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Research Article

Association Between Elevated Arsenic Exposure with Chronic Kidney Disease and Oxidative Stress in Subjects of the Contamination Area

Wasana Kayankarnna, Orathai Tangvarasittichai, Surapon Tangvarasittichai*

Chronic Diseases Research Unit, Department of Medical Technology, Faculty of Allied Health Sciences, Naresuan University, Phitsanulok 65000, Thailand

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ABSTRACT

Arsenic (As) is an important toxicant in environmental contamination that can cause diabetes mellitus, cardiovascular disease and cancer. The association between As exposure and chronic kidney disease (CKD) is unclear. We aim to examine the association between As exposure with CKD and oxidative stress in a total of 209 study residents of the As-contaminated villages. Individuals with high As exposure demonstrated CKD or renal insufficiency and increased oxidative stress. In bivariate correlation, urinary As was significantly correlated with CKD markers and malondialdehyde levels. Multiple forward stepwise linear regression analyses of the significant variables demonstrated that in these As exposure residents, independent predictors of As exposure were MDA (β = 0.412, r^2 =0.285, $p < 0.001$), NAG (β = 0.171, r^2 = 0.316, p = 0.007), and eGFR (β = -0.130, r^2 =0.330, p= 0.046). In conclusion, the present study demonstrated that As-exposure induced CKD or renal insufficiency concomitant with increased oxidative stress. It might be increased in morbidity and mortality of all degenerative diseases.

Keywords: Urinary arsenic, chronic kidney disease, estimated glomerular filtration rate, oxidative stress

INTRODUCTION

Arsenic (As) is one of the most toxic metals in the general population derived from the natural environment. It has been widely reported in many areas and cause life-threatening chronic diseases[1,2]. Arsenic exposure occurs from drinking water and food contaminated with large amounts of inorganic As[3,4]. The worst affected areas in the world are Bangladesh, West Bengal, India and Taiwan. People (over 100 million people) in 42 districts of southern Bangladesh and nine adjacent districts in West Bengal, India and people in central Taiwan[5,6] are exposed to groundwater arsenic concentrations that are greater than maximum limit of 50 µg/l[7]. In Ban Rai District, Uthai Thani central region of Thailand, the As contaminated areas were caused from the tin (Pb) mine clearing on the hill. These As contaminated areas are include the adjacent Dan Chang districts, Suphanburi Province were found higher levels of As in natural water and groundwater (range from 44 – 1,206 µg/l, Data from Department of Mineral Resources, Ministry of Natural Resources and Environmental and Bureau of Occupational and Environmental Diseases. Department of Disease Control, Ministry of Public Health, Thailand). Arsenic may increase oxidative stress by cycling between metals oxidation status such as As, Fe, Cu, etc., or by binding with sulfhydryl groups of proteins and enzymes and interferes with metabolism of glutathione (GSH), the major antioxidant agent responsible for metabolism and excretion of xenobiotics [8] and increasing inflammation, resulting in the accumulation of reactive oxygen species (ROS) in cells[9]. Long term exposure to As was found to be associated with increased multisystem chronic diseases, such as hypertension[10,11], metabolic syndrome[12], diabetes[13], respiratory disorders[14], and microvascular[15] and macrovascular[16] diseases related to diabetes and the most serious disease is cancer. The prevalence of chronic renal disease and type 2 diabetes mellitus were significantly increased in As contaminated areas[16]. However, the understanding of mechanism of As exposure caused renal dysfunction is unclear. Nordberg et al.[17] demonstrated increased urinary markers of renal dysfunction in response for As exposure such as β_2-microglobulin (B_2MG), N-acetyl-β-D-glucosaminidase (NAG), and microalbumin (MB) levels. Moreover, chronic kidney disease (CKD) is the major risk factor for cardiovascular disease[18,19]. The major risk factors of CKD are hypertension, diabetes[20] and obesity[21]. Environmental cadmium and lead exposures also play the major role in CKD development[22,23]. Many community-based studies of high As exposure residents were significantly higher in B_2MG, NAG, and MB levels than residents from the non-exposure residents[17,24-27]. There fore the aims of the present study to investigate the possible association between As exposure with development of CKD and concomitant with increased oxidative stress in these residents environmentally exposed to As.

