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Increases in Oxidized Low-Density Lipoprotein and Other Inflammatory and Adhesion Molecules With a Concomitant Decrease in High-Density Lipoprotein in the Individuals Exposed to Arsenic in Bangladesh

Md. Rezaul Karim,*,†,[1] Mashiur Rahman,*,[1] Khairul Islam,* Abdullah Al Mamun,* Shakhawoat Hossain,* Ekhtear Hossain,* Abdul Aziz,* Fouzia Yeasmin,* Smita Agarwal,* Md. Imam Hossain,* Zahangir Alam Saud,* Farjana Nikkon,* Mostaque Hossain,‡ Abul Mandal,§ Richard O. Jenkins,¶ Parvez I. Haris,¶ Hideki Miyataka,‖ Seiichiro Himeno,‖ and Khaled Hossain*,[2]

*Department of Biochemistry and Molecular Biology, Rajshahi University, Rajshahi-6205, Bangladesh; †Department of Applied Nutrition and Food Technology, Islamic University, Kushtia-7003, Bangladesh; ‡Department of Medicine, Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorders (BIRDEM), Dhaka, Bangladesh; §System Biology Research Centre, University of Skövde, SE-541-28 Skövde, Sweden; ¶Faculty of Health & Life Sciences, De Montfort University, Leicester, LE1 9BH, UK; and ‖Laboratory of Molecular Nutrition and Toxicology, Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Tokushima 770–8514, Japan

[1]These authors contributed equally to this work.
[2]To whom correspondence should be addressed. Fax: +88-0721-750064. E-mail: khossain69@yahoo.com.

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Elevated exposure to arsenic has been suggested to be associated with atherosclerosis leading to cardiovascular disease (CVD). However, biochemical events underlying the arsenic-induced atherosclerosis have not yet been fully documented. The aim of this study was to investigate the associations of circulating molecules involved in atherosclerosis with arsenic exposure in the individuals exposed to arsenic in Bangladesh. A total of 324 study subjects, 218 from arsenic-endemic areas and 106 from nonendemic areas in Bangladesh, were recruited. Drinking water, hair, nail, and blood samples were collected from the study subjects for analysis. Total cholesterol (TC), low-density lipoprotein (LDL), and high-density lipoprotein (HDL) levels were lower in arsenic-endemic subjects than those of nonendemic subjects. Oxidized LDL (Ox-LDL), C-reactive protein (CRP), intercellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1) levels were significantly higher in arsenic-endemic subjects than those in nonendemic subjects. All these circulating molecules showed significant correlations with arsenic exposure (water, hair, and nail arsenic concentrations), and all these relations were significant before and after adjusting for relevant covariates. Among the circulating molecules tested in this study, HDL, Ox-LDL, and CRP showed dose-response relationships with arsenic exposure. Ox-LDL/HDL ratios were increased with the increasing concentrations of arsenic in the water, hair, and nails. Furthermore, non-HDL cholesterol and TC/HDL ratios were significantly correlated with arsenic exposure before and after adjusting for relevant covariates. Thus, all the observed associations may be the major features of arsenic exposure–related atherosclerosis leading to CVD.

Key Words: **arsenic; atherosclerosis; adhesion molecules; Bangladesh; high-density lipoprotein; oxidized low-density lipoprotein.**

The ingestion of inorganic arsenic through drinking water causes significant health hazards in many countries of the world. The most alarming situation prevails in Bangladesh where significant numbers of arsenicosis cases have been reported in widespread areas, and tens of millions of additional people are currently at risk of arsenic toxicity (Chowdhury *et al*., 2000). Mounting evidence has demonstrated that arsenic exposure increases the morbidity and mortality of cardiovascular disease (CVD) (Chen *et al*., 2011; Cheng *et al*., 2010). Clinical manifestations of arsenic-induced CVD include hypertension, coronary heart disease, stroke, and peripheral arterial diseases (Rahman *et al*., 1999; Tseng *et al*., 2003; Wang *et al*., 2002, 2009).

The blood-circulating molecules including lipoproteins and inflammatory and adhesion molecules have been reported to be involved in the formation of atherosclerotic lesions, and many of them are predictive for atherosclerosis and CVD (Blankenberg *et al*., 2001; Hwang *et al*., 1997). Generally, lipid-related biomarkers such as triglyceride (TG), total cholesterol (TC), low-density lipoprotein (LDL), and high-density lipoprotein (HDL) have been used as markers or surrogates for atherosclerosis (deGoma *et al*., 2008). Recently, oxidized form of LDL (Ox-LDL), rather than total LDL, has created a great attention for its role in the development of atherosclerosis (Heinecke, 1998; Steinberg, 1995). The oxidation of LDL is shown to be involved in the initiation of atherosclerosis (Steinberg, 1997). The levels of Ox-LDL in plasma have been used as a potential marker for oxidative stress in vascular systems (Heinecke, 1998; Steinberg, 1997). On the other hand, many studies have shown that the levels of HDL

are inversely associated with the risk of atherosclerosis (Drexel, 2006; Mertens and Holvoet, 2001). The protective effects of HDL against CVD have been considered to be mediated by its ability to remove cholesterol from artery-wall foam cells via "reverse cholesterol transport." However, recent findings have suggested that multiple functions of HDL, including its antioxidant and anti-inflammatory activities, are also involved in antiatherogenic properties of HDL (Bandeali and Farmer, 2012). Thus, the blood levels of Ox-LDL and HDL may reflect the balance between oxidative or inflammatory stress and antioxidant or anti-inflammatory protections in vascular systems (Mertens and Holvoet, 2001).

Although many epidemiological studies have shown that arsenic exposure increases the risk of atherosclerosis, limited information is available on the changes in biochemical markers for atherosclerosis in individuals exposed to arsenic. Recently, two studies conducted on arsenic-exposed individuals in Bangladesh have reported that arsenic exposure is associated with the increased levels of soluble intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1), suggesting an arsenic-induced pathological activation of endothelial cells (Chen *et al*., 2007; Wu *et al*., 2012). However, no information is available regarding whether arsenic exposure increases Ox-LDL levels and/or decreases HDL levels, and their relationship with other indicators for pro-oxidative and pro-inflammatory events in the vasculature.

In previous studies, we investigated the dose-response relationship between arsenic-exposure metrics and blood biochemical markers such as cholinesterase (Ali *et al*., 2010), lactate dehydrogenase (Karim *et al*., 2010), serum hepatic enzyme activities (Islam *et al*., 2011), and Big endothelin-1 (Big ET-1) (Hossain *et al*., 2012), a precursor of endothelin-1, in the individuals exposed to arsenic in Bangladesh using three kinds of exposure metrics (drinking water, hair, and nails arsenic). Big ET-1 is a marker of endothelial damage/dysfunction, and endothelial dysfunction is the early event of atherosclerosis. The dose-dependent increase in the levels of plasma Big ET-1 with arsenic exposure and its association with hypertension prompted us to investigate the relationship between arsenic exposure and other biochemical markers related to atherosclerosis. Therefore, the aim of this study was to investigate the effects of arsenic exposure on the blood-circulating molecules including lipoproteins, and inflammatory and adhesion molecules in the individuals exposed to arsenic in Bangladesh.

