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Research Article

Elevated Arsenic Exposure Associated with Increased Cardiovascular Risk Factors in Residents of a Contamination Area

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ABSTRACT

Arsenic (As) is an important toxicant in environmental contamination. Residents with high as exposure demonstrated CKD or renal insufficiency, increased oxidative stress, also increased diabetes mellitus, cardiovascular disease and cancer. We aimed to examine the association between increased as exposure with cardiovascular risk factors in a sample of 209 residents of the As-contaminated villages of Uthai Thani Province in Thailand. Residents with high as exposure demonstrated significantly higher glucose, uric acid, microalbuminuria (MA), triglyceride (TG) levels, TG/high density lipoprotein- cholesterol (HDL-C) ratio and reduced HDL-C levels (p<0.05). In bivariate correlation, urinary as was significantly correlated with TG, HDL-C, TG/HDL-C ratio and MA levels. Overall findings showed that elevated urinary as appeared to increase risk of elevated TG/HDL-C ratio, elevated MA, elevated NAG, and reduced TAC; ORs and 95% CIs were 8.02 (2.75-23.40), 5.91 (2.24-15.61), 7.03 (2.73-18.09), and 9.23 (6.24-0.0001), respectively, after adjusting for BMI, age and sex. Our study revealed that elevated as exposure induces increased oxidative stress concomitant with CKD and associated with increased cardiovascular risk factors. These might be increased in morbidity and mortality from all degenerative diseases in the future.

Keywords: Urinary arsenic, oxidative stress, chronic kidney disease, glomerular filtration rate, cardiovascular disease risk factors

INTRODUCTION

Arsenic (As) contamination of groundwater and drinking water is a major public health problem worldwide. Arsenic has been classified as a group 1 carcinogen to humans by the International Agency for Research on Cancer. Many studies demonstrate the association between chronic as exposure with renal dysfunction and life-threatening chronic diseases[1,2]. Arsenic may induce oxidative stress by changing the oxidation states as do other metals such as Fe, Cu, etc. For example, the route of H_2O_2 production was proposed to involve the oxidation of As^{3+} to As^{5+} which results in the formation of H_2O_2 under physiological conditions [3]. Another by interaction with antioxidants, both As^{3+} and As^{5+} can interact and form complexes with GSH leading to increase GSSG formation and decrease of GSH levels[4]. And subsequence with increasing inflammation, resulting in ROS accumulation in cells[5]. There is indirect evidence that arsenic toxicity induces oxidative stress. Both acute and chronic arsenic toxicity in experimental rats are ameliorated by therapeutic and prophylactic efficacy of exogenous nutritional antioxidants such as ascorbic acid, α-tocopherol, methionine, thiamine and cysteine[6,7]. Our recent study[1] reported on residents in Ban Rai District, Uthai Thani Province, an as contaminated area. Study subjects suffered from increased oxidative stress and chronic kidney disease (CKD). Many studies have been demonstrated that long-term exposure too low/medium-As levels has effects on the heart, specially altered myocardial depolarization and cardiac arrhythmias[8,9], and as has been demonstrated to cause mild damage on vascular system. After a high as concentration exposure, severe hypertrophy of the ventricular wall was observed[10]. The prevalence of chronic renal disease and type 2 diabetes mellitus were significantly increased in as contaminated areas[11]. Therefore, the aims of the present study are to evaluate the association of as exposure with cardiovascular risk factors and concomitant with increased oxidative stress in those residents environmentally exposed to As.

MATERIALS AND METHODS

Study population

This cross-sectional study was the community-based on health investigation with 209 eligible volunteer subjects [63 (30.2%) male, 146 (69.8%) female] who were aged 11.0-81.0 years from Ban Rai District, Uthai Thani Province, an as contamination area. These residents resided in their present household in the contamination area for at least 10 consecutive years at the time of recruitment (during January 2014–January 2015). A questionnaire survey was conducted by trained health workers about demographic characteristics, occupational history, residence time, medical history of diabetes, hypertension, renal diseases, and cancers. Fifty-two

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subjects with known cancer, end stage renal failure, infection, any life threatening diseases and subjects who did not provide urine for as analysis were excluded from the study. The study protocol was approved by the Ethic committees of Naresuan University and the permission for research problem from the Uthai Thai Provincial Public Health. All subjects provided written informed consent before their blood and urine samples were collected.

