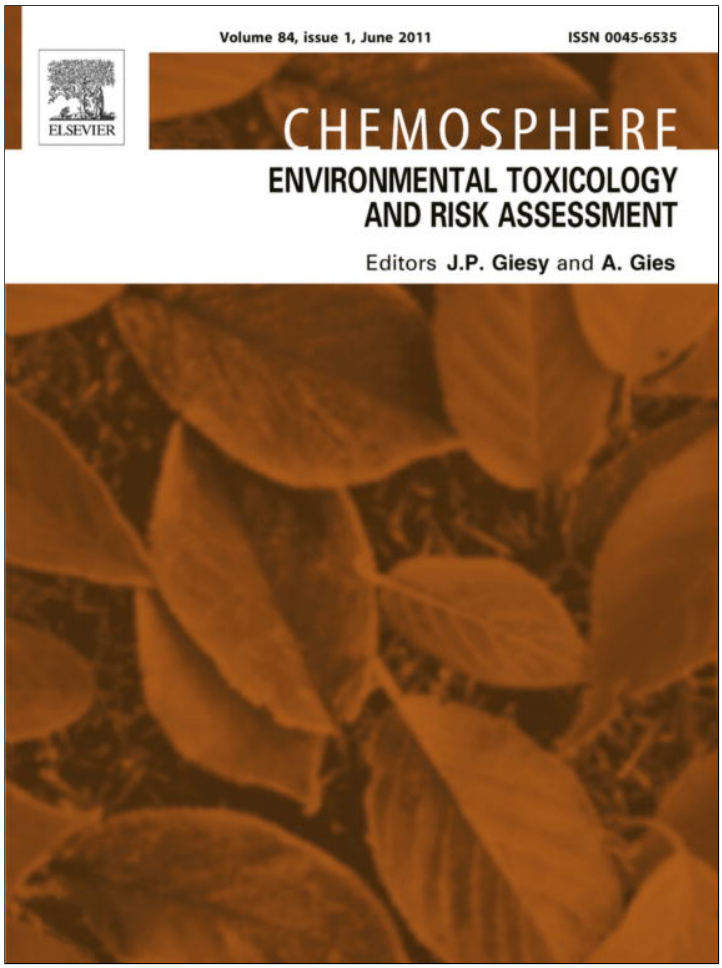

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The association between total urinary arsenic concentration and renal dysfunction in a community-based population from central Taiwan

Jein-Wen Chen [a], Hsiao-Yen Chen [a], Wan-Fen Li [a], Saou-Hsing Liou [a], Chien-Jen Chen [b,c], Jhuo-Han Wu [a], Shu-Li Wang [a,d,*]

[a] Division of Environmental Health and Occupational Medicine, National Health Research Institutes (NHRI), Miaoli 350, Taiwan
[b] Genomic Research Center, Academia Sinica, Taipei, Taiwan
[c] Graduate Institute of Epidemiology, College of Public Health, National Taiwan University, Taipei, Taiwan
[d] Institute of Environmental Medicine, College of Public Health, China Medical University Hospital, Taichung, Taiwan

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ABSTRACT

Arsenic (As) is an important environmental toxicant that can cause cancer and cardiovascular disease, but the relationship between As exposure and renal dysfunction is not clear. The aim of this study is to examine the association between As exposure and renal dysfunction in a community-based population in central Taiwan. One thousand and forty-three subjects were recruited between 2002 and 2005. The risk for type 2 diabetes was increased by 2-fold ($p < 0.05$) in subjects with total urinary As (U-As) > 75 μg g^{-1} creatinine as compared with subjects whose U-As was ⩽35 μg g^{-1} creatinine after the adjustment for potential confounders. The adjusted odds ratio for an abnormal β2 microglobulin (B2MG > 0.154 mg L^{-1}) was significantly higher in subjects with U-As > 35 μg g^{-1} creatinine as compared with the reference group adjusted for age, sex, living area, cigarette smoking, diabetes, and hypertension. The risk for abnormal B2MG and estimated glomerular filtration rate (eGFR < 90 mL min^{-1} (1.73 m^2)$^{-1}$) was both increased around 2-fold ($p < 0.05$) in subjects with U-As > 75 μg g^{-1} creatinine as compared with those with U-As ⩽ 35 μg g^{-1} creatinine adjusted for all the risk factors plus lead (Pb), cadmium and nickel. The prevalence of abnormal B2MG was 4.82 times higher in subjects with both over the median levels of U-As (85.1 μg L^{-1}) and urinary Pb (18.9 μg L^{-1}) as compared to both lower than the median ($p < 0.001$). These results indicate that U-As might relate to renal dysfunction even other important risk factors were taken into account. Follow-up studies for causal inference are warranted.

1. Introduction

Chronic arsenic (As) poisoning in the general population has been widely reported in many areas of the world and may cause life-threatening diseases (Yoshida et al., 2004; Baig et al., 2009). As exposure commonly occurs through drinking water contaminated with high amounts of inorganic forms of As (Smith et al., 2000; Kazi et al., 2009; Arain et al., 2009a). Environmental contamination with As and other metals is well documented in Taiwan. This study focused on Changhua County in central Taiwan because of its high As level. The study area had the highest contaminated level of As in groundwater based on a national survey in Taiwan between 2002 and 2005 (Taiwan-EPA, 2003–2006). Electroplating had seriously polluted agricultural soil over the last two decades through the discharge of wastewater into irrigation ditches (Chang et al., 2006).