MATERIALS AND METHODS

Study Areas and Subjects

Ethical permission was obtained from The University of Rajshahi Institute of Biological Sciences, Bangladesh (21/320-IAMEBBC/IBSc). The subjects who participated in this study gave their written consent. The arsenic-endemic and nonendemic areas and study subjects were selected as described previously (Ali *et al*., 2010; Hossain *et al*., 2012; Karim *et al*., 2010). Arsenic-endemic study areas were selected from the Northwest region of Bangladesh that included Marua in Jessore; Dutpatila, Jajri, Vultie, and Kestopur in Chuadanga; and Bheramara in Kushtia districts. Chowkoli, a village in Naogaon district with no history of arsenic contamination, was selected as nonendemic area. The adults (15–60 years of ages) who had lived for at least last 5 years in the arsenic-endemic and nonendemic areas were recruited for this study. During the sample collection process, we were blinded to arsenic levels in the drinking water and to those in the hair and nails of the study participants. Attempt was made to match, as much as possible, the following: age, sex, and socioeconomic parameters (occupation, monthly income and education) of arsenic-endemic and nonendemic study subjects. The ratios of endemic and nonendemic subjects were approximately 2:1, and male and female ratios in both endemic and nonendemic areas were also approximately 1:1. Endemic and nonendemic study subjects were individually matched on age (± 5 years). Further, both endemic and nonendemic study subjects were villagers, and other socioeconomic parameters such as occupation, monthly income, and education levels were almost closely matched.

Pregnant and lactating women, Hepatitis B positive, and the individuals who had a history of drug addiction, chronic alcoholism, prescription of hepatotoxic and antihypertensive drugs, malaria, kalazar, and hepatic, renal or cardiac diseases were excluded from this study. Of the 225 individuals recruited in arsenic-endemic areas, seven individuals were excluded according to the above-mentioned criteria. In nonendemic areas, four individuals were excluded from the 110 individuals recruited. Thus, the numbers of participants from arsenic-endemic and nonendemic areas were 218 and 106, respectively.

The interview of the study subjects was carried out by the trained members of the research team by visiting each household and using a standard questionnaire. Information obtained from the interview included the sources of water for drinking and daily household uses, water consumption history, socioeconomic status, occupation, food habit, cigarette smoking, alcohol intake, personal and family medical history, history of diseases, physiological complications, major diseases, previous physician's reports, and body mass index (BMI).

Hair and Nail Collections and Arsenic Analysis

Hair and nails of the study subjects were collected and washed by the method described previously (Ali *et al*., 2010). The washed samples were allowed to dry at 60°C overnight and digested with concentrated nitric acid using a hot plate at 70°C for 15 min and 115°C for 15 min. After cooling, the samples were diluted with 1.0% nitric acid containing yttrium (10 ppb). The concentrations of arsenic and yttrium in these samples were determined by Inductively Coupled Plasma Mass Spectroscopy (ICP-MS, HP-4500, Agilent Technologies, Kanagawa, Japan). All samples were determined in triplicate, and the average values were used. Accuracy of arsenic measurement was verified using a certified reference material (CRM) "human hair" (GBW09101, Shanghai Institute of Nuclear Research Academia Sinica, China). The average value of arsenic in human hair determined in triplicate followed by ICP-MS analysis was 0.61 ± 0.12 µg/g (reference value, 0.59 µg/g).

Water Collection and Arsenic Analysis

Water samples were collected from the tube wells which the study subjects used as a primary source of drinking water as described previously (Ali *et al*., 2010). Total arsenic concentration in water samples was determined by ICP-MS after the addition of a solution of yttrium (10 ppb in 1.0% nitric acid) as an internal standard for ICP-MS analysis. Accuracy of ICP-MS determination of water arsenic concentration was confirmed using "river water" (NMIJ CRM 7202-a No.347 National Institute of Advanced Industrial Science and Technology, Japan) as a CRM. The average value (mean ± SD) of arsenic in the "river water" determined in triplicate by ICP-MS analysis was 1.06 ± 0.04 µg/l (reference value, 1.18 µg/l).

Blood Pressure Measurement

The standard protocol for measuring blood pressure recommended by the World Health Organization was used in this study. After study subjects had rested for 20 min or longer, both systolic and diastolic blood pressures (SBP

and DBP) were measured three times with a mercury sphygmomanometer with subjects sitting. SBP and DBP were defined at the first and fifth phase Korotkoff sounds, respectively. The average of three measurements was used for the analysis. Hypertension was defined as a SBP of ≥ 140 mm Hg and a DBP of ≥ 90 mm Hg on three repeated measurements.

Collection of Plasma

The study participants were requested to fast overnight (10–12 h). Fasting blood samples (5–7ml) were collected in ethylenediaminetetraacetic acid (EDTA)-containing blood collection tubes from each individual by venipuncture. Whole blood was then placed immediately on ice and subsequently centrifuged at 1600 × g for 15 min at 4°C. Plasma supernatant was then taken and stored at −80°C.

Measurements of Plasma TG, TC, LDL, and HDL

The plasma levels of TG, TC, LDL, and HDL were measured by commercially available kits from Human Diagnostic, Germany, according to the manufacture's protocol with an analyzer (CHEM-5 V3, Erba, Mannheim, Germany).

Measurements of Plasma Ox-LDL, CRP, ICAM-1, and VCAM-1

Plasma levels of Ox-LDL, CRP, ICAM-1, and VCAM-1 were measured using commercially available enzyme-linked immunoassay kits for Ox-LDL (Mercodia, Uppsala, Sweden), CRP and VCAM-1 (R&D Systems, Inc. Minneapolis, MN), and ICAM-1 (Invitrogen Corporation, Camarillo, CA) according to the manufacture's protocols. A microplate reader (Mikura Ltd., UK) was used for the measurement of color development. All standards and samples were analyzed in duplicate.

Statistical Analysis

Statistical analysis for this study was performed using software of Statistical Packages for Social Sciences (SPSS version 17.0, SPSS Inc., Chicago, IL). Statistical analyses were performed after log transformation of the data due to the skewed distribution of the raw data. The differences in descriptive characteristics and the profiles of plasma circulating molecules between the study subjects of arsenic-endemic and nonendemic areas were analyzed by independent sample *t*-test and chi-square test. Spearman correlation coefficient tests were used to evaluate the correlations between the circulating molecules and the arsenic-exposure metrics (water, hair, and nail arsenic). Multivariate linear regression analyses were performed to assess the associations of arsenic-exposure metrics with circulating molecules before and after adjusting for age, sex, BMI, smoking, hypertension, occupation, education, and monthly income. To test the dose-response relationship, the study subjects of arsenic-endemic areas were split into tertile (low, medium, and high) groups based on the three concentrations of arsenic in water, hair, and nails. Nonendemic subjects were used as one group for the comparison. The dose-response relationships were analyzed by one-way ANOVA followed by Bonferroni multiple comparison test. A value of $p < 0.05$ was considered statistically significant.

