potentially useful measure for prediction of these events [49]. In a cross-sectional study of 463 subjects conducted in southwestern Taiwan with high levels of arsenic

exposure (median in villages ranges from 700 to 930 $\mu\text{g/L}$), there was a dose–response relationship between well-water arsenic and carotid atherosclerosis [50•]. Carotid atherosclerosis in this study was defined using three indices including presence of plaque or cIMT ≥ 1.0 mm, cIMT ≥ 1.0 mm, and presence of plaque. Measures of long-term arsenic exposure including the duration of consuming well water, the average arsenic concentration in well water, and the cumulative arsenic exposure were all significantly associated with the prevalence of carotid atherosclerosis in a dose–response manner [50•]. In a study from northeastern Taiwan with low-to-moderate arsenic exposure (median in villages from undetectable to 140 $\mu\text{g/L}$), the prevalence of carotid atherosclerosis was significantly increased with an adjusted odds ratio (OR) of 2.6 (95 % confidence interval [CI] 1.3–5.0) among the highest exposure group (>100 $\mu\text{g/L}$) compared with the lowest group (≤ 50 $\mu\text{g/L}$) [51].

Some recent studies conducted in Bangladesh with more precise exposure measurements at the individual level also evaluated the effect of low-to-moderate levels of arsenic exposure on subclinical atherosclerosis. In a pilot study of 66 healthy, normotensive, relatively young (mean age 35 years) participants selected from the Health Effects of Arsenic Longitudinal Study (HEALS) [52], a prospective cohort study of more than 20,000 participants in Arai hazar, Bangladesh, the adjusted ORs for cIMT >0.75 mm (a predictor of significant coronary artery disease [53]) were 1.1 (95 % CI 0.2–6.3) and 2.1 (95 % CI 0.4–10.5), comparing middle (12–144 $\mu\text{g/L}$) and highest (>144 $\mu\text{g/L}$) with the lowest levels of well-water arsenic (<14 $\mu\text{g/L}$), respectively. Although not statistically significant, probably due to a small sample size, this increasing trend in ORs with higher arsenic exposure was observed consistently with total urinary arsenic, even after adjusting for total urinary arsenic measured at the time of follow-up. A recent cross-sectional study of 959 subjects nested in the HEALS [54••] confirmed and extended these previous findings in the same population. Arsenic exposure measured in both well water and urine samples at baseline was positively associated with cIMT measured 7 years later, such that a 1-standard-deviation (SD) increase in urinary arsenic (357.9 $\mu\text{g/g}$ creatinine) and well-water arsenic (102 $\mu\text{g/L}$) were associated with an 11.7- μm (95 % CI 1.8–21.6) and 5.1- μm (95 % CI –0.2 to 10.3) increase in cIMT, respectively. These increases were also seen with an increasing proportion of urinary monomethylarsonic acid (MMA%), the most toxic arsenic metabolite, and every 10 % increase in MMA% was related to an increase of 12.1 μm (95 % CI 0.4–23.8) in cIMT. More recently, in a cross-sectional study of 199 children 3–14 years of age in México, total urinary arsenic was positively associated with increases in cIMT, with a 35- μm and 58- μm increase in cIMT among children exposed to 35–70 $\mu\text{g/L}$ and >70 $\mu\text{g/L}$ total urinary arsenic, respectively, compared with those exposed to <35 $\mu\text{g/L}$ [55]. Although cross-sectional, these data

Table 1 Epidemiologic studies of arsenic exposure and subclinical atherosclerosis

Study	Location	Study design	Study population	Arsenic measurement	Arsenic levels	Endpoints	Main findings
Wang et al. [50•]	Southwestern Taiwan	Cross-sectional	199 men and 264 women	Well water	Median in villages: 700–930 µg/L	Carotid atherosclerosis defined by three indices: presence of plaque or cIMT \geq 1.0 mm; cIMT \geq 1.0 mm; and presence of plaque	A dose-response relationship between three indices of long-term arsenic exposure and prevalence of carotid atherosclerosis after adjustment for age, sex, serum levels of total and LDL cholesterol, waist-to-hip ratio, diabetes mellitus, and hypertension
Wang et al. [62]	Southwestern Taiwan	Cross-sectional	280 men and 355 women	Well water	Median in villages: 700–930 µg/L	cIMT and prevalence of carotid plaque	A dose-response relationship of cumulative arsenic exposure with cIMT and prevalence of carotid plaque with adjustment for age, sex, hypertension, diabetes mellitus, serum total cholesterol and triglyceride level, BMI, cigarette smoking, and alcohol consumption
Hsieh et al. [98]	Northeastern Taiwan	Cross-sectional	863 subjects (384 cases and 479 controls)	Well water	Undetectable (<0.15 µg/L) to 3.59×10^3 µg/L in household	Carotid atherosclerosis defined by cIMT >1.0 mm and either plaque occurrence in at least two locations or stenosis occurrence >50 % on right or left side of extracranial carotid artery	Prevalence of carotid atherosclerosis was significantly increased comparing the highest with the lowest exposure levels with adjustment for age, sex, hypertension, diabetes mellitus, serum total cholesterol and triglyceride level, BMI, cigarette smoking, and alcohol consumption
Chen et al. [52••]	Bangladesh	Cross-sectional	66 young adults	Well water, urine	0.5–439 µg/L in well water; 6–209 µg/L in urine	cIMT >0.75 mm	Participants with a higher cIMT (>0.75 mm) in general had higher levels of arsenic exposure than those with a lower cIMT (≤ 0.75 mm), although the difference was not statistically significant
Chen et al. [54]	Bangladesh	Cross-sectional	959 participants	Well water, urine	Median in well water: 41 µg/L	cIMT	A marginally positive association between baseline well-water arsenic and cIMT assessed on average 7 years later and a dose-response relationship of baseline urinary arsenic and urinary MMA% with cIMT during follow-up
Osorio-Yáñez et al. [55]	México	Cross-sectional	199 children aged 3–14 years	Urine	3–135 µg/L in drinking water; mean total urinary arsenic: 74.3 µg/L	cIMT	Minimum cIMT was significantly associated with total urinary arsenic after adjustment for atherogenic factor, BMI-z score, age, and plasma ADMA