Exposure to inorganic As was found to be associated with increased rates of various chronic diseases, including diabetes (Navas-Acien et al., 2006), hypertension (Chen et al., 1995; Navas-Acien et al., 2005), metabolic syndrome (Wang et al., 2007), respiratory disorders (Arain et al., 2009a,b), and other macrovascular (Wang et al., 2003) and microvascular (Chiou et al., 2005) diseases related to diabetes. The prevalence of type 2 diabetes and renal disease were significantly increased in areas where there was increased exposure to As (Wang et al., 2003). However, little is understood regarding renal dysfunction caused by exposure to As as a function of diabetic status. Nordberg et al. (2005) assessed increased levels of urinary β2-microglobulin (B2MG), N-acetyl-h-glucosaminidase (NAG), and albumin (ALB) as markers of renal dysfunction, which was increased in response to As exposure (Nordberg et al., 2005). In a community-based study of high As levels in soil from Guizhou province in China, and the levels of B2MG, NAG, and ALB in residents from the polluted area were significantly higher than in residents from the

* Corresponding author at: Division of Environmental Health and Occupational Medicine, National Health Research Institutes (NHRI), Miaoli 350, Taiwan. Tel.: +886 37 246 166x36509; fax: +886 37 587 406.
E-mail address: slwang@nhri.org.tw (S.-L. Wang).

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non-polluted areas ($p < 0.01$) (Hong et al., 2004). A recent report by Wang et al. (2009) indicated that chronic As exposure had a significant adverse impact on kidney function of residents from the Xngjing Autonomous Region (Wang et al., 2009).

The chronic kidney disease (CKD) with an estimated glomerular filtration rate (eGFR) < 60 mL min^{-1} (1.73 m^2)$^{-1}$ in Taiwan is 11.9%. However, only 3.5% of patients were aware of their disorder (Wen et al., 2008). The present study aimed to examine the association between total urinary arsenic (U-As) exposure and renal dysfunction in a community-based population. Total U-As is an established biomarker of inorganic As exposure in the absence of seafood intake (Hughes, 2006; Navas-Acien et al., 2008). Renal function was evaluated by both B2MG and eGFR. GFR is commonly thought to be a good index of renal dysfunction (Levey et al., 1999) but is difficult to measure in clinical practice. Therefore, an eGFR derived from serum creatinine level like most clinicians was used. The urinary levels of other metals that are known to be related to renal dysfunction such as lead (Pb), cadmium (Cd), and nickel (Ni) were also measured and their effects were considered in multivariable analyses.

2. Materials and methods

2.1. Study population

We have reported in our previous study about the study areas and subjects (Chang et al., 2006). Population census data obtained from the Ministry of Interior, Taiwan, were utilized to select the study population. Eligible subjects were aged 35–64 years and resided in their present household for at least 5 consecutive years at the time of recruitment. The density of metal-related factories was 4.2 factories km^{-2} (2855 factories (686.95 km^2)$^{-1}$) in Changhua County situated in central Taiwan (IDBMOEA, 2002). Fourteen townships were selected as study area during the period of 2002–2005 (Fig. 1). Of the 1086 stratified random sample by 3 age bands (35–44, 45–54, 55–64 years old) and 2 genders, 28 did not provide 5 ml sufficient amount of urine for As analysis, and another 15 were rejected because their occupational and life-style records were incomplete. Thus, the final study group used for data analysis contained 1043 subjects.

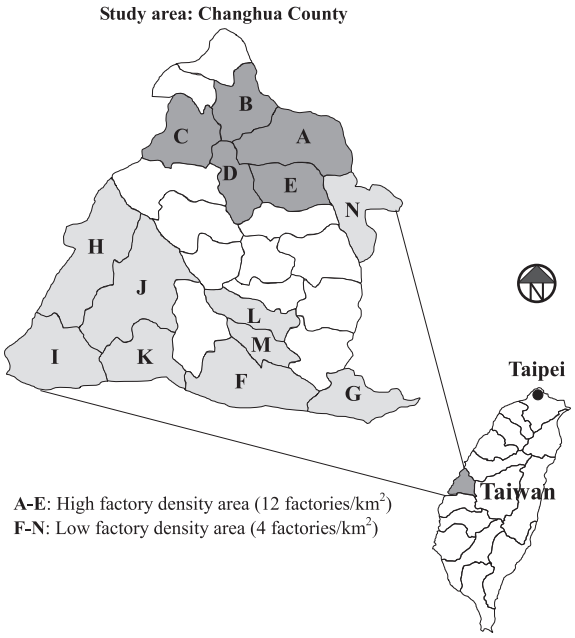

Fig. 1. The location of study areas. A–N were the towns recruited subjects, in which A: Changhua City, B: Hemei, C: Lugang, D: Sioushuei, E: Huatan, F: Hsicou, G: Ershuei, H: Fangyuan, I: Dacheng, J: Erhlin, K: Chutang, L: Tienwei, M: Beidou, and N: Fenyuan Town.