RESULTS

Descriptive Characteristics of the Participants

Table 1 shows the characteristics of the study subjects in the arsenic-endemic (n = 218) and nonendemic areas (n = 106). Because attempts were made to match age, sex, and socioeconomic parameters (occupation, monthly income, and education) between arsenic-endemic and nonendemic study subjects, no significant differences were observed in those parameters between the two study groups. No female was found to be a smoker. None of the study subjects drank alcohol. The average level of BMI of the study subjects in arsenic-endemic areas was slightly lower than that in nonendemic area. Arsenic concentrations in drinking water, hair, and nails of the arsenic-endemic subjects were approximately 75, 17, and 7.5 times higher, respectively, than those in nonendemic subjects. The average levels of DBP and SBP in arsenic-endemic study subjects were significantly higher than that in nonendemic subjects.

Levels of Plasma Circulating Molecules Related to Atherosclerosis

Table 2 shows the plasma levels of TG, TC, LDL, HDL, Ox-LDL, CRP, ICAM-1, and VCAM-1 in arsenic-endemic and nonendemic study subjects. No significant difference was

TABLE 1
Descriptive Characteristics of the Study Subjects in Arsenic-Endemic and Nonendemic Areas

Parameters	Nonendemic	Arsenic-endemic	*p*
Total subjects (*n*)	106	218	
Sex (*n*)			
Male	54	118	
Female	52	100	
Age	35.04 ± 10.93	37.55 ± 11.71	0.059[a]
Duration of residence (years)	28.43 ± 12.76	31.35 ± 13.95	0.06[a]
As concentration in drinking water (μg/l)	2.30 ± 2.77	173.46 ± 156.59	<0.001[a]
As concentration in hair (μg/g)	0.33 ± 0.25	5.63 ± 6.41	<0.001[a]
As concentration in nail (μg/g)	1.25 ± 1.32	9.27 ± 6.79	<0.001[a]
SBP (mm Hg)	110.47 ± 14.43	120.76 ± 17.45	<0.001[a]
DBP (mm Hg)	70.10 ± 9.51	78.65 ± 10.82	<0.001[a]
Occupation (*n*, [%])			
Male			
Farmers	44 (81.48)	99 (83.9)	
Business	1 (1.9)	3 (2.5)	
Students	4 (7.4)	4 (3.4)	
Tailors	1 (1.9)	3 (2.5)	
Others[b]	4 (7.6)	9 (7.5)	
Female			0.242[c]
Housewives	47 (90.4)	91 (91)	
Farm workers	2 (3.8)	3 (3)	
Students	0	3 (3)	
Others[d]	3 (5.8)	3 (3)	
Education (*n*, [%])			
No formal education	60 (56.6)	118 (54.1)	
Primary	40 (37.7)	77 (35.3)	0.504[c]
Secondary	5 (4.7)	21 (9.6)	
Higher	1 (0.9)	2 (0.9)	
Income/month (U.S.$)	23.21 ± 5.46	23.96 ± 8.37	0.332[a]
Hypertension (*n*, [%])			
Yes	2 (1.9)	30 (13.8)	<0.01[c]
No	104 (98.1)	188 (86.2)	
Smoking in male (*n*, [%])			
Yes	21 (38.89)	44 (37.29)	0.841[c]
No	33 (61.11)	74 (62.71)	
BMI (kg/m^2)	21.20 ± 2.75	20.5 ± 3.11	<0.05[a]

Note. Data were presented as mean ± SD. As, arsenic.
[a]*p*-values were from the independent sample *t*-test.
[b]Others included village doctor, carpenter, rickshaw puller, security guard, and retired worker.
[c]*p*-values were from the chi-square test.
[d]Others included farmer and laborer.

found in plasma TG levels between the two groups. The levels of TC, LDL, and HDL were significantly lower in arsenic-endemic subjects than in nonendemic subjects. On the other hand, the levels of Ox-LDL, CRP, ICAM-1, and VCAM-1 were significantly higher in arsenic-endemic group than in nonendemic group.

Relationship Between Arsenic-Exposure Metrics and Plasma Circulating Molecules

Table 3 shows the correlation between arsenic-exposure metrics (water, hair, and nail arsenic concentrations) and plasma circulating molecules involved in atherosclerosis. No significant correlation was observed between arsenic-exposure metrics and plasma TG levels. Plasma levels of TC, LDL, and HDL showed significantly negative correlations with arsenic-exposure metrics. On the contrary, plasma levels of Ox-LDL, CRP, ICAM-1, and VCAM-1 showed significantly positive correlations with arsenic exposure. We next examined the correlation of Ox-LDL/HDL ratios with arsenic-exposure metrics. Interestingly, Ox-LDL/HDL ratios were found to be strongly correlated with arsenic-exposure metrics (Table 3 and Fig. 1). All the associations of plasma circulating molecules with arsenic-exposure metrics were statistically significant before and after adjusting for age, sex, BMI, smoking, hypertension, occupation, education, and monthly income (Table 4 and Supplementary table 1).

TABLE 2
Levels of Plasma Circulating Molecules Related to Atherosclerosis in the Study Subjects From Arsenic-Endemic and Nonendemic Areas

Parameters	Nonendemic	Arsenic-endemic	*p*
TG (mg/dl)	103.6 ± 47	98.3 ± 39.3	0.312
TC (mg/dl)	147.3 ± 35.2	127.2 ± 32.1	<0.001
LDL (mg/dl)	88.7 ± 31.9	73.6 ± 31.1	<0.001
HDL (mg/dl)	42.9 ± 14.1	30.9 ± 10.1	<0.001
Ox-LDL (U/l)	39.7 ± 11	51.5 ± 19.6	<0.001
CRP (mg/l)	0.78 ± 0.88	1.92 ± 2.47[a]	<0.001
ICAM-1 (ng/ml)	371.4 ± 112.2	529.3 ± 154.3	<0.001
VCAM-1 (ng/ml)	420.3 ± 129.9[b]	606 ± 222.7	<0.001

Note. Data were presented as mean ± SD. Differences were analyzed by independent sample *t*-test.
[a]Data were missing on CRP for 11 study subjects in arsenic-endemic areas.
[b]Data were missing on VCAM-1 for eight study subjects in nonendemic area.

