

Associations between urinary excretion of cadmium with alpha-1 microglobulin and microalbuminuria: a cross-sectional study in northwestern Thai population

Sujitra Sikaphan¹ Ratchaneekorn Boonthum² Siriwan Leudang¹ Sittiporn Parmen^{1*}

¹Toxicology Center, National Institute of Health, Department of Medical Sciences, Ministry of Public Health, Nonthaburi Province, Thailand.

²Regional Medical Sciences Center 2, Department of Medical Sciences, Ministry of Public Health, Phitsanulok Province, Thailand.

ARTICLE INFO

Article history:

Received January 2021

Received in revised form March 2021

Accepted as revised April 2021

Available online April 2021

Keywords:

Alpha-1 microglobulin, microalbuminuria,
urinary cadmium

ABSTRACT

Background: In Thailand, a high Cadmium (Cd) levels were observed in the northwestern population, especially in Mae Sot District, Tak Province. However, the association between urinary cadmium level and changes in renal biomarkers after long-term expose has not been studied, especially in case of low-level Cd exposures.

Objectives: The main focus of this study was to investigate the associations between urinary cadmium levels and renal biomarkers in the northwestern Thai population.

Materials and methods: A total of 817 persons (males=309, females=508) living in Cd-polluted area of Mae Sot district were participated in this study. The urinary cadmium level was analyzed using graphite furnace atomic absorption spectrometry (GFAAS). Two renal biochemical markers were selected, namely urinary alpha-1-microglobulin and microalbuminuria.

Results and Conclusion: The geometric mean concentration for urinary cadmium was 3.67(±3.08) µg/gm creatinine and 4.83(±3.82) µg/gm creatinine in adult males and females, respectively. Based on correlation analysis, the urinary cadmium level was positively correlated with age, years of residence, microalbuminuria and urinary alpha-1 microglobulin in both male and female. While a significant inverse correlation was found between the urinary cadmium level and BMI. Linear regression analysis yielded that alpha-1-microglobulin and microalbuminuria were significantly increased with increasing of urinary cadmium levels. Interestingly, the prevalence of increased alpha-1 microglobulin was higher than the prevalence of increased microalbuminuria in subjects with low urinary cadmium levels. The findings of this study indicate that urinary alpha-1 microglobulin showed a best of biomarker for monitoring the low-level of Cd exposure in both populations.

Introduction

Cadmium (Cd) pollution has become an issue of public health concern and associated with nephrotoxic effects as well as noncommunicable diseases such as cardiovascular disease, diabetes mellitus.¹⁻³ Primary sources of Cd exposure are natural components (soils, air, water), cigarette smoke,

dietary intake and anthropogenic activities from industrial production such as batteries, plastic and synthetic materials.⁴⁻⁶ Continuous exposure to Cd has been linked to bioaccumulation in living organisms and potential adverse health effects in human especially people living in contaminated areas.^{5,7,8} In the northwest of Thailand, the cadmium contaminated areas were located in Mae Sot District, Tak Province. Major contamination source is believed to be a zinc mine nearby two creeks of Mae Tao and Mae Ku which had been operated more than 20 years.⁹ As a result of this mining activities, increasing cadmium levels have been recorded in the areas by heavy metal monitoring scheme.¹⁰ Several studies have shown a degree of cadmium contaminated from food

* Corresponding author.

Author's Address: Toxicology Center, National Institute of Health, Department of Medical Sciences, Ministry of Public Health, Nonthaburi Province, Thailand.

** E-mail address: sittiporn.p@dmsc.mail.go.th

doi:

E-ISSN: 2539-6056

chain to local population living in the areas.^{7-9,11,12}

Cadmium toxicity refers to the level of tubular dysfunction, which is diagnosed by increased urinary excretion of low molecular weight proteins such as alpha-1 microglobulin (α_1 -MG), beta-2 microglobulin (β_2 -MG), retinol-binding protein (RBP), metallothionein (MT), microalbuminuria and kidney injury molecule-1(KIM-1).^{5, 7, 11-14} These biomarkers can reflect the early changes and the late stage of renal function. Using the German Commission on Human Biological Monitoring (HBM) recommended two different reference values for Cd in urine for the general population based on toxicology and epidemiology studies, HBM I (2 $\mu\text{g/gm}$ creatinine) and HBM II (5 $\mu\text{g/gm}$ creatinine).¹⁵ The concentrations below the lower HBM I level are not considered to be a risk for the general population, while concentrations above HBM II indicate an increased risk of adverse health effects in susceptible individuals of the general population.¹⁵