ADMA asymmetric dimethylarginine, BMI body mass index, cIMT carotid artery intima-media thickness

Table 2 Epidemiologic studies of arsenic exposure and electrocardiographic abnormalities

Study	Location	Study design	Study population	Arsenic measurement	Arsenic levels	Endpoints	Main findings
Wang et al. [62]	Southwestern Taiwan	Cross-sectional	280 men and 355 women	Well water	Median in villages: 700–930 µg/L	QTc prolongation	A dose–response relationship between three measures of arsenic exposure and the prevalence of QTc prolongation with adjustment for age, sex, hypertension, diabetes mellitus, serum total cholesterol and triglyceride level, BMI, cigarette smoking, and alcohol consumption
Ahmad et al. [59]	Bangladesh	Cross-sectional	50 each in exposed with or without skin lesions and unexposed	Well water	100–800 µg/L in well water, mean 493 µg/L	QTc prolongation	Prevalence of QTc prolongation was significantly higher in arsenic-exposed group with skin lesions than in those without skin lesions or not exposed to arsenic
Yildiz et al. [60]	Turkey	Cross-sectional	40 men each in exposed and unexposed	Well water	422–1,066 µg/L in villages, mean 659 µg/L	QTc	QTc was significantly higher in men exposed to arsenic compared with those unexposed
						QTd	QTd was significantly higher in men exposed to arsenic compared with those unexposed. The proportion of men with a QTd >50 msec was significantly higher in the exposed group than in the unexposed
						TDR	TDR was significantly higher in men exposed to arsenic compared with those unexposed
Mumford et al. [61•]	Inner Mongolia	Cross-sectional	168 men and 145 women	Well water	≤21, 100–300, 430–690 µg/L in households	QTc prolongation	A significant monotonic relationship between well-water arsenic and the prevalence of QTc prolongation with adjustment for age, sex, BMI, and age/BMI interaction
Mordukhovich et al. [63]	USA	Cross-sectional	226 elderly men	Toenail	Median in toenail: 0.069 µg/g, interquartile range: 0.052–0.11 µg/g	QT and QTc	Toenail arsenic is positively associated with QT and QTc interval durations
Chen et al. [64•]	Bangladesh	Cross-sectional	1,715 adults	Well water, urine	0.1–790 µg/L in well water, mean 95 µg/L	QTc prolongation	A positive association between baseline arsenic exposure and the QTc prolongation assessed on average 6 years later. This association

Table 2 (continued)

Study	Location	Study design	Study population	Arsenic measurement	Arsenic levels	Endpoints	Main findings
Wang et al. [65]	Southwestern Taiwan	Cross-sectional	280 men and 355 women	Well water	Median in villages: 700–930 µg/L	QT _D	appeared to be present in women but not in men Cumulative arsenic exposure was dose-dependently associated with QT _D with adjustment for age, sex, hypertension, diabetes mellitus, serum total cholesterol and triglyceride level, BMI, cigarette smoking, and habitual alcohol consumption

BMI body mass index, *QT_c* heart-rate corrected QT, *QT_D* QT dispersion, *TDR* transmural dispersion of repolarization

strongly support a role for arsenic exposure in subclinical atherosclerosis, at both high and low-to-moderate levels.