TABLE 3
Correlations of Arsenic Exposure Metrics With Plasma Circulating Molecules

	Water arsenic	Hair arsenic	Nail arsenic
TG	−0.068	−0.019	−0.040
TC	−0.218**	−0.209**	−0.207**
LDL	−0.117*	−0.137*	−0.155**
HDL	−0.387***	−0.368***	−0.369***
Ox-LDL	0.361***	0.403***	0.327***
CRP	0.354***	0.339***	0.277***
ICAM-1	0.371***	0.376***	0.334***
VCAM-1	0.313***	0.372***	0.300***
Ox-LDL/HDL	0.440***	0.446***	0.419***

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Dose-Response Relationship of Arsenic Exposure With Circulating Molecules

Dose-response relationships were tested in nonendemic and tertile (low, medium, and high) groups of arsenic-endemic subjects. Results in Table 5 showed that there were some general trends in changing TC, LDL, HDL, ICAM-1, and VCAM-1 in the low, medium, and high arsenic-exposure groups. TC, LDL, HDL, ICAM-1, and VCAM-1 were significantly changed in the higher (low, medium, and high) exposure groups compared with the nonendemic group with the exceptions of LDL levels in the high groups of water, hair, and nail arsenic concentrations. Among the plasma biomarkers, Ox-LDL, and CRP showed relatively good shape of dose-response relationships with arsenic exposure. Gradual increasing tendencies of Ox-LDL levels were observed in the higher exposure groups compared with the lower groups of water and nail arsenic concentrations, but these differences were only significant in the higher exposure groups compared with the nonendemic groups. Ox-LDL levels were increased in the higher exposure groups of hair arsenic concentrations, and the levels were significantly higher in the medium and high groups compared with the nonendemic group. Additionally, Ox-LDL levels were significantly different for high versus low and high versus medium groups of hair arsenic concentrations. CRP levels were also gradually increased in the higher exposure gradients except the medium group of hair arsenic concentrations. CRP levels in the endemic tertile groups of water, hair, and nail arsenic concentrations were also significantly different from the nonendemic group with the exception of the low groups of water and nail arsenic concentrations. Moreover, CRP levels were significantly different for medium versus low, high versus medium, and high versus low of water arsenic concentrations.

Correlations of Plasma Circulating Molecules Changed by Arsenic Exposure

Finally, we investigated the correlations between the levels of Ox-LDL, HDL, and Ox-LDL to HDL ratios with those of HDL, CRP, ICAM-1, and VCAM-1 (Supplementary table 2). Plasma levels of Ox-LDL showed a negative correlation with HDL and positive correlations with CRP, ICAM-1, and VCAM-1. The ratios of Ox-LDL to HDL also showed significantly positive correlations with CRP, ICAM-1, and VCAM-1. On the other hand, HDL showed significantly negative correlations with CRP, ICAM-1, and VCAM-1.

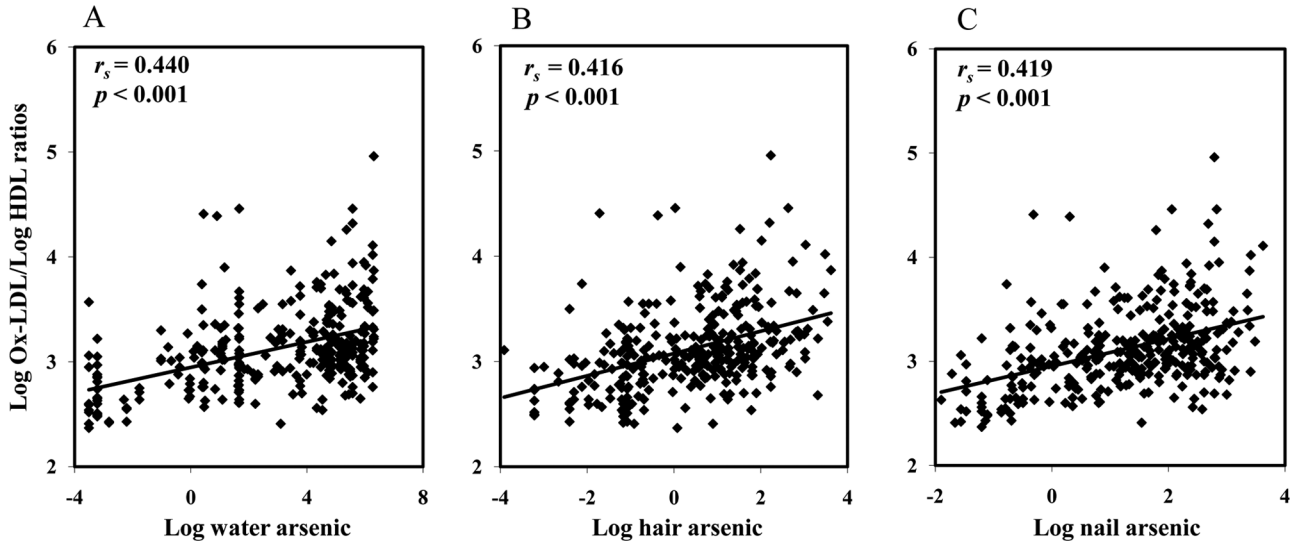

FIG. 1. Correlation between Ox-LDL/HDL ratios and water (A), hair (B), and nail (C) arsenic concentrations. Log-transformed values of arsenic concentrations, Ox-LDL, and HDL were used.

Associations of Non-HDL Cholesterol and TC-to-HDL Cholesterol With Arsenic Exposure

Next, we tested the associations of arsenic exposure with non-HDL (TC minus HDL) cholesterol and TC/HDL ratios (Supplementary table 3). Multivariate analysis suggested that arsenic exposure were significantly associated with the increased levels of non-HDL and TC/HDL ratios before and after adjusting for age, sex, BMI, smoking, hypertension, occupation, education, and monthly income.

DISCUSSION

In this present study, we demonstrated the characteristic changes in biochemical indicators for atherosclerosis; low levels of TC, LDL, and HDL, and high levels of Ox-LDL, CRP, ICAM-1, and VCAM-1 among the residents in arsenic-endemic areas in Bangladesh compared with the nonendemic residents. Significant associations of arsenic exposure with those biochemical indicators were found. Further, in dose-response relationship, although there were some general trends in changing plasma biomarkers in the higher exposure gradients compared with the lower exposure gradients, but the significant changes of the plasma markers were largely limited to nonendemic versus each tertile of endemic subjects. Relatively, Ox-LDL and CRP showed good shape of dose-response relationships with hair and water arsenic concentrations, respectively.

Accumulating evidence has shown that arsenic exposure enhances the risk of vascular diseases in Bangladesh and in Taiwan (Chen *et al*., 2011; Tseng *et al*., 2003). Development of atherosclerosis is the fundamental underlying mechanism of CVD. Preclinical manifestations of atherosclerosis as examined by carotid artery plaques and thickness have been observed in the residents exposed to arsenic both in Taiwan (Wang *et al*., 2009) and in Bangladesh (Chen *et al*., 2006). However, very few studies have reported the changes in biochemical indicators for atherosclerosis in human exposed to arsenic (Chen *et al*., 2007; Wu *et al*., 2012).