Blood and urine samples collection

Fasting venous blood was collected from all participants. Plasma glucose (Glu), serum blood urea nitrogen (BUN), uric acid (UA), total cholesterol (TC), triglycerides (TG), and high density lipoprotein cholesterol (HDL-C) were measured by using enzymatic colorimetric method. Low density lipoprotein cholesterol (LDL-C) concentrations were calculated with Friedewald's formula in specimens with TG levels\400 mg/dl. TG/HDL-C ratio was calculated by dividing TG by HDL-C. Urinary-calcium (U-Cal) concentration was determined based on the colorimetric assay with o-cresolphthalein complexone to form purple color of calcium-o-cresolphthalein complex. Serum and urine creatinine (CT) concentration was determined based on the Jaffe reaction procedures with an auto-analyzer (Archited c4000, Abbott Laboratories, Illinois, USA) at the laboratory of Medical Technology Unit, Uthai Thani Hospital. Serum sample for MDA and TAC determination was immediately separated and stored at -70°C without the addition of exogenous antioxidants before MDA and TAC analysis. Urine samples were collected in polyethylene bottles after the subject's physical examination, wherein anthropometric measurements and blood taken. The urine sample from each subject was divided into three aliquots (3–5 ml each); one for urine microscopic examination and other aliquots were frozen and stored at −70 ◦C without the any addition for later analysis of as, creatinine and NAG concentrations.

Sample analysis

Urinary arsenic determination

Urinary as concentration was determined by a Graphite Furnace Atomic Absorption Spectrometer (GFAAS) (Varian SpectrAA-800, USA) at the laboratory service of the Nakhonsawan Provincial Public Health. All specimens were analyzed in duplicate. The laboratory has been certified and participated as the member of the toxicological analyses in biological materials by the External Quality Assessment Scheme of Medical Sciences Center, Thailand. The within-run assay coefficients of variation ranged from 2.5% to 11.5%. Additionally, in an external quality assurance program from the external quality assessment scheme of Medical Sciences center of Thailand, laboratory measures were within 10% of reference means for urinary cadmium ($r^2 = 0.98$).

Triglycerides/ high density lipoprotein cholesterol ratio

Dyslipidemia in patients with metabolic disorders including obesity, metabolic syndrome, and T2DM is characterized by elevated triglycerides (TG), reduced high density lipoprotein cholesterol (HDL-C) and small dense low density lipoprotein (LDL)-particles, elevated triglyceride-rich remnant lipoprotein (TGRLs), and an increased circulating insulin concentration[12]. In these concepts, insulin resistance and CVD risk are estimated by using TG/HDL-C ratio[13].

Malondialdehyde (MDA) assay

After thawing the samples, MDA level was determined by using the thiobarbituric acid substances (TBARS) assay, a spectroscopic techniques descripted in our previous report[14]. The method is based on the formation of red (pink) chromophore following the reaction of TBA with MDA and the other breakdown products of peroxidized lipids called MDA. One molecule of MDA reacts with 2 molecules of TBA to yield a pink pigment with absorption maximum at 532 nm.

Total antioxidants capacity (TAC) assay

The assay is based on the reaction of metmyoglobin with hydrogen peroxide to form ferryl myoglobin, a free radical species. A chromogen 2, 2'-amino-di- [3-ethylbenzthiazole sulphonate] is incubated with ferryl myoglobin to produce a radical cation which has a relatively stable blue-green color that can be measured at 600 nm. Antioxidants in the added serum can suppress this color production to a degree proportional to their concentration. The assay was calibrated using 6-hydroxy-2, 5, 8-tetramethylchroman-2- carboxylic acid (Trolox), and results were expressed as mmol/l trolox equivalent [15]. The within–run coefficient of variation for the TAC assay in control material assay was 4.8% (n=10).

Microalbumin assay

Microalbumin concentration in urine was measured by an immunoturbidimetric assay in which anti-albumin antibodies react with the microalbumin in the sample to form antigen-antibody complexes which, following agglutination, are measured as turbidimetrically. The urinary albumin excretion (UAE) <30 mg/gram creatinine (gCT) was defined as a nomoalbuminuria, 30-300 mg/gCT as a microalbumununa (MA), and >300 mg/gCT as a macroalbuminuria[16,17].

N-acetyl-β-D-glucosaminidase (NAG) assay

The assay method was as described by Horak et al.[18]. The NAG in urine is added to an enzyme reaction mixture that consists of the substrate (p-nitrophenyl-N-acetyl- β -D-glucosaminide) dissolved in sodium citrate buffer (pH 4.4). During incubation at 37 ºC, NAG hydrolysis of the substrate liberates p-nitrophenylate ion. The reaction is stopped by adding 2-amino-2-methyl-1-propanol (AMP) buffer (pH 10.25), and the reaction product is measured by spectrophotometry at 405 nm. Urine NAG activity is proportional to the absorbance of the liberated p–nitrophenylate ion, after correction for absorbance of a urine "blank" sample. The within–run and between-run coefficient of variation for NAG assay in control material assay was 3.14% and 4.11% (n=10).