Electrocardiographic Abnormalities

The QT interval on an electrocardiogram (ECG) is defined as the time from the onset of the QRS complex to the end of the T wave. It represents the duration of ventricular electrical systole, including depolarization and repolarization. Its prolongation indicates nonuniform recovery of myocardial excitability and has been shown to lower the ventricular fibrillation threshold and increase susceptibility to ventricular arrhythmia and sudden cardiac death [56]. The prolongation of heart-rate corrected QT interval (QT_c) has been associated with an increased risk of cardiovascular and all-cause mortality in a broad range of clinical populations as well as in healthy subjects in population-based studies [57, 58]. The effects of arsenic on QT_c prolongation have been the subject of extensive investigation in cross-sectional studies. The first study from Bangladesh showed that the prevalence of prolonged QT_c (QT_c ≥430 msec) was significantly higher in arsenic-exposed individuals (mean 493 µg/L, range 100–800 µg/L in well water) with skin lesions than in those without skin lesions or not exposed to arsenic [59]. In a study from Turkey, QT_c was significantly higher in men (*n*=40) chronically exposed to high levels of arsenic in drinking water (mean 659 µg/L, range 422–1,066 µg/L in villages) compared with men (*n*=40) not exposed to arsenic [60]. In spite of the significant difference in QT_c, the non-corrected QT intervals were actually within normal ranges in both groups [60]. Another study of 313 adults (168 men and 145 women) in Inner Mongolia with well-water arsenic measured at the household level reported a monotonic relationship between well-water arsenic and the prevalence of QT_c prolongation (QT_c ≥450 msec). However, the exposure categories were wide and incomplete (<21, 100–300, 430–690 µg/L) [61]. A recent study of 280 men and 355 women in southwestern Taiwan reported a positive association between measures of long-term arsenic exposure and the prevalence of QT_c prolongation in a dose–response manner [62]. In a US general population of elderly men with much lower levels of arsenic exposure, an interquartile range (0.059 µg/g) increase in toenail arsenic, a biomarker of long-term arsenic exposure, was associated with a 3.8-msec increase in QT (95 % CI 0.82–6.8) and a 2.5-msec increase in QT_c (95 % CI 0.11–4.9) [63]. However, the effects of arsenic exposure on conventional dichotomized QT or QT_c prolongation were not examined and therefore it was not clear whether these increases are clinically relevant. Most recently, a larger study of 1,715 adults in the HEALS in Bangladesh with a wide range of arsenic exposure levels (mean 95 µg/L, range 0.1–790 µg/L in well water) found a positive relationship

Table 3 Epidemiologic studies of arsenic exposure and markers of endothelial dysfunction, inflammation, oxidative stress, and lipids

Study	Location	Study design	Study population	Arsenic measurement	Arsenic levels	Endpoints	Main findings
Chen et al. [70]	Bangladesh	Cross-sectional	115 adults with skin lesions	Well water, urine	3–864 µg/L in well water	Plasma sICAM-1 and sVCAM-1	Baseline well-water arsenic was positively associated with both baseline sVCAM-1 and sICAM-1 levels and more strongly with sVCAM-1, with increasing values in increasing well-water arsenic quartiles. Baseline urinary arsenic was also positively associated with sICAM-1 and sVCAM-1 at baseline; however, only the association with sICAM-1 was significant
Wu et al. [71••]	Bangladesh	Cross-sectional	668 adults	Well water, urine	0.1–500.6 µg/L in well water	Plasma MMP-9, MPO, PAI-1, sE-Selectin, sICAM-1, and sVCAM-1	A positive association between arsenic exposure, measured by well-water arsenic or urinary arsenic, and plasma levels of sICAM-1. PAI-1 levels were elevated in the highest quartile of well-water arsenic (73.5–500.6 µg/L), and the increased levels of PAI-1 were stronger among individuals with BMI
Karim et al. [72]	Bangladesh	Cross-sectional	324 exposed and 106 unexposed	Well water, hair, nail	Mean in well water: 173 µg/L	Plasma Ox-LDL, CRP, sICAM-1, and sVCAM-1	Plasma levels of Ox-LDL, CRP, sICAM-1 and sVCAM-1 were significantly higher among those residing in an arsenic-endemic area than less-exposed counterparts in a nonendemic area, and were significantly correlated with arsenic exposure measured from water, hair, and nail
Banerjee et al. [85]	West Bengal	Cross-sectional	50 exposed and 41 unexposed	Well water, urine	Mean in well water: 218 µg/L	Serum MPO activity	MPO activities were significantly higher in exposed group than the unexposed group; however, no further dose–response relation was found among the exposed group
Osorio-Yáñez et al. [55]	México	Cross-sectional	199 children aged 3–14 years	Urine	3–135 µg/L in drinking water; mean total urinary As: 74.3 µg/L	Plasma ADMA	InAs% was significantly associated with plasma ADMA in multivariable regression analysis
Karim et al. [72]	Bangladesh	Cross-sectional	324 exposed and 106 unexposed	Well water, hair, nail	Mean in well water: 173 µg/L	Plasma TG, TC, LDL, and HDL	TC, LDL, and HDL levels were lower among those residing in an arsenic-endemic area than less exposed counterparts in a nonendemic area, and were significantly correlated with arsenic exposure measured from water, hair, and nail

Table 3 (continued)

Study	Location	Study design	Study population	Arsenic measurement	Arsenic levels	Endpoints	Main findings
Tseng et al. [86]	Southwestern Taiwan	Cross-sectional	533 adults	Well water	Median in villages: 700–930 µg/L	Serum TC, TG, HDL, LDL, ApoA1, and ApoB	Cumulative arsenic exposure, duration of drinking well water, and duration of living in arseniasis hyperendemic villages were positively associated with serum levels of TC and LDL. Cumulative arsenic exposure was marginally inversely related to HDL
Nabi et al. [87]	Bangladesh	Cross-sectional	115 patients with arsenicosis and 80 unexposed	Well water	3–875 µg/L, mean 218 µg/L, median 156 µg/L	Serum TC, TG, HDL, and LDL	Arsenic-exposed subjects had significantly lower levels of TC, HDL, and LDL as compared with the unexposed subjects