Recently, the significance of biochemical indicators for pro-oxidative and pro-inflammatory lesions such as Ox-LDL, ICAM-1, VCAM-1, and CRP rather than traditional lipid markers such as TC and LDL has been highlighted for assessing the development of atherosclerosis. This study for the first time provided evidence that plasma Ox-LDL levels were increased in the individuals exposed to arsenic. Modified LDL such as Ox-LDL interacts with monocytes and macrophages to form foam cells in the artery (Aviram, 1999). Furthermore, Ox-LDL is shown to induce the production of pro-inflammatory cytokines and adhesion molecules, thereby promoting the development of atherosclerosis via activation of endothelial cells and smooth muscle cells (Galle *et al*., 2006). The increase in plasma Ox-LDL levels among arsenic-endemic individuals suggests either enhanced oxidative stress or reduced protection against the oxidation of LDL, or both. In addition, the inverse correlation between Ox-LDL and HDL found in this study (Supplementary table 2) suggests that the decrease level of HDL may not show enough protection against the oxidation of LDL to Ox-LDL that may cause the elevation of Ox-LDL levels in plasma. In support of this notion, antioxidative effects of HDL on the oxidation of LDL have been reported (Mertens and Holvoet, 2001; Podrez, 2010). On the other hand, LDL levels were shown to be lower in arsenic-endemic subjects than in nonendemic subjects (Table 2). Traditionally, high LDL and low HDL levels have been implicated in the development of atherosclerosis. However, recent studies have shown that the

TABLE 4
Association Between Arsenic Exposure Metrics and Plasma Circulating Molecules in All Study Individuals Through Multivariate Linear Regression Analysis

	Independent variable			
	Water arsenic		Hair arsenic	
	IQR[a]		IQR[b]	
Dependent variable	Before adjustment	After adjustment	Before adjustment	After adjustment
TC				
β-Coefficient (95% CI)	−0.023 (−0.033, −0.013)	−0.025 (−0.035, −0.015)	−0.034 (−0.053, −0.016)	−0.038 (−0.057, −0.02)
Change by IQR (95% CI)[c]	−0.12 (−0.13, −0.11)	−0.13 (−0.14, −0.12)	−0.05 (−0.07, −0.03)	−0.056 (−0.075, −0.036)
LDL				
β-Coefficient (95% CI)	−0.022 (−0.039, −0.005)	−0.028 (−0.045, −0.011)	−0.036 (−0.067, −0.005)	−0.04 (−0.078, −0.015)
Change by IQR (95% CI)	−0.115 (−0.132, −0.097)	−0.146 (−0.164, −0.128)	−0.053 (−0.084, −0.021)	−0.058 (−0.09, −0.027)
HDL				
β-Coefficient (95% CI)	−0.056 (−0.069, −0.044)	−0.054 (−0.068, −0.041)	−0.090 (−0.114, −0.065)	−0.085 (−0.11, −0.059)
Change by IQR (95% CI)	−0.292 (−0.305, −0.278)	−0.281 (−0.295, −0.268)	−0.131 (−0.157, −0.106)	−0.124(−0.157, −0.099)
Ox-LDL				
β-Coefficient (95% CI)	0.043 (0.031, 0.055)	0.041 (0.029, 0.053)	0.088 (0.067, 0.11)	0.086 (0.064, 0.108)
Change by IQR (95% CI)	0.224 (0.212, 0.236)	0.214 (0.202, 0.225)	0.129 (0.107, 0.150)	0.126 (0.104, 0.147)
CRP				
β-Coefficient (95% CI)	0.136 (0.085, 0.188)	0.139 (0.084, 0.193)	0.27 (0.175, 0.364)	0.276 (0.177, 0.374)
Change by IQR (95% CI)	0.709 (0.658, 0.759)	0.724 (0.669, 0.779)	0.394 (0.3, 0.489)	0.403 (0.305, 0.501)
ICAM-1				
β-Coefficient (95% CI)	0.045 (0.033, 0.058)	0.042 (0.029, 0.055)	0.096 (0.073, 0.119)	0.091 (0.068, 0.114)
Change by IQR (95% CI)	0.234 (0.221, 0.248)	0.219 (0.205, 0.233)	0.14 (0.117, 0.164)	0.133 (0.109, 0.156)
VCAM-1				
β-Coefficient (95% CI)	0.039 (0.025, 0.052)	0.036 (0.023, 0.05)	0.09 (0.066, 0.114)	0.086 (0.062, 0.110)
Change by IQR (95% CI)	0.203 (0.189, 0.217)	0.188 (0.174, 0.201)	0.131 (0.108, 0.155)	0.125 (0.102, 0.149)
Ox-LDL/HDL				
β-Coefficient (95% CI)	0.061 (0.047, 0.074)	0.058 (0.044, 0.072)	0.106 (0.081, 0.131)	0.100 (0.074, 0.127)
Change by IQR (95% CI)	0.318 (0.304, 0.332)	0.302 (0.288, 0.316)	0.155 (0.129, 0.18)	0.146 (0.121, 0.172)

Note. Log-transformed dependent and independent variables were used. Age, sex, BMI, smoking, hypertension, occupation, education, and monthly income were adjusted as covariates. CI, confidence interval; IQR, interquartile range.
[a]Water IQR (2.98, 186)
[b]Hair IQR (0.393, 4.703).
[c]Change in outcome associated with an IQR increase in arsenic.

low level of HDL is an independent risk factor for coronary heart disease (deGoma *et al*., 2008; Ginsberg, 2000). Although in this study we did not provide any direct evidence, but the decreased levels of HDL in arsenic-exposed individual may be associated with the development of atherosclerosis. A cohort-based further investigation is needed to show whether low HDL levels are significantly implicated with the development of atherosclerosis in arsenic-exposed individuals.

ICAM-1 and VCAM-1 expressed in endothelial cells play important roles in the recruitment and transendothelial migration of leukocytes, leading to the initiation of atherosclerosis (Galkina and Ley, 2007). Reactive oxygen species, pro-inflammatory cytokines, CRP, and Ox-LDL have been shown to induce the expression of ICAM-1 and VCAM-1 in the endothelial cells, resulting in the elevation of soluble forms of ICAM-1 and VCAM-1 in blood plasma (Cook-Mills *et al*., 2011; Zhang *et al*., 2012). The studies conducted previously in the different areas of Bangladesh showed slight but significant increases in soluble ICAM-1 and VCAM-1 levels in plasma of arsenic-endemic residents (Chen *et al*., 2007; Wu *et al*., 2012). In this present study, however, plasma ICAM-1 and VCAM-1 levels were increased in concert with those of Ox-LDL and CRP among arsenic-endemic individuals. These results provide much clearer insight into the interaction of pro-oxidative and pro-inflammatory events induced by arsenic exposure and the expression of ICAM-1 and VCAM-1 in endothelial cells.