Renal Function

All participants had no clinically identified renal organ damage, defined as a serum creatinine level lower than 1.2 mg/dl l (106.0 μmol/l) and serum BUN level lower than 20 mg/dl (7.14 mmol/l). Estimated GFR was calculated by the Modification of Diet in Renal Disease (MDRD) equation[19]. The formula is:

eGFR = 186 *[plasma creatinine - 1.154] *(age) -0.203 *(0.742 if female) *(1.210 if African-American). Five

eGFR stages were used: Stage I was normal eGFR (≥90 ml/min/1.73 m^2); Stage II was mildly eGFR (60–89 mL/min/1.73 m^2); Stage III was moderately eGFR (30–59 ml/min/1.73 m^2); Stage IV was severely eGFR (<30 ml/min/1.73 m^2), and Stage V was end-stage renal disease: eGFR (<15 ml/min/1.73 m^2). An eGFR lower than 60 ml/min/1.73 m^2 (moderately eGFR) was defined as chronic kidney disease (CKD)[20].

Statistical analysis

All data are presented as median and interquartile range. A Mann-Whitney U-test was used to analyze the differences between non-As-exposure and As-exposure. Because, there is no normal range of U-As level recommended for general populations. Therefore, we used the U-As exposure cut-off point as ≥35μg/L suggested by ACGIH [21] in the following analyses of the present study. Correlation between as exposure index with renal toxicity (eGFR, NAG and microalbumin levels), lipid peroxidation and total antioxidant capacity were analyzed with Spearman's rho correlation test. Odds ratios (OR) from logistic regression analyses were used to estimate the risk of hypertriglyceridemia, lower HDL-C, estimated insulin resistance, microalbuminuria, MDA and CKD that was associated with elevated urinary as concentration. The results of all statistical analyses were evaluated for statistical significance using *p*-value <0.05 and the 95% confidence intervals (CI). All analysis was performed using the SPSS computer program version 13.0 (SPSS, Chicago, IL).

RESULTS

In our previous study[1] demonstrated CKD, decreased TAC and increased oxidative stress in elevated as exposure group as shown in Table 1. In the present study, we demonstrated that residents with elevated as exposure group also had significantly increased cardiovascular risk factors including higher in oxidative stress, elevated TG, TG/HDL-C ratio, urine MAgCT levels and lower HDL-C, eGFR and TAC levels. Urinary As was significantly correlated with UA (r =0.330, *p*<0.001), TG (r=0.534, *p*<0.001), HDL-C (r=-0.201, *p*=0.004), TG/HDL-C (r=0.473, *p*<0.001), U-Cal (r =0.261, *p*<0.001), NAG/g CT (r =0.457, *p*<0.001), MAgCT (r=0.459, *p*<0.001), eGFR (r = -0.592, *p*<0.001) and MDA (r =0.636, *p*<0.001), TAC (r= -0.530, *p*<0.001) and the correlation of the other variables is shown in Table 2. Multiple logistic regression analyses were used to test for association between elevated urinary as with TG/HDL-C ratio, MAgCT, NAG/g CT and reduced TAC after adjusting for their covariates. The risk of increased TG/HDL-C ratio (as estimated insulin resistance) OR = 8.02 (95% confidence interval (CI): 2.75-23.40), the risk for microalbuminuria OR = 5.91 (95% CI: 2.24-15.61), elevated NAG OR = 7.03 (95% CI: 2.73-18.09) and reduced TAC OR = 9.23 (95% CI: 6.24-0.0001) that was associated with elevated urinary as concentration after adjusting for BMI, age and gender as shown in Table 3. These results demonstrate that elevated as exposure is associated with increased risk of insulin resistance and CVD as demonstrated by hypertriglyceridemia, lower HDL-C levels, estimated insulin resistance, renal tubular damage and increased oxidative stress (reduced TAC).