ADMA asymmetric dimethylarginine, *BMI* body mass index, *CRP* C-reactive protein, *HDL* high-density lipoprotein, *HDL* low-density lipoprotein, *MMP-9* matrix metalloproteinase-9, *MPO* myeloperoxidase, *Ox-LDL* oxidized low-density lipoproteins, *PAI-1* plasminogen activator inhibitor-1, *sICAM-1* soluble intercellular adhesion molecule 1, *sVCAM-1* soluble vascular adhesion molecule 1, *TC* total cholesterol, *TG* triglyceride

between past and persistent arsenic exposure measured at baseline and QTc prolongation (QTc \geq 450 msec in men and \geq 460 msec in women) assessed during follow-up [64•]. The adjusted OR for QTc prolongation was 1.17 (95 % CI 1.01–1.35) and 1.18 (95 % CI 0.97–1.43) associated with a 1-SD increase in well-water arsenic (108.7 µg/L) and total urinary arsenic (270.7 µg/g creatinine), respectively. The positive association was stronger in women, with adjusted ORs of 1.24 (95 % CI 1.05–1.47) and 1.24 (95 % CI 1.01–1.53) for a 1-SD increase in well-water arsenic and total urinary arsenic, respectively, compared with 0.99 (95 % CI 0.73–1.33) and 0.86 (95 % CI 0.49–1.51) in men.

QT interval dispersion (QTD) is defined as the inter-lead difference between the longest and shortest QTc intervals in the standard 12-lead ECG. Although QTD provides only an imperfect estimate of the degree of heterogeneity of ventricular repolarization, several studies have suggested that QTD helps identify patients at an increased risk of ventricular arrhythmias or clinical events in a variety of clinical settings as well as predicts all-cause and cardiovascular mortality in the general population [58]. In the Turkey study [60], QTD and transmural dispersion of repolarization (TDR), the interval between the peak and the end of the T wave and a better marker of cardiac repolarization, were significantly higher in the exposed group than the unexposed. In a recent study of 280 men and 355 women conducted in the high-exposed area of Taiwan [65], cumulative arsenic exposure was dose-dependently associated with QTD, and prolongation of QTD, defined as QTD \geq 65 msec, predicted all-cause and cardiovascular mortality in this population [65]. Taken together, these data suggest that arsenic exposure may increase QT interval and QTD as one of the underlying mechanisms for increased CVD risk. ECG may be useful for early detection of cardiac toxicity induced by arsenic exposure and for evaluation of populations at high risk for cardiovascular events. Future studies are needed to further examine the associations of lower levels of arsenic exposure with QTD and its prolongation.

Markers of Endothelial Dysfunction, Inflammation, and Oxidative Stress

Adhesion of circulating leukocytes to the endothelium and subsequent transmigration into the arterial wall is an early step in atherosclerotic plaque formation. This process is mediated by cell adhesion molecules (CAMs), such as selectins, intercellular adhesion molecule-1 (ICAM-1), and vascular adhesion molecule-1 (VCAM-1), expressed on the surface of activated endothelial cells in response to inflammatory stimuli. The selectins, including E-selectin and P-selectin, mediate transient rolling of the leukocytes along the endothelium, while stronger attachment is mediated by ICAM-1 and

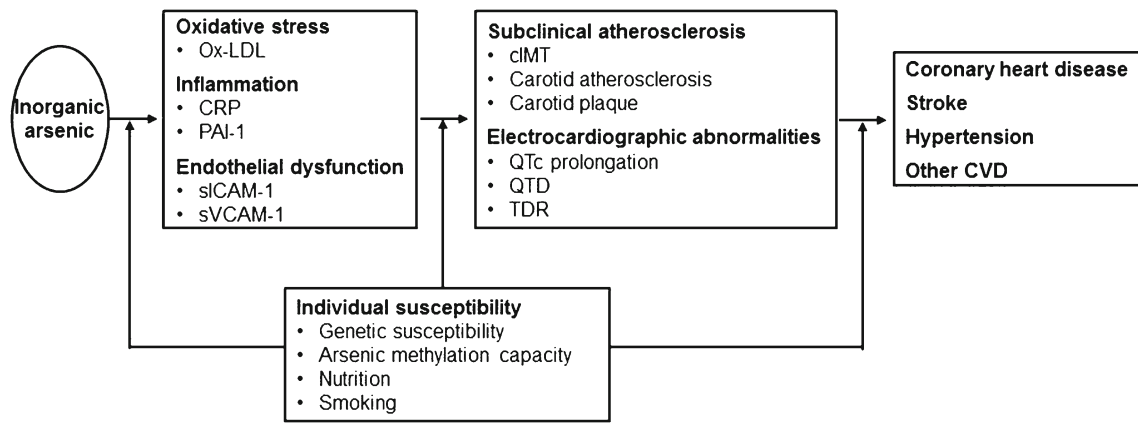


Fig 1 Schematic depiction of epidemiologic evidence on possible mechanisms underlying the cardiovascular effects of arsenic exposure. Epidemiologic studies have demonstrated that arsenic may lead to cardiovascular diseases via its effects on subclinical endpoints which may be modified by individual susceptibility. *Ox-LDL* oxidized low-density