MATERIALS AND METHODS

Study population

*Author for Correspondence

Table 1: Comparison of the clinical characteristics of the As exposure (< 35.0 μg/L) and non-As exposure (≥ 35.0 μg/L) in the contamination area.

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
MB (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

* median (interquartile)

Table 2: Demonstration of the bivariate correlation of all variables in population of As exposure

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		UCal	0.180	0.009
	UCal	0.261	<0.001	eGFR	Glu	-0.414	<0.001
	MBgCT	0.459	<0.001		UA	-0.313	<0.001
	NAG	0.457	<0.001		UCal	-0.198	0.004
	MDA	0.636	<0.001		MBgCT	-0.420	<0.001
Glu	BUN	0.175	0.011		NAG	-0.424	<0.001
	CT	0.195	0.005	MDA	Glu	0.418	<0.001
	UA	0.154	0.026		UA	0.258	<0.001
	UCal	0.181	0.009		eGFR	-0.507	<0.001
CT	UA	o.228	0.001		UCal	0.288	<0.001
MBgCT	Glu	0.221	0.001		MBgCT	0.378	<0.001
	UA	0.185	0.007		NAG	0.432	<0.001
	NAG	0.361	<0.001				

This cross-sectional study was the community-based on health evaluation. Data reported As contamination area in Ban Rai District, Uthai Thani from Department of Mineral Resources and Bureau of Occupational and Environmental Diseases, Thailand. A total of 209 eligible volunteer subjects [63 (30.2%) male, 146 (69.8%) female] who were aged 11.0-81.0 years and 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. We excluded the 52 subjects with known cancer, end stage renal failure, infection, any life threatening diseases and subjects did not provide urine for As analysis 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, blood and urine samples for their health check.

Blood and urine samples collection: Fasting venous blood was collected from all participants. Plasma glucose (Glu), serum blood urea nitrogen (BUN) and uric acid (UA) were measured by using enzymatic colorimetric method, serum and urine creatinine (CT) concentration was measured based on the Jaffe reaction procedures with an auto-analyzer (Archited c4000, Abbott laboratories, Illinois,

Table 3: Multiple forward stepwise linear regression analyses of the significant variables demonstrated that eGFR, NAG and MDA were independent predictors of increased As exposure in these patients

Variables	β	R^2	Adjusted R^2	*p*-value
MDA	0.412	0.285	0.282	<0.001
NAG	0.171	0.316	0.310	0.007
eGFR	-0.130	0.330	0.320	0.046

USA) at 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.

Sample analysis

Urinary arsenic determination: Urinary arsenic 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 (CV) 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 ($r^2 = 0.98$).

Malondialdehyde (MDA) assay: After thawing the samples, MDA level was determined by using the thiobarbituric acid substances (TBARS) assay, a spectroscopic techniques as our previously report[28]. The method is based on the formation of red (pink) chromophore following the reaction of TBA with the breakdown products of lipid peroxidation 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[29]. The within–run coefficient of variation for the TAC assay in control material assay was 4.8% (n=10).

Microalbumin (MB) assay: Microalbumin concentration in urine was measured by an immunoturbidimetric assay in which anti-albumin antibodies react with the antigen (MB) in the sample to form antigen/antibody complexes which, following agglutination, are measured turbidimetrically. The MB <30 mg/g CT was defined as a nomoalbuminuria, 30-300 mg/g as a microalbuminuria, and >300 mg/g as a macroalbuminuria.

N-acetyl-β-D-glucosaminidase (NAG) assay: The assay method was as described by Horak et al.[30]. 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 clinically normal renal function, defined as a serum creatinine level below 1.2 mg/dl l (106.0 μmol/l) and serum BUN level below 20 mg/dl (7.14 mmol/l). Estimated GFR was calculated by the Modification of Diet in Renal Disease (MDRD) equation [31]. 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)[32].

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[33] 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. Clinical variables that correlated with As exposure in these subjects were tested as independent variables by using multivariate forward stepwise linear regression analysis. Statistical analysis was done with SPSS (v 17.0 SPSS Inc, Chicago, USA). All *p*-value <0.05 were considered as significant.