DISCUSSION

The present study reported that residents with elevated as (≥35 μg/L) had increased oxidative stress and CKD or were renal insufficient as indicated by lower eGFR level and higher excretion of urinary NAG. These as exposure subjects demonstrated nephrotoxicity including renal tubular damage and CKD and also increased in UA, TG, TG/HDL-C ratio, MAgCT and decreased HDL-C level concomitance with increased oxidative stress. Many research studies have reported significant increased oxidative stress and decreased antioxidant activities in patients with renal dysfunction and in the early stages of CKD[22,23]. Co-increased oxidative stress from as exposure and from CKD stages accelerated the oxidative stress condition in the residents of as exposure areas. Oxidative stress, an extra risk factor, may accelerate atherosclerosis and glomerulosclerosis. It may have a pivotal role in vascular injury and the progression of atherosclerosis. The main atherosclerosis amplification occurs by direct oxidative damage the endothelial cells leading to defect in vascular function, ageing and apoptosis[3,24]. These results support the idea that ROS and/or increased lipid peroxidation play the critical role in As-induced cells, tissues or organs damage, induced degenerative diseases and carcinogenesis. Arsenic toxicity caused from increased ROS production and by reduced antioxidant activity including various enzymatic and nonenzymatic factors[25,26] especially in reduced glutathione ability and its cofactor (for enzymes GPx and GST) induced oxidative stress[27].

The association between renal insufficiency and increased risk of mortality and mobility of cardiovascular complications has been consistently noted[28]. Similarly, the subclinical elevations of urinary albumin excretion (UAE) are associated with the higher risk of subsequent development of nephropathy in type 2 diabetes mellitus patients[29], and the increased UAE was associated with higher CV risk and increased the mortality and mobility in both diabetic and non-diabetic individuals[30,31]. Many research studies indicated that MA is a marker of early nephropathy[32], and is also associated with cardiovascular risk factors including smoking, obesity, aging, dyslipidemia, hypertension and diabetes[32]. As in the discussion above, MA has been used as a marker of cardiovascular disease and the risk factor found in the patients with insulin resistance syndrome[33,34]. In the present study, we demonstrated increased oxidative stress, reduced TAC, hypertriglyceridemia, reduced HDL-C levels and higher TG/HDL-C ratio in subjects with as exposure. The TG/HDL-C ratio has been demonstrated as a marker for estimated insulin resistance[14], and may show oxidative stress induced insulin resistance[35,36]. Oxidative stress plays the major role in insulin signals disruption and adipocytokines dysregulation to cause insulin resistance[35,37]. An insulin resistance state is demonstrated by dyslipidemia, called the lipid triad which consists of hypertriglyceridemia, lower HDL-C levels and increased

Table 1: Comparison of the clinical characteristics of residents in the contamination area based on their As exposure levels.

Variables	As <35µg/L group (n=129)	As ≥35µg/L group (n=80)	*p*-value
Age (years)	53.0 (41.5-60.0) *	58.0 (50.5-64.0) *	0.030
WC (cm)	83.0 (76.5-90.0)	82.0 (76.3-90.0)	0.841
BMI (kg/m^2)	23.2 (23.3-28.6)	24.1 (21.5-27.0)	0.402
As (µg/L)	13.30 (0.50-22.45)	60.70 (45.45-93.68)	<0.001
Glu (mg/dl)	86.0 (79.0-90.0)	95.0 (88.0-102.0)	<0.001
BUN(mg/dl)	13.0 (11.0-16.0)	12.5 (10.0-14.0)	0.251
CT (mg/dl)	0.84 (0.77-1.04)	0.84 (0.77-1.00)	0.726
UA (mg/dl)	5.1 (4.3-5.8)	6.2 (4.93-7.3)	<0.001
UCal (mg/dl)	6.10 (3.50-11.45)	10.20 (6.90-15.00)	<0.001
eGFR ((ml/min/1.73 m^2))	82.92 (71.19-98.89)	56.13 (48.14-62.72)	<0.001
NAG (U/gCT)	5.08 (1.88-11.17)	18.41 (6.84-32.88)	<0.001
MA (mg/gCT)	29.65 (16.25-58.36)	114.01 (75.36-252.02)	<0.001
MDA (µmol/l)	2.54 (2.16-2.91)	3.49 (3.20-3.88)	<0.001
TAC (µmolTroloxEquiv/L)	0.412(0.329-0.481)	0.279(0.238-0.481)	<0.001
TC (mg/dl)	203.0 (177.5-235.0)	211.0(191.5-235.3)	0.374
TG (mg/dl)	140.0 (99.5-170.0)	226.0(195.0-279.8)	<0.001
HDL-C (mg/dl)	55.0 (46.0-62.0)	47.0(40.0-57.0)	0.001
LDL-C (mg/dl)	120.8 (98.4-150.0)	115.6(90.1-139.2)	0.198
TG/HDL-C ratio	2.54 (1.67-3.43)	4.93 (3.53-6.59)	<0.001