lipoproteins, *CRP* C-reactive protein, *PAI-1* plasminogen activator inhibitor-1, *sICAM-1* soluble intercellular adhesion molecule 1, *sVCAM-1* soluble vascular adhesion molecule 1, *cIMT* carotid artery intima-media thickness, *QTc* heart-rate corrected QT, *QTd* QT dispersion, *TDR* transmural dispersion of repolarization, *CVD* cardiovascular disease

VCAM-1 [66]. Circulating markers of systemic inflammation and endothelial dysfunction, such as the soluble parts of CAMs (sE-selectin, sICAM-1, and sVCAM-1), have been associated with risk of fatal and nonfatal cardiovascular events [67–69]. In a cross-sectional analysis of 115 adults (20–65 years of age) with arsenic-related skin lesions from Araihasar, Bangladesh, there was a positive association of arsenic exposure at moderate levels (range 3–864 $\mu\text{g/L}$), measured as both well-water arsenic and total urinary arsenic, with plasma levels of sICAM-1 and sVCAM-1 [70]. The same group subsequently conducted independent separate cross-sectional analyses and confirmed a positive association between lower arsenic exposure (median 23 $\mu\text{g/L}$, range 0.1–500.6 $\mu\text{g/L}$) and plasma levels of sVCAM-1 [71••]. However, no significant association was observed for plasma levels of sE-selectin and sICAM-1. Unlike the study of skin lesion patients, this study consisted of mostly healthy subjects free of skin lesions at baseline with a much lower exposure level and therefore excludes the possibility that the observed association may be a consequence of skin lesions. Although the biological explanation for the variability in associations across these CAMs is not clear, the findings support the link between arsenic exposure and elevated concentrations of certain markers of endothelial dysfunction and suggest a potential mechanism underlying the effect of long-term arsenic exposure on CVD. In another study among adults aged 15–60 years old from a northwest region of Bangladesh, circulating levels of sICAM-1 and sVCAM-1 were significantly higher among participants residing in an arsenic-endemic area (mean 173 $\mu\text{g/L}$ in well water, $n=218$) compared with participants from a nonendemic area (mean 2.3 $\mu\text{g/L}$, $n=106$); in addition, sICAM-1 and sVCAM-1 were positively and significantly correlated with arsenic exposure as measured in water, hair, and nail [72]. Additional studies are needed to evaluate the relationship between different CAM molecules and arsenic

exposure levels and determine the specificity of the association with some of the CAMs, but not with others.

Studies have also evaluated other intermediate markers. Oxidation of low-density lipoproteins (LDLs) plays a crucial role in the initiation and progression of atherosclerosis [73] and the levels of oxidized LDL (Ox-LDL) in plasma have been used as a biomarker for oxidative stress in vascular systems [74]. Circulating levels of Ox-LDL have been positively related to presence and incidence of CVD [75–77]. The recent Bangladesh study indicated that arsenic exposure in water and hair was dose-dependently related to increases in plasma levels of Ox-LDL, as well as increases in plasma levels of C-reactive protein (CRP) [72], a circulating acute-phase reactant that reflects active systemic inflammation. These markers were increased in concert with increases in plasma levels of sICAM-1 and sVCAM-1 in this study [72]. These findings are consistent with the experimental evidence that CRP and Ox-LDL induces the expression of ICAM-1 and VCAM-1 in endothelial cells, although proper mediation analyses were not conducted [78]. These data provide insight into the interaction of oxidative stress, inflammatory responses, and endothelial dysfunction in the development of arsenic-induced cardiovascular effects.

In the cross-sectional study of Bangladesh [71••], in addition to the CAMs, the associations between arsenic exposure and plasma levels of plasminogen activator inhibitor-1 (PAI-1), matrix metalloproteinase-9 (MMP-9), and myeloperoxidase (MPO) were also assessed. PAI-1 is a member of the plasminogen activator inhibitors that interact with plasminogen activators to keep the balance between the coagulation and fibrinolytic systems of endothelial cells that are involved with thrombosis. In vitro, sodium arsenite has been shown to increase PAI-1 expression of human microvascular endothelial cells and thus lead to reduced fibrinolytic activity [75]. In mice, PAI-1 expression was time- and dose-dependently increased in cardiac

tissue with exposure to 50–250 $\mu\text{g/L}$ arsenic in drinking water, and plasma PAI-1 levels were increased in mice exposed to >500 $\mu\text{g/L}$ arsenic [79]. Elevated PAI-1 levels appear to increase the risk of atherothrombotic events and may also promote the progression of CVD [80]. PAI-1 levels were elevated in the highest quartile of well-water arsenic (73.5–500.6 $\mu\text{g/L}$), and the association between arsenic exposure and PAI-1 levels was stronger among individuals with higher body mass index [71••]. The findings thus do not preclude that high levels of arsenic exposure may be related to increased levels of PAI-1, particularly in susceptible populations. Additional studies are needed to evaluate the association between arsenic exposure and PAI-1, especially at low-to-moderate levels.