Emerging evidence has suggested an important role of CRP both as a powerful predictor of CVD and as a player in the development of atherosclerosis (Blake *et al*., 2003). The circulating CRP, as well as endogenously produced CRP, is known to induce the release of pro-inflammatory cytokines from monocytes (Ballou and Lozanski, 1992) and promote the expression of ICAM-1 and VCAM-1 in endothelial cells (Wadham *et al*., 2004). Furthermore, CRP accelerates the monocyte adhesion

TABLE 5
Dose-Response Relationship Between Arsenic Exposure Levels in Water, Hair, and Nails, and the Levels of Plasma Circulating Molecules

Dependent variable	Independent variable	Nonendemic	Low	Medium	High	*p*-value (*F*-test)
TC (mg/dl)	Water As	147.3 ± 35.2	124.5 ± 32.7[a]	128.6 ± 26.9[a]	128.4 ± 36.3[a]	<0.001
	Hair As		128.5 ± 34.5[a]	122.6 ± 26.5[a]	130.4 ± 34.7[a]	<0.001
	Nail As		126.6 ± 34.6[a]	125.8 ± 32.4[a]	129.0 ± 29.7[a]	<0.001
LDL (mg/dl)	Water As	88.69 ± 31.86	68.4 ± 29.0[a]	71.8 ± 26.8[a]	80.4 ± 35.7	<0.001
	Hair As		70.0 ± 30.0[a]	70.2 ± 25.1[a]	80.5 ± 36.6	<0.001
	Nail As		73.2 ± 33.8[a]	71.8 ± 29.0[a]	75.8 ± 30.6	<0.001
HDL (mg/dl)	Water As	42.87 ± 14.09	32.1 ± 9.9[a]	31.2 ± 9.9[a]	29.4 ± 10.3[a]	<0.001
	Hair As		31.1 ± 9.2[a]	31.3 ± 10.7[a]	30.3 ± 10.4[a]	<0.001
	Nail As		32.2 ± 10.0[a]	30.3 ± 8.5[a]	30.3 ± 11.5[a]	<0.001
Ox-LDL (U/l)	Water As	39.7 ± 11.0	48.9 ± 14.7[a]	49.0 ± 15.1[a]	56.5 ± 26.0[a]	<0.001
	Hair As		44.4 ± 12.1	50.3 ± 15.5[a]	59.5 ± 25.5[a,b,c]	<0.001
	Nail As		49.3 ± 16.3[a]	49.9 ± 15.7[a]	55.4 ± 24.9[a]	<0.001
CRP (mg/l)	Water As	0.78 ± 0.88	1.15 ± 1.53	1.75 ± 1.96[a,b]	2.82 ± 3.28[a,b,c]	<0.001
	Hair As		1.74 ± 2.22[a]	1.37 ± 1.59 [a]	2.64 ± 3.19[a]	<0.001
	Nail As		1.39 ± 1.68	1.99 ± 2.29[a]	2.33 ± 3.14[a]	<0.001
ICAM-1 (ng/ml)	Water As	371.4 ± 112.2	518.1 ± 155.2[a]	520.3 ± 135.0[a]	549.2 ± 170.3[a]	<0.001
	Hair As		548.9 ± 160.1[a]	519.3 ± 137.2[a]	520.5 ± 164.5[a]	<0.001
	Nail As		530.9 ± 177.8[a]	533.5 ± 143.2[a]	523.8 ± 141.5[a]	<0.001
VCAM-1 (ng/ml)	Water As	420.3 ± 129.9	589.7 ± 184.8[a]	604.1 ± 216.3[a]	623.7 ± 261.1[a]	<0.001
	Hair As		605.1 ± 220.1[a]	588.4 ± 201.3[a]	624.2 ± 245.7[a]	<0.001
	Nail As		602.1 ± 237.6[a]	627.3 ± 200.1[a]	589.1 ± 229.9[a]	<0.001

Note. Data were presented as mean ± SD. Statistically significant association between exposure level and the levels of circulating molecules in one-way ANOVA was examined by *F*-test, followed by Bonferroni multicomparison test between each group of exposure level. As levels in water; nonendemic (0.03–13.17 μg/l; $n = 106$), low (0.46–69.4 μg/l; $n = 72$), medium (76–205 μg/l; $n = 72$), and high (214–546 μg/l; $n = 74$). As levels in hair; nonendemic (0.03–1.62 μg/g; $n = 106$), low (0.25–2.37 μg/g; $n = 71$), medium (2.45–4.95 μg/g; $n = 73$), and high (5–37.24 μg/g; $n = 74$). As levels in nail; nonendemic (0.15–8.13 μg/g; $n = 106$), low (0.53–5.14 μg/g; $n = 72$), medium (5.21–10.65 μg/g; $n = 72$), and high (10.67–37.42 μg/g; $n = 72$). As, arsenic.

[a]Significantly difference from nonendemic group.
[b]Significantly different from "low" group.
[c]Significantly different from "medium" group.

to endothelial cells and the uptake of Ox-LDL by endothelial cells via the activation of LOX-1, which is a receptor for Ox-LDL (Li *et al*., 2004). Thus, the increases in plasma levels of ICAM-1 and VCAM-1 concomitantly with CRP in arsenic-endemic residents suggest that arsenic-induced inflammatory events are involved in pathological activation of ICAM-1 and VCAM-1 in the endothelial cells.

In this present study, we found that the levels of HDL were inversely correlated with the levels of Ox-LDL, CRP, ICAM-1, and VCAM-1 (Supplementary table 2). Recently, it has been indicated that antiatherogenic activities of HDL are attributable to much broader functions of HDL than expected before (Podrez, 2010). In addition to the well-documented role in reverse cholesterol transport, HDL has been shown to possess antioxidative, anti-inflammatory, and antithrombotic effects. The results of this study are consistent with the reported functions of HDL in the protection against LDL oxidation (Kontush and Chapman, 2010) and the reduction in CRP-induced expression of adhesion molecules in endothelial cells (Wadham *et al*., 2004). In this study, we found the positive correlations between arsenic-exposure metrics and ratios of Ox-LDL/HDL (Table 3, Fig. 1). Ratios of Ox-LDL/HDL are also positively correlated with plasma CRP, ICAM-1, and VCAM-1 levels (Supplementary table 2). Recently, Ox-LDL/HDL has been recognized as the most useful and independent biochemical marker for atherosclerosis (Lankin *et al*., 2011). Therefore, increased Ox-LDL/HDL ratios with the increasing levels of arsenic exposure and their correlations with other inflammatory and adhesion molecules observed in this study may provide important clues to developing future CVD in individuals living in arsenic-endemic areas. Further, non-HDL cholesterol and ratios of total cholesterol to HDL cholesterol have been suggested to be predictors of cardiovascular diseases (von Mühlen *et al*., 2003). In this study, we also found that non-HDL cholesterol and TC/HDL ratios were increased with the increasing concentrations of arsenic in the water, hair, and nails (Supplementary table 3). All these observed associations were independent of age, sex, BMI, smoking, hypertension, occupation, education, and monthly income. These results further provide evidence that arsenic exposure may be a potent risk factor for CVD.