* median (interquatile)

Table 2: Bivariate correlation of all variables in residents of As exposure area

Correlation between markers		Correlation coefficient		Correlation between markers		Correlation coefficient	
		r	*p*-value			r	*p*-value
As	Glu	0.341	<0.001	NAG	Glu	0.280	<0.001
	eGFR	-0.592	<0.001		UA	0.207	0.003
	UA	0.330	<0.001		TG	0.349	<0.001
	TG	0.534	<0.001		HDL-C	-0.238	0.001
	HDL-C	-0.201	0.004		TG/HDL-C	0.356	<0.001
	TG/HDL-C	0.473	<0.001		UCal	0.180	0.009
	UCal	0.261	<0.001	MAgCT	Glu	0.221	0.001
	MAgCT	0.459	<0.001		UA	0.185	0.007
	NAG	0.457	<0.001		TG	0.347	<0.001
	MDA	0.636	<0.001		HDL-C	-0.172	0.013
	TAC	-0.530	<0.001		TG/HDL-C	0.314	<0.001
Glu	BUN	0.175	0.011		TAC	-0.341	<0.001
	CT	0.195	0.005		NAG	0.361	<0.001
	UA	0.154	0.026	CT	UA	o.228	0.001
	TG	0.340	<0.001		TC	0.154	0.026
	HDL-C	-0.168	0.015		TG	0.237	0.001
	TG/HDL-C	0.318	<0.001		HDL-C	-0.159	0.022
	UCal	0.181	0.009		TG/HDL-C	0.241	<0.001
eGFR	Glu	-0.414	<0.001	MDA	Glu	0.418	<0.001
	UA	-0.313	<0.001		UA	0.258	<0.001
	TG	-0.424	<0.001		TG	0.562	<0.001
	HDL-C	0.225	0.001		HDL-C	-0.228	0.001
	TG/HDL-C	-0.398	<0.001		TG/HDL-C	0.504	<0.001
	UCal	-0.198	0.004		eGFR	-0.507	<0.001
	MAgCT	-0.420	<0.001		UCal	0.288	<0.001
	NAG	-0.424	<0.001		MAgCT	0.378	<0.001
					NAG	0.432	<0.001

small, dense, low density lipoprotein cholesterol (sdLDL-C)[38]. These results may indicate that residents with as exposure were in the insulin resistance state as higher in TG/HDL-C ratio.

There are many studies providing evidences that the cardiovascular system may be affected by consumed and inhaled environmental inorganic As[39,40]. Wang et al.[11] demonstrated an increased incidence of blood vessels

Table 3: Association of elevated As-exposure with elevated TG/HDL-C ratio, microalbuminurea, elevated NAG, and reduced TAC after adjusted with their variables

Variables	Elevated As exposure		
	OR	95% confident interval)	*p*-value
Elevated TG/HDL-C ratio	8.02	2.75-23.40	<0.001
Elevated microalbumin	5.91	2.24-15.61	<0.001
Elevated NAG	7.03	2.73-18.09	<0.001
Reduced TAC	9.23	6.24-0.0001	<0.001
BMI	0.99	0.89-1.09	0.869
Age	0.95	0.92-0.98	0.002
Sex	0.86	0.33-2.22	0.753

diseases in Taiwanese populations in arsenic-contaminated areas (>0.35 mg l/l). Another study from the USA demonstrated a significant increase in the number of deaths from arteriosclerosis, aneurysm and other related diseases were found in the areas of arsenic contaminated drinking water (>20 μg/1)[41]. All of these cardiovascular disease risk factors are the same risk factors for type 2 diabetes mellitus (T2DM)[42], and result in significantly increased prevalence of chronic renal disease and T2DM in as contaminated areas[11]. Limitations of the present study are the single time measurement, the area based study and the small sample size. Moreover, other important biochemical markers were not analyzed. The future follow-up study in these as exposure people will, especially focus on chronic metabolic diseases and as carcinogenic effects to establish the causal inference and future prevention.

Conclusions

The present study demonstrated that As-exposure (≥35 μg/L) increased oxidative stress, reduced TAC, increased UMAE, induced CKD or renal insufficiency to cause insulin resistance and dyslipidemia (elevated TG and reduced HDL-C levels). These results indicate that elevated as exposure is associated with increased cardiovascular risk factors in the residents of the contamination area.

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