2.2. Data and specimen collections

Prior to data collection, informed consent was obtained from each participant. The study protocol was approved by the Human Subjects Review Board of National Health Research Institutes in Taiwan. Well-trained interviewers used a structured questionnaire to record the data on demographic factors, residential and occupational history from participants at home or a health care unit. Subjects were asked not to eat seafood for three days before the test, and venous blood was collected after $\geqslant$10-h fast. Subjects received health examinations at local health care units, where fasting blood and urine samples were collected. Subjects also received a general health checkup, including blood pressure measurements, from a general/internist physician. Urine and plasma specimens were stored at 4 °C immediately after collection and then be analyzed within 3 h. Fasting plasma glucose and lipids were quantified on the same day in the central laboratory of Changhua Christian Hospital, a medical center that has served local residents for several years.

2.3. Sample analysis

U-As concentrations were measured by an ELAN 6100 inductively coupled plasma-mass spectrometry (ICP-MS, Perkin Elmer, Shelton, CT, USA). Using ICP-MS normally generate better sensitivity with lower limit of detection than atomic absorption spectrometry (Montaser, 1998). A standard reference material, MEDICHEM Metalle U Level 1 (Daignostica and Verfahrensentwicklung Kringserasse, Steinenbronn, Germany), was used for quality control evaluation. The method of detection limit (MDL) of As, Pb, Cd, and Ni were 0.045, 0.038, 0.063, and 0.066 μg L^{-1}, respectively. There were 3.2% (for As), 2.9% (for Pb), 5.8% (for Cd), and 4.7% (for Ni) of samples that had concentrations below the MDL. When the concentrations were below MDL, samples were assigned a value of 50% of the MDL which is a conventional approach and also adopted by NHANES III (The Third National Health and Nutrition Examination Survey) (Paschal et al., 2000). Fasting plasma samples were analyzed in the central laboratory of Changhua Christian Hospital for concentrations of glucose, triglycerides, cholesterol, and low- and high-density lipoproteins using a Beckmen SYNCHRON LX20 System (Beckmen Coulter, CA, USA). Urinary B2MG and urinary creatinine levels were determined in the same medical laboratory. B2MG levels were measured by MEIA (microparticle enzyme immunoassay) and creatinine levels were measured by the Modified Jaffe Method (CREA-HR-1, Wako Pure Chemical Industries, Osaka, Japan).

2.4. Indices of renal function

The B2MG, creatinine concentrations and eGFR were used to assess renal function. The formula developed by the "Modification of Diet in Renal Disease (MDRD) Study Group" is widely used to estimate GFR from serum creatinine measurements for systematically evaluating patients with chronic renal disease (Levey et al., 1999). The eGFR equation was:

$$\text{eGFR} = 186 \times \text{serum creatinine}^{-1.154} \times \text{age}^{-0.203} (\times 0.742 \text{ if female}) \quad \text{(Levey et al.,1999)} \qquad (1)$$

In clinical definition, chronic kidney disease was defined as kidney damage or GFR < 60 mL min^{-1} (1.73 m^2)$^{-1}$ for 3 months or more (Levey et al., 2005); however, present study did not had more than one GFR estimate in different times due to the limitation of

cross-sectional study. In order to detect early signs of renal dysfunction associated with As, 90 mL min^{-1} (1.73 m^2)$^{-1}$ was chosen as another cut-off value. Abnormal B2MG was defined as B2MG > 0.154 mg L^{-1} according to the criteria of the medical laboratory of Changhua Christian Hospital. In addition, diabetes mellitus (DM) was defined as a fasting plasma glucose concentration $\geqslant$126 mg dL^{-1} or treatment with diabetic therapy. Hypertension was defined as a diastolic blood pressure $\geqslant$90 or systolic blood pressure $\geqslant$140 mm Hg, or on anti-hypertension therapy.

2.5. Statistical analyses

The data of the U-As, urinary Pb (U-Pb), Cd (U-Cd), and Ni (U-Ni) concentrations were not normally distributed and skewed to the right. Thus, geometric means (GM) along with 95% confidence intervals were calculated to prevent over-estimation from using arithmetic means. When the log transformed data were normally distributed, Student's *t*-test or analysis of variance (ANOVA) was utilized to test difference among the groups. For transformed data that were not normally distributed, nonparametric methods were employed. If concentrations of metals below the MDL, the concentrations were recorded to 1/2 × MDL in the data analyses (Paschal et al., 2000). Associations between categorical variables were tested using a Chi-square test. Kendall's tau-c trend test was used for trend analysis of demographic factors, lifestyles, and renal dysfunction biomarkers by U-As. The American Conference of Governmental Industrial Hygienists (ACGIH) recommendation for the biological exposure index of U-As concentration is 35 μg L^{-1} (ACGIH, 1996). The cut-off of U-As level suggested by ACGIH was used to examine the association between U-As levels and renal function indices. Univariate and multivariate logistic regression analyses were performed to predict the presence of renal dysfunction and diabetes based on U-As level. For smoking status, people smoke at least 1 cigarette per day more than six months was defined as cigarette smoker. When fasting plasma glucose $\geqslant$126 mg dL^{-1} or on diabetes therapy, it was defined as diabetes, and if systolic blood pressure $\geqslant$90 or diastolic blood pressure $\geqslant$140 mm Hg or on anti-hypertension therapy was defined as hypertension. All analyses were performed using SPSS version 15.0.