CVD is a major cause of mortality worldwide. Even a small increased risk associated with arsenic exposure can cause a large number of excess deaths. Therefore, arsenic-exposure-related CVD could be of a public health concern in countries whose population is exposed to elevated concentration of arsenic. The major strengths of this study were as

follows: (1) wide range of arsenic concentrations in the drinking water, hair, and nails of the study subjects that provided strong dose-response relationship between arsenic exposure and biochemical markers for atherosclerosis; and (2) all associations were shown using three ways of exposure metrics (water, hair, and nail arsenic). Drinking water arsenic is recognized as external exposure metric, whereas hair and nail arsenic are recognized as internal exposure metrics. Arsenic levels in nail and hair samples have been reported to provide the integrated measure for arsenic exposure (Agahian *et al*., 1990; Karagas *et al*., 1996). One centimeter of hair reflects approximately 1 month of exposure. On the other hand, nail captures historical exposure to arsenic from several months to a year. Therefore, correlations of biochemical markers for atherosclerosis with these three exposure metrics reduced the possibilities of misclassification of exposure and effects of other confounders on the observed associations. Although this study represented an extensive epidemiological research, the present study should be interpreted with cautions. First, we adjusted age, sex, BMI, smoking, hypertension, occupation, education, and monthly income as covariates (Table 4 and Supplementary tables 1 and 3), but there may be some other additional factors that might influence the results. Second, this study was designed to be cross sectional but not prospective. A cohort-based study is required in future for precise cause and effect relationship between circulating molecules related to atherosclerosis and arsenic exposure. Third, the most of the study subjects had poor socioeconomic conditions, and their BMIs were in the lower end of normal range of BMI. Thus, the results of the current study may not be generalizable to other study populations because of the different distribution of risk factors for atherosclerosis that may influence the effect of arsenic exposure. Nevertheless, this study suggests that exposure to arsenic causes pro-oxidative and pro-inflammatory conditions that may lead to the development of atherosclerosis.

In summary, this study has demonstrated that arsenic exposure are associated with the elevation of plasma Ox-LDL, CRP, ICAM-1, and VCAM-1 levels with the concomitant reduction of HDL levels. This study has also showed the positive correlations of the Ox-LDL/HDL ratios, non-HDL-cholesterol, and TC/HDL ratios with arsenic-exposure metrics. All these associations may be the hallmark features of arsenic-induced pro-oxidative and pro-inflammatory events leading to atherosclerosis. The biochemical indicators determined in this study strongly suggest that the residents in arsenic-endemic areas are at risk for atherosclerosis and future development of CVD.

SUPPLEMENTARY DATA

Supplementary data are available online at http://toxsci.oxfordjournals.org/.

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REFERENCES

Agahian, B., Lee, J. S., Nelson, J. H., and Johns, R. E. (1990). Arsenic levels in fingernails as a biological indicator of exposure to arsenic. *Am. Ind. Hyg. Assoc. J*. **51,** 646–651.

Ali, N., Hoque, M. A., Haque, A., Salam, K. A., Karim, M. R., Rahman, A., Islam, K., Saud, Z. A., Khalek, M. A., Akhand, A. A., *et al.* (2010). Association between arsenic exposure and plasma cholinesterase activity: A population based study in Bangladesh. *Environ. Health* **9,** 36.

Aviram, M. (1999). Macrophage foam cell formation during early atherogenesis is determined by the balance between pro-oxidants and anti-oxidants in arterial cells and blood lipoproteins. *Antioxid. Redox Signal*. **1,** 585–594.

Ballou, S. P., and Lozanski, G. (1992). Induction of inflammatory cytokine release from cultured human monocytes by C-reactive protein. *Cytokine* **4,** 361–368.

Bandeali, S., and Farmer, J. (2012). High-density lipoprotein and atherosclerosis: The role of antioxidant activity. *Curr. Atheroscler. Rep*. **14,** 101–107.

Blake, G. J., Rifai, N., Buring, J. E., and Ridker, P. M. (2003). Blood pressure, C-reactive protein, and risk of future cardiovascular events. *Circulation* **108,** 2993–2999.

Blankenberg, S., Rupprecht, H. J., Bickel, C., Peetz, D., Hafner, G., Tiret, L., and Meyer, J. (2001). Circulating cell adhesion molecules and death in patients with coronary artery disease. *Circulation* **104,** 1336–1342.

Chen, Y., Graziano, J. H., Parvez, F., Liu, M., Slavkovich, V., Kalra, T., Argos, M., Islam, T., Ahmed, A., Rakibuz-Zaman, M., *et al.* (2011). Arsenic exposure from drinking water and mortality from cardiovascular disease in Bangladesh: Prospective cohort study. *BMJ* **342,** d2431.

Chen, Y., Hakim, M. E., Parvez, F., Islam, T., Rahman, A. M., and Ahsan, H. (2006). Arsenic exposure from drinking-water and carotid artery intima-medial thickness in healthy young adults in Bangladesh. *J. Health. Popul. Nutr*. **24,** 253–257.

Chen, Y., Santella, R. M., Kibriya, M. G., Wang, Q., Kappil, M., Verret, W. J., Graziano, J. H., and Ahsan, H. (2007). Association between arsenic exposure from drinking water and plasma levels of soluble cell adhesion molecules. *Environ. Health Perspect*. **115,** 1415–1420.

Cheng, T. J., Ke, D. S., and Guo, H. R. (2010). The association between arsenic exposure from drinking water and cerebrovascular disease mortality in Taiwan. *Water Res*. **44,** 5770–5776.

Chowdhury, U. K., Biswas, B. K., Chowdhury, T. R., Samanta, G., Mandal, B. K., Basu, G. C., Chanda, C. R., Lodh, D., Saha, K. C., Mukherjee, S. K., *et al.* (2000). Groundwater arsenic contamination in Bangladesh and West Bengal, India. *Environ. Health Perspect*. **108,** 393–397.

Cook-Mills, J. M., Marchese, M. E., and Abdala-Valencia, H. (2011). Vascular cell adhesion molecule-1 expression and signaling during disease: Regulation by reactive oxygen species and antioxidants. *Antioxid. Redox Signal*. **15,** 1607–1638.

deGoma, E. M., Leeper, N. J., and Heidenreich, P. A. (2008). Clinical significance of high-density lipoprotein cholesterol in patients with low low-density lipoprotein cholesterol. *J. Am. Coll. Cardiol*. **51,** 49–55.