MMP-9 is located at the shoulder region of plaques and areas of foam cell accumulation and plays an important role in several stages of atherosclerosis [81]. MMP-9 expression was time-dependently increased in mice chronically exposed to 50–250 $\mu\text{g/L}$ arsenic in drinking water [79]. MPO has been implicated as a catalyst for LDL oxidation [82] and contributes to the overall reactive oxygen species burden in the physiological milieu. Positive associations of circulating levels of MMP-9 and MPO with CVD have been observed in cohort studies [83, 84]. However, there was no association of either well-water arsenic or urinary arsenic with plasma levels of MMP-9 and MPO in 668 participants selected from the HEALS in Araihasar, Bangladesh [71••]. A recent study in West Bengal ($n=91$) showed that individuals chronically exposed to arsenic (mean 218 $\mu\text{g/L}$ in well water) had significantly higher MPO activities in serum than the unexposed group; however, no further dose–response relation was found among the exposed group [85]. The only study among children from México found that the proportion of InAs was significantly associated with asymmetric dimethylarginine (ADMA), an endogenous inhibitor of nitric oxide synthase that could lead to endothelial dysfunction and cardiovascular events [55]. However, total arsenic levels were not associated with ADMA. The biological importance of these markers in mediating the cardiovascular effects of arsenic exposure needs further investigation.

Lipid Profiles

Few studies have evaluated the effect of arsenic exposure on traditional lipid markers of atherosclerosis, namely, total cholesterol (TC), LDL, high-density lipoprotein (HDL), and triglyceride. Generally, abnormal lipid profiles such as a large amount of TC, especially LDL, and triglyceride, and lower levels of HDL are associated with a higher risk of CVD. In a study of southwestern Taiwan, cumulative arsenic exposure was positively associated with serum levels of TC and LDL; however, this study did not adjust for potential confounding factors such as age [86]. A later study from Bangladesh

showed that arsenic-exposed subjects (median 156 $\mu\text{g/L}$, range 3–875 $\mu\text{g/L}$) had significantly lower levels of TC, HDL, and LDL as compared with the unexposed subjects [87]. A subsequent Bangladesh study observed that serum levels of TC, LDL, and HDL were lower in arsenic-endemic subjects than those of nonendemic subjects, and all these markers were inversely related to arsenic exposure in well water, nail, and hair [72]. No association with triglyceride was observed in any of these studies. Thus, data on the associations between arsenic exposure and lipid profiles are inconsistent, and whether these lipid markers may serve as causal intermediates between arsenic exposure and CVD are far from clear. For instance, in the study of southwestern Taiwan [86], the prevalence of peripheral vascular disease did not differ by lipid levels. Likewise, another study from the same region found no significant association between these lipids and arsenic-related ischemic heart disease [88]. Conversely, another study found a dose–response relationship between serum levels of TC, HDL, LDL, and triglyceride, and carotid atherosclerosis in this high-exposure area of Taiwan [50•]. In the pediatric population of México, plasma lipids were not correlated with total urinary arsenic [55], indicating that arsenic-mediated early effects on atherogenesis may be independent of the lipid profiles.

Susceptibility to Arsenic-Induced Subclinical Endpoints

The cardiovascular effects of arsenic exposure may be complicated by varied individual susceptibilities. Differences in arsenic methylation capacity are believed to be an important susceptibility factor. InAs in drinking water is present as either As^{V} or As^{III} . As^{V} is first reduced to As^{III} , followed by methylation to monomethylarsonic acid (MMA^{V}). After the reduction of MMA^{V} to MMA^{III} , a second methylation can occur to generate dimethylarsinic acid (DMA^{V}), which can be further reduced to DMA^{III} . MMA^{III} is believed to be more toxic than other metabolites and DMA, being more readily excreted in urine and expelled from body. The composition of arsenic metabolites, which are often expressed as percentages of all arsenic species in urine (i.e., InAs%, MMA%, DMA%) or as ratios (i.e., MMA/InAs, DMA/MMA), is indicative of methylation capacity. Incomplete arsenic methylation capacity, often indicated by a higher urinary MMA% or a lower DMA/MMA, has been associated with an increased risk of skin cancer [89, 90], bladder cancer [91, 92], and skin lesions [93]. In the Bangladesh study [54], the association between well-water arsenic and cIMT was stronger among participants with a higher urinary MMA% and MMA/InAs or a lower DMA/MMA [54]. However, there is no evidence that the positive association between high arsenic exposure (median 700–930 $\mu\text{g/L}$) and carotid atherosclerosis differed by DMA/MMA or MMA/InAs [88]. The data suggest that susceptibility

to subclinical atherosclerosis because of lower methylation capacity may be more critical at lower levels of exposure. However, further studies are needed to confirm this hypothesis.