3. Results

Table 1 shows the demographic factors and related biochemical variables by tertiles of U-As concentration in the study population. B2MG, U-Pb, and U-Cd levels increased along with increased tertiles of U-As concentrations. Smoking status was also found positively associated with U-As level. While, eGFR decreased with increasing U-As concentration. Table 2 shows the demographic factors and related biochemical variables according to B2MG concentration and eGFR. Blood pressure, fasting plasma glucose, and serum creatinine were significantly higher in the groups with abnormal renal function indices (B2MG > 0.154 mg L^{-1} and eGFR < 60 mL min^{-1} (1.73 m^2)$^{-1}$) as compared with the normal group after adjustment for age. A significant association was also found between renal function indices (B2MG and eGFR) and diabetes or hypertension status.

There is no normal range of U-As level recommended for general populations. Therefore, we used the biological exposure index of U-As (35 μg L^{-1}) suggested by ACGIH (1996) as the cut-off point in the following analyses. Table 3 shows the results of multiple logistic regression analyses for diabetes by groups of various U-As levels, with subjects whose levels were $\leqslant$35 μg L^{-1} serving as the reference group. The adjusted odds ratios (ORs) for diabetes mellitus (DM) showed an increasing trend according to As levels using ACGIH cut-off points (Table 3). The ORs for DM were 2.08 and 2.22 for U-As levels of >75–200 and >200 μg g^{-1} creatinine, respectively, as compared with U-As levels $\leqslant$35 μg g^{-1} creatinine, after adjustments

Table 1
Demographic factors and related biochemical variables by tertiles of urinary arsenic (U-As) concentration.

Characteristics (N = 1043)	U-As (μg L^{-1})			*p*-Value[a]
	<61.66 (n = 341)	61.66–112.2 (n = 371)	>112.2 (n = 331)	
Continuous variables				
Age (y)[b]	51.2 ± 8.5	50.4 ± 8.6	50.7 ± 8.4	0.37
Body mass index (kg m^{-2})[b]	24.8 ± 3.5	25.3 ± 3.5	24.9 ± 3.3	0.08
Fasting plasma glucose (mg dL^{-1})[b]	102.0 ± 33.8	102.6 ± 31.5	102.4 ± 31.7	0.97
Systolic blood pressure (mm Hg)[b]	130.6 ± 21.2	130.7 ± 22.0	130.9 ± 21.1	0.97
β2 microglobulin (mg L^{-1}) [c]	0.2 (0.08–0.41)	0.3 (0.02–0.7)	0.5 (0.03–0.9)	0.04[*,e]
Serum creatinine (mg dL^{-1})[b]	0.9 ± 0.7	0.9 ± 0.2	1.0 ± 0.6	0.11
Estimated glomerular filtration rate (eGFR, mL min^{-1} (1.73m^2)$^{-1}$)[b,d]	79.4 ± 15.2	77.4 ± 12.9	73.6 ± 11.9	0.02[*,e]
Urinary lead (μg L^{-1})[e]	17.1 (15.9–18.5)	17.7 (16.3–19.2)	23.6 (21.5–25.8)	0.002[**,e,f]
Urinary cadmium (μg L^{-1})[e]	0.9 (0.8–1.0)	0.9 (0.8–1.1)	1.1 (1.0–1.3)	0.007[**,e,f]
Urinary nickel (μg L^{-1})[e]	4.3 (3.9–4.7)	4.3 (3.9–4.7)	4.5 (4.2–4.9)	0.33
	Percentage (n)			
Categorical variables				
Male (%)	37.2% (127)	47.2% (175)	56.5% (187)	<0.001[***]
Cigarette smoker (%)[g]	14.4% (49)	20.5% (76)	29.6% (98)	<0.001[***]
Diabetes (%)[h]	7.9% (27)	8.6% (32)	8.5% (28)	0.10
Hypertension (%)[i]	41.3% (141)	41.2% (153)	42.9% (142)	0.22

[a] ANOVA with Scheffe post hoc test for continuous variables, Kendall's tau-c trend test for categorical variables.
[b] Mean ± SD or geometric mean ± geometric SD.
[c] Geometric mean (95% confidence interval).
[d] Estimated glomerular filtration rate (eGFR, mL min^{-1} (1.73 m^2)$^{-1}$) = 186 × (serum creatinine (mg dL^{-1}))$^{-1.154}$ × age (y)$^{-0.203}$(×0.742 if female).
[e] 1st tertile vs. 3rd tertile.
[f] 2nd tertile vs. 3rd tertile.
[g] Cigarette smoker: at least 1 cigarette per day more than 6 months.
[h] diabetes: fasting plasma glucose $\geqslant$126 mg dL^{-1} or on diabetes therapy.
[i] hypertension: systolic and diastolic blood pressure $\geqslant$90 or 140 mm Hg, respectively, or on anti-hypertension therapy.
[*] $p < 0.05$.
[**] $p < 0.01$.
[***] $p < 0.001$.

Table 2
Demographic factors and related biochemical variables according to renal dysfunction indices (β2 microglobulin (B2MG) and estimated glomerular filtration rate (eGFR)).