Drexel, H. (2006). Reducing risk by raising HDL-cholesterol: The evidence. *Eur. Heart J*. **8,** 23–29.

Galkina, E., and Ley, K. (2007). Vascular adhesion molecules in atherosclerosis. *Arterioscler. Thromb. Vasc. Biol*. **27,** 2292–2301.

Galle, J., Hansen-Hagge, T., Wanner, C., and Seibold, S. (2006). Impact of oxidized low density lipoprotein on vascular cells. *Atherosclerosis* **185,** 219–226.

Ginsberg, H. N. (2000). Insulin resistance and cardiovascular disease. *J. Clin. Invest*. **106,** 453–458.

Heinecke, J. W. (1998). Oxidants and antioxidants in the pathogenesis of atherosclerosis: Implications for the oxidized low density lipoprotein hypothesis. *Atherosclerosis* **141,** 1–15.

Hossain, E., Islam, K., Yeasmin, F., Karim, M. R., Rahman, M., Agarwal, S., Hossain, S., Aziz, A., Al Mamun, A., Sheikh, A., *et al.* (2012). Elevated levels of plasma Big endothelin-1 and its relation to hypertension and skin lesions in individuals exposed to arsenic. *Toxicol. Appl. Pharmacol*. **259,** 187–194.

Hwang, S. J., Ballantyne, C. M., Sharrett, A. R., Smith, L. C., Davis, C. E., Gotto, A. M. Jr, and Boerwinkle, E. (1997). Circulating adhesion molecules VCAM-1, ICAM-1, and E-selectin in carotid atherosclerosis and incident coronary heart disease cases: The Atherosclerosis Risk In Communities (ARIC) study. *Circulation* **96,** 4219–4225.

Islam, K., Haque, A., Karim, R., Fajol, A., Hossain, E., Salam, K. A., Ali, N., Saud, Z. A., Rahman, M., Rahman, M., *et al.* (2011). Dose-response relationship between arsenic exposure and the serum enzymes for liver function tests in the individuals exposed to arsenic: A cross sectional study in Bangladesh. *Environ. Health* **10,** 64.

Karagas, M. R., Morris, J. S., Weiss, J. E., Spate, V., Baskett, C., and Greenberg, E. R. (1996). Toenail samples as an indicator of drinking water arsenic exposure. *Cancer Epidemiol. Biomarkers Prev*. **5,** 849–852.

Karim, M. R., Salam, K. A., Hossain, E., Islam, K., Ali, N., Haque, A., Saud, Z. A., Yeasmin, T., Hossain, M., Miyataka, H., *et al.* (2010). Interaction between chronic arsenic exposure via drinking water and plasma lactate dehydrogenase activity. *Sci. Total Environ*. **409,** 278–283.

Kontush, A., and Chapman, M. J. (2010). Antiatherogenic function of HDL particle subpopulations: focus on antioxidative activities. *Curr. Opin. Lipidol*. **21,** 312–318.

Lankin, V., Viigimaa, M., Tikhaze, A., Kumskova, E., Konovalova, G., Abina, J., Zemtsovskaya, G., Kotkina, T., Yanushevskaya, E., and Vlasik, T. (2011). Cholesterol-rich low density lipoproteins are also more oxidized. *Mol. Cell. Biochem*. **355,** 187–191.

Li, L., Roumeliotis, N., Sawamura, T., and Renier, G. (2004). C-reactive protein enhances LOX-1 expression in human aortic endothelial cells: Relevance of LOX-1 to C-reactive protein-induced endothelial dysfunction. *Circ. Res*. **95,** 877–883.

Mertens, A., and Holvoet, P. (2001). Oxidized LDL and HDL: Antagonists in atherothrombosis. *FASEB J*. **15,** 2073–2084.

Podrez, E. A. (2010). Anti-oxidant properties of high-density lipoprotein and atherosclerosis. *Clin. Exp. Pharmacol. Physiol*. **37,** 719–725.

Rahman, M., Tondel, M., Ahmad, S. A., Chowdhury, I. A., Faruquee, M. H., and Axelson, O. (1999). Hypertension and arsenic exposure in Bangladesh. *Hypertension* **33,** 74–78.

Steinberg, D. (1995). Role of oxidized LDL and antioxidants in atherosclerosis. *Adv. Exp. Med. Biol*. **369,** 39–48.

Steinberg, D. (1997). Lewis A. Conner Memorial Lecture. Oxidative modification of LDL and atherogenesis. *Circulation* **95,** 1062–1071.

Tseng, C. H., Chong, C. K., Tseng, C. P., Hsueh, Y. M., Chiou, H. Y., Tseng, C. C., and Chen, C. J. (2003). Long-term arsenic exposure and ischemic heart disease in arseniasis-hyperendemic villages in Taiwan. *Toxicol. Lett*. **137,** 15–21.

von Mühlen, D., Langer, R. D., and Barrett-Connor, E. (2003). Sex and time differences in the associations of non-high-density lipoprotein cholesterol versus other lipid and lipoprotein factors in the prediction of cardiovascular death (The Rancho Bernardo Study). *Am. J. Cardiol*. **91,** 1311–1315.

Wadham, C., Albanese, N., Roberts, J., Wang, L., Bagley, C. J., Gamble, J. R., Rye, K. A., Barter, P. J., Vadas, M. A., and Xia, P. (2004). High-density lipoproteins neutralize C-reactive protein proinflammatory activity. *Circulation* **109,** 2116–2122.

Wang, C. H., Chen, C. L., Hsiao, C. K., Chiang, F. T., Hsu, L. I., Chiou, H. Y., Hsueh, Y. M., Wu, M. M., and Chen, C. J. (2009). Increased risk of QT prolongation associated with atherosclerotic diseases in arseniasis-endemic area in southwestern coast of Taiwan. *Toxicol. Appl. Pharmacol*. **239,** 320–324.

Wang, C. H., Jeng, J. S., Yip, P. K., Chen, C. L., Hsu, L. I., Hsueh, Y. M., Chiou, H. Y., Wu, M. M., and Chen, C. J. (2002). Biological gradient between long-term arsenic exposure and carotid atherosclerosis. *Circulation* **105,** 1804–1809.

Wu, F., Jasmine, F., Kibriya, M. G., Liu, M., Wójcik, O., Parvez, F., Rahaman, R., Roy, S., Paul-Brutus, R., Segers, S., *et al.* (2012). Association between arsenic exposure from drinking water and plasma levels of cardiovascular markers. *Am. J. Epidemiol*. **175,** 1252–1261.

Zhang, Y. C., Wei, J. J., Wang, F., Chen, M. T., and Zhang, M. Z. (2012). Elevated levels of oxidized low-density lipoprotein correlate positively with C-reactive protein in patients with acute coronary syndrome. *Cell Biochem. Biophys*. **62,** 365–372.