RESULTS

The clinical characteristics of the As-exposure group and non-As-exposure group are shown in Table 1. Residents of the As-exposure and non-As-exposure group were not significantly difference in BMI and WC. Our present study demonstrated As-exposure group was significantly younger than non-As-exposure group, the inference is that may depend on the individual eating and drinking habit but not the result only the residential time. Subjects with elevated As-exposure group had significantly higher urinary As, NAG, microalbuminuria, urine calcium, UA and lower eGFR than non-As-exposure group (p<0.05), no significantly difference in BUN and CT levels. In bivariate correlation, urinary As showed the correlation with Glu (r =0.341, p<0.001), UA (r =0.330, p<0.001), U-Cal (r =0.261, p<0.001), NAG/g CT (r =0.457, p<0.001), eGFR (r = -0.592, p<0.001) and MDA (r =0.636, p<0.001) and the correlation of the other variables is shown in Table 2. We used multiple forward stepwise linear regression analysis to test the effects of variables in the association of these variables with elevated urinary As subjects. Statistics are listed in Table 3. These results demonstrate that MDA, NAG and eGFR showed association with elevated As exposure, which remained highly significant after adjusting for any clinical or laboratory confounding variables [MDA (β = 0.412, r^2 = 0.285, p<0.001; NAG (β = 0.171, r^2 = 0.316, p =0.007; eGFR (β = -0.130, r^2 =0.330, p = 0.046].

DISCUSSION

The present study demonstrated increasing excretion of NAG in urine and lower eGFR could be observed in individuals with elevated As (≥35 μg/L). These As exposure subjects demonstrated nephrotoxicity including renal tubular dysfunction or CKD and hypercalciuria. Urinary NAG and eGFR levels were better early markers for renal tubular dysfunction and CKD and sensitive than CT and BUN. The data also demonstrated urinary As exposure level associated with CKD concomitant with oxidative stress after adjusting for their covariates. The association between urinary As and CKD found in the present study is consistent with the many studies results in the high As-exposure area[17,34]. Kidney is one of major target organ of arsenic toxicity. It is involved in arsenic absorption and accumulation in kidney. In acute arsenic exposure, the highest arsenic concentrations can be detected in kidneys and liver[35]. Arsenic-induced oxidative stress in kidney and liver microsomes damaged cellular lipids, proteins and caused antioxidant and xenobiotic metabolizing enzyme activity reduction in rats[36]. Chronic renal insufficiency is mainly seen in acute poisoning by arsenic toxicity with oliguria and anuria progressing to renal failure. We also found increased oxidative stress in As exposure subjects that demonstrated by increased levels of MDA levels, a lipid peroxidation marker. Oxidative stress has been implicated in the important role in the pathogenesis of various chronic and degenerative diseases[37]. Arsenic may increase oxidative stress by cycling between metals oxidation status such as As, Fe, Cu, etc., or by binding with sulfhydryl groups of proteins and enzymes and interferes with metabolism of glutathione (GSH), the major antioxidant agent responsible for metabolism and excretion of xenobiotics[8] and increasing inflammation, resulting in the accumulation of reactive oxygen species (ROS) in cells[9]. Moreover, the As–GSH complexes can inhibit several GSH-dependent enzymes, such as glutathione reductase[38]. The co-occurrence in present study, more evidences demonstrated that CKD is also associated with oxidative stress from increased oxidant production and decreased antioxidant defenses. There are many potential sources to increased oxidative stress in CKD patients including uremic toxins, angiotensin II, proinflammatory cytokines, iron overload, and decreased in antioxidants levels[39]. Arsenic toxicity can cause acute renal failure and chronic renal insufficiency. The actual injury may cause from the direct effect of arsenic on tubule cells or hypotensive shock, myoglobinuric tubular injury. The kidney function is the main route of arsenic excretion and to filter waste products that build up in the blood. Sublethal arsenic toxicity has resulted in CKD or renal insufficiency and necrosis[40]. Limitation of the present study is the use only urinary As level to reflect the toxic form of As exposure and small sample size. The study is limit only single time area based recruitment. The future follow-up study of renal disease and other metabolic disorders incidence relation with the As exposure would be helpful to establish the causal inference and future prevention.

CONCLUSIONS

In the present study, As-exposure (≥35 μg/L) induced CKD or renal insufficiency concomitant with increased oxidative stress and the multivariate statistical demonstrated the association between As-exposure with MDA, NAG and eGFR levels. It may play a central role of the other degenerative diseases.

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