Characteristics (N = 1043)	B2MG		p-Value[a,b]	eGFR		p-Value[a,b]
	(>0.154 mg L^{-1}, n = 249)	(≦0.154 mg L^{-1}, n = 794)		(<60 mL min^{-1} (1.73 m^2)$^{-1}$, n = 94)	(≧60 mL min^{-1} (1.73 m^2)$^{-1}$, n = 949)	
Continuous variables						
Age (y)[c]	53.2 ± 8.1	50.0 ± 8.4	<0.001***	56.4 ± 7.6	50.2 ± 8.4	<0.001***
Body mass index (kg m^{-2})[c]	24.9 ± 3.4	25.0 ± 3.5	0.53	26.2 ± 3.3	24.9 ± 3.4	0.01*
Systolic blood pressure (mm Hg)[c]	138.4 ± 24.6	128.4 ± 19.7	0.01*	140.4 ± 22.7	129.8 ± 21.3	0.04*
Fasting plasma glucose (mg dL^{-1})[c]	107.5 ± 38.1	100.7 ± 30.1	0.01**	113.8 ± 46.4	101.2 ± 30.4	0.03*
Serum creatinine (mg dL^{-1})[c]	1.1 ± 0.1	1.0 ± 0.2	0.008**	1.6 ± 1.6	0.9 ± 0.2	0.01**
Urinary arsenic (μg L^{-1})[d]	79.4 (75.7–83.6)	69.2 (64.3–93.0)	0.01**	81.3 (77.6–85.1)	69.5 (64.2–93.8)	0.19
Urinary cadmium (μg L^{-1})[d]	1.0 (0.9–1.1)	1.0 (0.9–1.1)	0.81	1.0 (0.9–1.1)	0.9 (0.8–1.1)	0.31
Urinary lead (μg L^{-1})[d]	19.5 (18.5–20.5)	16.0 (14.5–17.5)	0.008**	19.9 (18.0–20.0)	16.2 (14.3–18.4)	0.10
Urinary nickel (μg L^{-1})[d]	4.5 (4.2–4.7)	4.0 (3.6–4.4)	0.06	4.3 (4.1–4.5)	4.7 (3.9–5.5)	0.38
	Percentage (%)			Percentage (%)		
Categorical variables						
Male (%)	63.5	44.2	<0.001***	57.4	46.0	0.02*
Cigarette smoker (%)	28.9	19.0	0.001**	24.5	21.1	0.26
Diabetes (%)[e]	13.3	8.4	0.019*	23.4	8.2	<0.001***
Hypertension (%)[f]	56.2	37.3	<0.001***	60.6	37.1	<0.001***

[a] Student's t-test for normal distributed and Mann–Whitney test for non-normal distributed continuous variables, chi-square test for categorical variables.
[b] Age-adjustment.
[c] Mean ± SD or geometric mean ± geometric SD.
[d] Geometric mean (95% confidence interval).
[e] Diabetes: fasting plasma glucose ⩾126 mg dL^{-1} or on diabetes therapy.
[f] Hypertension: systolic and diastolic blood pressure ⩾90 or 140 mm Hg, respectively, or on anti-hypertension therapy.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

Table 3
Odds ratios (ORs) for diabetes mellitus according to American Conference of Governmental Industrial Hygienists (ACGIH) cut-off points of urinary arsenic (U-As) concentration (n = 1043).

U-As (μg g^{-1} creatinine)	With diabetes mellitus [n (percentage)][a,e]	ORs[b]	ORs[c]	ORs[d,e]
⩽35 (n = 133)	6 (4.5%)	1	1	1
>35–75 (n = 315)	26 (8.3%)	2.13 (0.86–3.39)	1.97 (0.69–2.67)	1.95 (0.56–2.66)
>75–200 (n = 484)	45 (9.3%)	2.30 (1.32–4.89)*	2.11 (1.31–4.16)*	2.08 (1.05–3.69)*
>200 (n = 111)	10 (9.8%)	2.77 (1.42–5.27)q**	2.34 (1.38–4.67)*	2.22 (1.21–4.09)*

[a] Diabetes mellitus: fasting plasma glucose ≧126 mg dL^{-1} or on diabetes therapy.
[b] Adjusted by age, sex, living area, and cigarette smoking.
[c] Adjusted by age, sex, living area, cigarette smoking, and hypertension.
[d] Adjusted by age, sex, living area, cigarette smoking, hypertension, lead, cadmium, and nickel.
[e] Trend test for increasing diabetes prevalence with U-As level, p = 0.07 by X^2, and for ORs[d], p = 0.09 by logistic regression.
* $p < 0.05$.
** $p < 0.01$.