Evidence is also building that genetic polymorphisms may contribute to individual susceptibility. In this regard, the HEALS group recently conducted the largest and most comprehensive candidate gene-based investigation of gene–environment interactions in arsenic-induced CVD risk. A total of 360 single nucleotide polymorphisms (SNPs) and 18 genes were genotyped, and the investigators identified novel SNPs in *ICAM-1* and *VCAM-1* that had significant interactions with well-water arsenic in CVD risk, which remained robust after adjustment for multiple testing [94]. Regarding subclinical cardiovascular endpoints, the majority of evidence comes from a series of studies conducted in Taiwan that found the prevalence of arsenic-related carotid atherosclerosis was modifiable by genetic polymorphisms related to oxidative stress, inflammation, and arsenic metabolism [95–99]. However, these studies have included a limited number of genetic variants and did not have enough sample size to evaluate the effect modification at low levels of exposure. Recently, using a candidate gene approach, the HEALS group conducted a cross-sectional study of 1,078 participants to evaluate whether the association between arsenic exposure and cIMT differs by the 360 SNPs in the 18 genes related to arsenic metabolism, oxidative stress, inflammation, and endothelial dysfunction [100]. The investigators identified several novel SNPs in *arsenic-3-methyltransferase (AS3MT)*, a gene encoding the arsenic methyltransferase capable of methylating InAs to MMA and MMA to DMA, which may synergistically interact with arsenic exposure in subclinical atherosclerosis. Importantly, the at-risk genotypes of these SNPs were positively related to urinary MMA% and negatively related to urinary DMA%, indicating that the effect modification of these SNPs may be through its direct effect on levels of MMA% and DMA% in urine. Supporting this, a recent genome-wide association study (GWAS) from the HEALS identified five SNPs in *AS3MT* that were associated with poor conversion of MMA to DMA, one of which was also associated with an increased risk of skin lesions [101].

Summary and Conclusions

Epidemiologic evidence is accumulating to elucidate the mechanisms by which arsenic causes CVD, and we identified a fairly clear association of arsenic exposure with markers of endothelial dysfunction such as sICAM-1 and sVCAM-1, subclinical atherosclerosis, and QT prolongation — all of which have been implicated as predictors for future cardiovascular events. The association was present both in studies with high levels of arsenic exposure and in studies with low-

to-moderate exposure levels. The evidence of a causal effect of arsenic exposure on other intermediate endpoints, such as markers of oxidative stress and inflammation, QTD, and lipid profiles, however, is not as well established by the small number of studies, the heterogeneity in exposure assessment across studies, and inconsistency across studies. In addition, there are other emerging subclinical cardiovascular endpoints, such as F2-isoprostanes (F2-IsoPs), flow-mediated dilation (FMD), peripheral arterial tonometry (PAT), brachial artery distensibility (BAD), and circulating levels of adiponectin that have not been evaluated. Also, the available data are scarce for in utero and early-life exposure, and much remains to be done before we can fully understand susceptibility to the cardiovascular effects of arsenic exposure.

Studies are lacking on the investigation of arsenic exposure in relation to other emerging subclinical cardiovascular endpoints or markers. F2-IsoPs are specific products of lipid peroxidation that are excreted and detectable in urine and have been positively associated with CVD risk factors in children and adults [102]. Arsenic increases lipid peroxides in cell systems and animal models [103]; in vitro work also observed dose-dependent increases in the levels of 8-isoprostane, the most thoroughly investigated F2-IsoPs, in response to As^{III} exposure [104]. Evidence has suggested that dysregulated production of adipokines by adipose tissue may play a role in the pathogenesis of CVD. Adiponectin, one of the most studied adipokines, has anti-inflammatory and anti-oxidative properties and, in contrast to other adipokines, is protective against obesity and obesity-related disorders [105]. Arsenic exposure suppressed differentiation of human mesenchymal stem cells to adipocytes, leading to decreased expression of adiponectin [106]. In addition, arsenic added to differentiated adipocytes inhibited adiponectin generation [107], although no significant effects of the arsenic exposure on circulating adiponectin in vivo was observed. However, epidemiologic studies on arsenic exposure in relation to F2-IsoPs or adiponectin are lacking. Importantly, noninvasive assessment of endothelial dysfunction has recently received increasing attention. Despite evidence of the effect of arsenic exposure on endothelial dysfunction from experimental studies, no epidemiologic studies have evaluated the association between arsenic exposure and measures of endothelial dysfunction, such as PAT, which measures pulsatile arterial volume changes before and after induced ischemia by nonultrasound-based finger plethysmography to determine endothelial function, or ultrasound-based FMD, a method that has been validated for evaluating endothelial function. Taken together, additional studies are needed to systemically evaluate the effects of arsenic exposure on these emerging subclinical endpoints of CVD.