for age, sex, living area, cigarette smoking, hypertension, Pb, Cd, and Ni. Table 4 shows the results of multiple logistic regression analysis using three different regression models for an abnormal B2MG (>0.154 mg L^{-1}) and eGFR (<60 and <90 mL min^{-1} (1.73 m^2)$^{-1}$ are both shown) based on U-As levels. The ORs for an abnormal B2MG excretion were 1.69, 2.11, and 2.04 for U-As levels of >35–75, >75–200, and >200 μg g^{-1} creatinine, respectively, as compared to the groups with B2MG excretions ⩽35 μg g^{-1} creatinine, after adjustment for age, sex, living area, cigarette smoking, diabetes, hypertension status, Pb, Cd, and Ni. When considering a similar approach by non-adjusted U-As concentration (μg L^{-1}), the results were the same as previous ones. Subjects with U-As levels >75 μg g^{-1} creatinine had a significantly higher OR of eGFR < 90 mL min^{-1} (1.73 m^2)$^{-1}$ than those with U-As levels ⩽35 μg g^{-1} creatinine. However, when eGFR < 60 mL min^{-1} (1.73 m^2)$^{-1}$ was considered as the cut-off value for renal dysfunction, subjects with U-As levels >200 μg g^{-1} creatinine had a significantly higher OR of renal dysfunction than those with U-As levels ⩽35 μg g^{-1} creatinine. Fig. 2 shows that the prevalence of abnormal B2MG was 4.82 times higher in subjects with both over the median levels of U-As (85.1 μg L^{-1}) and U-Pb (18.9 μg L^{-1}) as compared to both lower than the median ($p < 0.001$).

4. Discussion

The association between U-As and renal dysfunction found in this study is consistent with the results of a cross-sectional study in an area of high exposure to As in Guizhou, China (Nordberg et al., 2005). They reported the combined exposure of individuals to inorganic As and Cd resulted in a higher prevalence of renal dysfunction than the separate exposure to either metal. Hsueh et al. (2009) reported that total U-As was associated significantly with CKD in a dose–response relationship, especially with a total As level than 20.7 compared with 11.8 μg g^{-1} creatinine or less. Since U-As is normally used as biomarker of current As exposure, the present study focused on total U-As levels with a consideration of other metals such as Pb, Cd, and Ni. However, multiple logistic regression analysis with the urinary levels of other metals entered as covariates showed that only As was significantly associated with

Table 4
Odds ratios (ORs) for renal dysfunction (elevated β2 microglobulin (B2MG) or reduced estimated glomerular filtration rate (eGFR)) according to American Conference of Governmental Industrial Hygienists (ACGIH) cut-off points of urinary arsenic (U-As) concentration (n = 1043).

U-As (μg g^{-1} creatinine)	B2MG > 0.154 mg L^{-1} [n (percentage)][d]	ORs[a]	ORs[b]	ORs[c,d]
⩽35 (n = 133)	14 (10.5%)	1	1	1
>35–75 (n = 315)	66 (21.0%)	2.61 (1.61–6.36)**	2.29 (1.13–5.55)**	1.69 (0.94–3.64)
>75–200 (n = 484)	137 (28.3%)	3.82 (1.66–6.62)**	3.44 (1.55–6.11)**	2.11 (1.23–4.98)*
>200 (n = 111)	32 (28.8%)	3.28(1.78–6.56)***	3.24(1.56–6.24)**	2.04(1.11–4.37)*
	eGFR < 60 mL min^{-1} (1.73 m^2)$^{-1}$ [n (percentage)]			
⩽35 (n = 133)	6 (4.5%)	1	1	1
>35–75 (n = 315)	31 (9.8%)	1.34 (0.78–2.23)	1.15 (0.63–1.89)	1.11 (0.56–1.80)
>75–200 (n = 484)	48 (9.9%)	0.91 (0.51–1.79)	0.76 (0.44–1.86)	0.68 (0.42–1.33)
>200 (n = 111)	10 (9.0%)	2.39 (1.11–5.44) *	2.12 (1.08–5.45) *	1.98 (0.95–4.99) *
	eGFR < 90 mL min^{-1} (1.73 m^2)$^{-1}$ [n (percentage)]			
⩽35 (n = 133)	98 (73.9%)	1	1	1
>35–75 (n = 315)	267 (84.8%)	1.80 (0.66–2.39)	1.67 (0.59–1.98)	1.45 (0.49–1.88)
>75–200 (n = 484)	403 (83.3%)	2.50 (1.32–3.89)*	2.21 (1.21–3.86)*	2.15 (1.06–3.78)*
>200 (n = 111)	96 (86.5%)	3.08 (1.42–7.27)**	2.31 (1.18–4.67)*	2.16 (1.11–3.49)*

[a] Adjusted for age, sex, living area, and cigarette smoking.
[b] Adjusted for age, sex, living area, cigarette smoking, diabetes, and hypertension.
[c] Adjusted for age, sex, living area, cigarette smoking, diabetes, hypertension, lead, cadmium, and nickel.
[d] Trend test for increasing rate of abnormal β2 microglobulin with U-As level, p = 0.05 by X^2, and for ORs [c], p = 0.05 by logistic regression.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

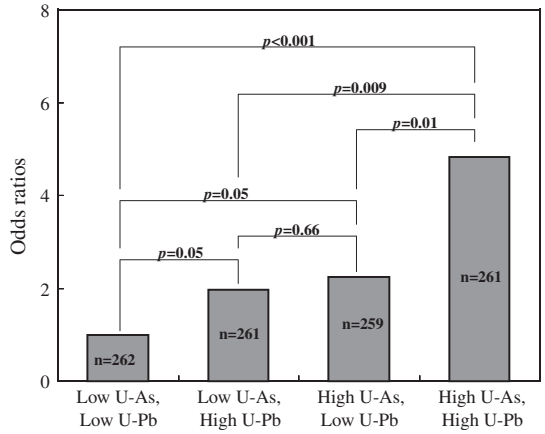

Fig. 2. Logistic regression of abnormal β2-microglobulin (B2MG ⩾ 0.154 mg L^{-1}) by median of urinary arsenic (U-As: 85.13 μg L^{-1}) and urinary lead (U-Pb: 18.9 μg L^{-1}). The model was adjusted for age, sex, living area, cigarette smoking, diabetes, hypertension, cadmium, and nickel.

renal dysfunction. In the present study, renal tubular dysfunction, indicated by an elevated excretion of B2MG, occurred in subjects with high levels of U-As. Recently, Buchet et al. (2003) reported the renal effects of co-exposure to As and Cd in a Chinese population. In this study, U-As was claimed as a significant predictor of renal tubular and glomerular dysfunction (Buchet et al., 2003). In the present study, among the renal-toxic metals (such as As, Pb, Cd, and Ni) it was found that total U-As showed significant associations with B2MG and eGFR after adjustment for all potential confounding variables including Pb, Cd, and Ni. In a governmental report (Taiwan-EPA, 2003–2006), several metals in agriculture soil of study areas exceed the criteria of Taiwan, including As, Pb, Ni, and Hg due to seriously polluted by electroplating and metal-related factories. From questionnaire, around 13% of subjects living near metal-related factories (within 50 m), in which 34% of the factories were electroplating and lead-acid batteries factory. It is likely that residents in this area were exposed to multi-elements. In fact, urinary metal measurement showed a high correlation between U-As with other metals of Pb, Cd, and Ni. We have evaluated the effects of other metals by similar statistical approaches and found that Cd and Ni did not show significant ORs with diabetes, abnormal B2MG, and eGFR. It was found only Pb showed significant ORs for abnormal B2MG in the upper tertile as compared with lower tertile (p < 0.05, data not shown but in Supplemental Table A and Table B). In Table 2, B2MG was also found significantly different between lower and upper groups of U-Pb, indicating that Pb exposure may also have adverse effects on renal tubular function. Thus, in the present study, the contribution of Pb to the observed renal dysfunction cannot be ruled out. When evaluating the effect of renal tubular or glomerular dysfunction by U-As, to consider the effect by U-Pb is needed.

For U-Cd the correlation was not significant, this may be because the study areas were not highly contaminated with Cd; thus, the subjects may have ingested low levels of Cd with little influence on renal function. U-Cd concentration in present study was 0.96 μg g^{-1} creatinine (0.94 μg g^{-1}), similar to that of the control area in China (Hong et al., 2004; Nordberg et al., 2005) with U-Cd concentrations 0.86 and 2.16 μg g^{-1} creatinine in control and exposed areas, respectively. The U-Cd level of present study was higher than that of the subjects in NHANES III (0.48 μg g^{-1} creatinine or 0.57 μg g^{-1}) (Paschal et al., 2000). In present study, subjects were living within contaminated areas in central Taiwan, and their U-As level was significantly associated with renal dysfunction.

Evidence of renal toxicity by As exposure (kidney cancer excepted) was not frequently available from animal models using relatively high dose of As. A previously study reported that, depending on the serum marker used (serum creatinine or cystatin C), the increase in GFR ranged from 7% to 11% in children who had blood Pb levels in the upper quartile of the population (>55 μg L^{-1}; mean blood Pb, 78.4 μg L^{-1}) (de Burbure et al., 2006). According to an experimental study focused on (Courtois et al., 2003), the initial mechanism may well depend on Pb-induced production of reactive oxygen species that upregulate cyclooxygenase (COX-2) expression in the vascular smooth muscle. Oxidative stress has been identified as an important mechanism in As-induced decreased kidney function through accumulation of As in kidney tissue, and reduced glutathione in treated animal (Sinha et al., 2008). Wang et al. (2009) reported that kidney is susceptible to secondary damage resulting from disease such as diabetes and hypertension (Wang et al., 2009). Further studies are needed.

U-As levels tend to vary greatly between studies. A study carried out in Bangladesh (Lindberg et al., 2008) found that the respective 5th and 95th percentiles of U-As concentration were 20 and

Table 5
Total urinary arsenic levels from different countries.