As mentioned, the differences in arsenic exposure across studies may have limited the causal inference. In the studies from southwestern Taiwan, arsenic exposure was measured

ecologically using the median level of well-water arsenic in a village where the participants resided. The inaccuracy in exposure assessment could lead to an underestimation of arsenic's effect. Such a study design may not be appropriate for investigating effects of arsenic exposure at lower levels, as the misclassification of exposure may mask the association and even lead to biased results. In the US, the heterogeneity of drinking water resources, the limited exposure range, and the relatively high migration within the nation pose challenges for epidemiologic studies to track the change in arsenic exposure at the individual level. In rural Bangladesh, typically all members of a household drink water from a single tube well for decades in the absence of interventions. Soon after the HEALS was established, remediation efforts began to reduce the levels of arsenic exposure in the highly exposed cohort participants and their immediate family members. Therefore, it is possible to track changes in exposure in the families. With repeated measurements of arsenic exposure every 2 years since 2000, the HEALS provides an unprecedented opportunity to evaluate the cardiovascular effects of both continuing arsenic exposure and reduction of exposure. Research on the influence of change in exposure status is crucial to elucidate whether remediation is beneficial to arsenic-induced CVD and other chronic diseases.

Importantly, recent evidence of growing concern indicates that dietary intake of arsenic can significantly contribute to an individual's overall exposure level [3–5], especially in populations with limited exposure to arsenic via drinking water. Children who reported consuming rice had urinary arsenic concentrations that were nearly 40 % higher than those who did not consume rice [4], and rice consumption has been associated with total urinary arsenic, as well as InAs, MMA, and DMA in pregnant women [5]. In the US, an analysis of diet, water, and urinary arsenic data concluded that 75 % of InAs exposure was attributed to diet in households with arsenic in drinking water at concentrations of 10 $\mu\text{g/L}$ or lower [108]. Thus, exposure to InAs from diet should be considered when estimating the health effects of total arsenic exposure. Studies that take advantage of biomarkers of arsenic exposure, such as levels in urine, blood, toenails, and hair, as a measure of exposure from all sources can more precisely examine the effects at lower levels such as those common to the US.

There is highly suggestive evidence that early-life exposure to arsenic causes detrimental pregnancy and birth outcomes, impaired development in infancy and childhood, and increased risks of adverse health effects including cancer and CVD later in life [109]. Importantly, the literature indicates that cardiovascular effects may begin in utero or in early childhood and CVD risk factors measured in adolescence or early adulthood are predictive of CVD up to 50 years later [110]. The examination of the relationship between arsenic exposure in early childhood and intermediate phenotypes for CVD during adolescence and young adulthood may help

establish a causal role of early-life arsenic exposure in the development of CVD in later life and provide avenues for early intervention. As indicated above, however, epidemiologic studies on this topic are very limited. A life-course approach offers a way to conceptualize how underlying environmental determinants of health, experienced at different life-course stages, can differentially influence the risk of chronic diseases [111, 112].

Although it is recognized that many common diseases are a result of complex interplays of genetic and environmental factors, studies of gene-environment interactions in CVD and its subclinical surrogates relevant to arsenic exposure are just emerging. The few existing studies have used a candidate gene approach irrespective of the sample sizes and the number of SNPs included. While this approach has such strengths as biological plausibility and clear *a priori* hypotheses, it is always open to bias given the undefined or even controversial functional significance of some SNPs, the multifaceted nature of mechanistic pathways, and the existence of genes or SNPs that may have important roles in disease etiology but are not yet known. An alternative approach is to use GWAS, which allows for an unbiased screening of genetic variants, or whole genome sequencing that has the advantage of discovering rare variants. Future GWAS with innovative analysis approaches are needed to assess the interaction between arsenic exposure and genetic factors in CVD-related outcomes. Aside from arsenic methylation capacity and genetics, other factors may also influence susceptibility to the health effects of arsenic. For instance, recent experimental findings suggest that gut microbes might be involved in presystemic arsenic metabolism; i.e., arsenic methylation that occurs before arsenic enters the internal environment of our bodies, by converting As^{V} to As^{III} and forming MMA^{V} and MMA^{III} [113, 114]. Whether differences in the gut microbial composition may influence arsenic methylation capacity and subsequently contribute to susceptibility to the subclinical and clinical cardiovascular consequences of arsenic exposure represents a critical gap in our knowledge. A systemic evaluation of these emerging factors along with other well known factors such as nutrition, smoking, and co-exposures is needed.

More than 200 million persons worldwide might be chronically exposed to arsenic in drinking water at concentrations above the World Health Organization (WHO) and US Environmental Protection Agency (EPA) standard of 10 $\mu\text{g/L}$ [115, 116]. Millions more are exposed to arsenic through their diet and no standards for arsenic in foods currently exist. Since CVD is the leading cause of mortality worldwide and arsenic exposure at low-to-moderate levels is more prevalent, a small increased risk associated with arsenic exposure can cause a large number of excess deaths. Prospective analyses of populations with available data on both intermediate endpoints and clinical disease outcomes may be useful to further support

biological plausibility of the putative causal pathways linking arsenic exposure to CVD.

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Compliance with Ethics Guidelines

Conflict of Interest Fen Wu, Peter Molinaro, and Yu Chen declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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