Country	Exposure status	Sample size	Arsenic levels in urine	References
Denmark	A group of unexposed Danish cohort	Male: 89	Male: 15.3 ± 12.4 μg L^{-1}	Kristiansen et al. (1997)
		Female: 93	Female:11.3 ± 10.4 μg L^{-1}	
UK	Healthy unexposed British subjects	23	12.3 (0.9–1080) μg L^{-1}	White and Sabbioni (1998)
German	The population of German Environmental Survey II (1990–1992)	4001	6.3 (6.08–6.51)[a] μg L^{-1}	Seifert et al. (2000)
Chile	Subjects who had long-term exposure to very high levels of arsenic in drinking water (735–762 μg L^{-1})	Fathers: 11	Father: 464 (184–1026) μg L^{-1}	Chung et al. (2002)
		Mothers: 11	Mother: 466 (161–773) μg L^{-1}	
		Sons: 14	Sons: 566 (55–1320) μg L^{-1}	
		Daughters: 8	Daughter: 427 (260–736) μg L^{-1}	
UK	Healthy volunteers	Asian: 21	Somali Black-African population: 7.2 μg g^{-1} creatinine	Brima et al. (2006)
		Somali: 22	Asian: 20.6 μg g^{-1} creatinine	
		White: 20	White groups: 24.5 μg g^{-1} creatinine	
US	US general population	788	7.1 (3.6–13.9) μg L^{-1}[b]	Navas-Acien et al. (2008)
China	Subjects with low-arsenic and high-arsenic exposure through food intake	High: 10	High: 58.3 (56.2–60.5) μg g^{-1} creatinine	Li et al. (2008)
		Low: 35	Low: 23.4 (20.9–25.8) μg g^{-1} creatinine	
China	Subjects in a high-arsenic-exposed area and control study site	Exposed subject: 113	Exposed subjects: 192.2 ± 22 μg g^{-1} creatinine	Wang et al. (2009)
		Control site: 30	Control site: 63.6 ± 5.9 μg g^{-1} creatinine	
Bangladesh	Infants 7 months-of-age who were born in an area of high-arsenic-contaminated tube wells	1799	8 weeks gestation: 81 (37–207)[b] μg L^{-1}	Tofail et al. (2009)
			30 weeks gestation: 84 (42–230)[b] μg L^{-1}	
Bangladesh	The study subjects were 10,402 residents, who had consumed contaminated well water	Male: 4138	Male: 131.7 ± 137.8 μg L^{-1}; 229.8 ± 249.0 μg g^{-1} creatinine	Heck et al. (2009)
		Female: 6264	Female: 132.3 ± 152.2 μg L^{-1}; 297.6 ± 289.4 μg g^{-1} creatinine	
Taiwan (present study)	Subjects live in an area where electroplating has seriously polluted agricultural soils through the discharge of wastewater into irrigation ditches	Male: 489	Male: 91.2 (85.1–97.7) μg L^{-1}; 80.1 (72.8–87.3) μg g^{-1} creatinine	
		Female: 554	Female: 74.1 (69.2–77.6) μg L^{-1}; 88.6 (80.8–96.3) μg g^{-1} creatinine	

[a] Geometric mean (95% confidence interval).
[b] Median (interquartile range).

453 μg L^{-1}, whereas the median (77 μg L^{-1}) was similar to the median in present study. Table 5 shows the results of epidemiologic studies in several countries compared with the present study. The total U-As concentrations varied widely in different countries. The U-As concentrations were >100 μg L^{-1} when individuals were exposed to a high concentration of As (such as in Chile, China, and Bangladesh). However, the U-As concentrations of healthy individuals that were not exposed to high As levels were lower (Denmark, UK, German, and US). The higher U-As levels found in men in this study (Table 5) were also reported for residents in the arseniasis endemic area in southwestern Taiwan and in the general population in a Danish study (Kristiansen et al., 1997). However, some studies shown in Table 5 show an opposite result, with U-As levels higher in females than males. Further investigations are required to determine the possible confounding variables that affect U-As concentration. Important issues that need evaluation are whether different lifestyles result in different levels of exposure. Many epidemiologic studies on As poisoning from the consumption of As-contaminated water have been reported worldwide (Yoshida et al., 2004; Arain et al., 2009a). However, there are relatively few reports on the dose–response relationship between As exposure and As-related adverse health effects, because it is often difficult to evaluate individual levels of exposure to As.

A limitation of the present study is the use of total U-As level to reflect toxic form of inorganic As exposure. Compared to the time-consuming As species analysis, total U-As can be measured in a short period of time for a large number of samples, which is essential for many study. However, this measure does not account for variation in As uptake and metabolism between individuals. In spite of this shortcoming, total U-As is still considered a reasonable biomarker for inorganic As exposure. A significant dose–response relationship between total U-As and eGFR in non-diabetic subjects (data not shown) was also found. The present study may be limited also to single time recruitment, thus it is difficult to establish a causal relationship between U-As concentration and renal dysfunction. A future follow-up study of renal disease incidence according to the As exposure would be helpful to establish the temporality for causal inference.

5. Conclusions

In the present study, the rate of abnormal B2MG increased with the U-As concentrations in a dose-dependent manner. Renal dysfunction rates significantly increased when the U-As rose above 75 μg g^{-1} creatinine, for both tubular (B2MG > 0.154 mg L^{-1}) and glomerular function (eGFR < 90 mL min^{-1} (1.73 m^2)$^{-1}$). A

significantly higher OR of DM in subjects with a U-As $\geqslant 75\ \mu g\ g^{-1}$ creatinine was found as compared to the reference group with a U-As $\leqslant 35\ \mu g\ g^{-1}$ creatinine. The prevalence of abnormal B2MG was 4.82 times higher in subjects with both over the median levels of U-As ($85.1\ \mu g\ L^{-1}$) and U-Pb ($18.9\ \mu g\ L^{-1}$) as compared to both lower than the median ($p \leqslant 0.001$). These results indicate that U-As might relate to renal dysfunction, and the contribution of Pb to the observed renal dysfunction cannot be ruled out. Further studies are needed by using follow-up approach or experiment of renal tubular damage by As.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chemosphere.2011.02.091.

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