In the present study, we investigated the relationships between two renal biomarkers, namely alpha-1 microglobulin and microalbuminuria with urinary excretion of cadmium from 817 subjects living around the cadmium contaminated area of Mae Sot District, Tak Province. Based on the cut-off values, the level of microalbuminuria below 20 mg/L indicate normal albuminuria whereas level of urinary alpha-1 microglobulin above 15 mg/gm creatinine is assumed tubular dysfunction.^{16, 17}

Materials and methods

Study subject and study site

The study was conducted in northwest Thai population at Tak Province. A total of 817 subjects were recruited including 309 males and 508 females. This study was approved by the Ethical Review Committee for Research in Human Subjects at Department of Medical Sciences, Ministry of Public Health, Thailand (Protocol No. 2/2014). All participants signed a written informed consent form. Demographics information, health behavior, education and occupation were administered.

Urine sample collection and urinary cadmium determination

Urine samples were collected from all subjects. Urine volume was measured and 30-50 mL was collected in 100 mL polypropylene tube before being stores at 4-8 °C for alpha-1 microglobulin and -20°C for creatinine as well as urinary Cd until analysis.

Biochemical markers and urinary Cd analyses

Analysis of the urine samples were performed at Toxicology center, National Institute of Health (Department of Medical Sciences, Ministry of Public Health). Using an automated analyzer (Beckman coulter, AU 480 chemistry System), the following urinary analyses were analyzed, according to manufacturer's instructions: creatinine according to the kinetic Jaffe method (compensated, rate blanked), microalbuminuria measured by turbidimetry and urinary alpha-1 microglobulin based on immunological agglutination.

Urinary cadmium levels were analyzed using graphite furnace atomic absorption spectrometry (GFAAS) (Perkin Elmer, Analyst 600). Urine sample and standard were diluted 1: 5 in modifier (0.1% triton x-100, 0.2% ammonium dihydrogen phosphate and 0.2% magnesium nitrate). The electrodeless discharge lamp (EDL) was used with the 228.8 nm wavelength cadmium line.

Data and statistical analysis

Statistical analysis were carried out on all 817 urine samples. Values below the limit of quantitation (LOQ) were treated as half of this limit, i.e. 0.25 $\mu\text{g/gm}$ creatinine. Pearson's correlation coefficient was used to measures the statistical association between the two variables. Linear regression analysis was performed to determine the relationships between urinary Cd and two renal biomarkers as well as demographic parameters. The statistical analysis was analyzed using SPSS Statistics (version 27.0.1.0, IBM). Reference values used for urinary cadmium were published by the German Commission on Human Biological Monitoring.¹⁵ In adults >25 years old, urinary cadmium concentrations below the lower HBM I level (2 $\mu\text{g/gm}$ creatinine) are not considered to be a risk of advance health effects, whereas concentrations above HBM II (5 $\mu\text{g/gm}$ creatinine) are increased risk of adverse health effects in susceptible individuals in the general population.¹⁵ For two renal biomarkers, the cut-off values microalbuminuria levels above 20 mg/L indicate abnormal albuminuria and the cut-off values of urinary alpha-1 microglobulin above 15 mg/gm creatinine was assumed tubular dysfunction.¹⁶

Results

Descriptive profile

Characteristics of the study population are summarized in Table 1. A total of 817 subjects (309 males, 508 females) were included for statistical analysis. Subjects were in their mid-50s and the mean years of residence was 54 years (SD 16, with ranges of 3 to 91 years) in males and 53 years (SD 14, with ranges of 3 to 83 years) in females. Geometric mean level of urinary cadmium was 3.67 \pm 3.08 $\mu\text{g/gm}$ creatinine in males and 4.83 \pm 3.82 $\mu\text{g/gm}$ creatinine in females. The urinary-Cd levels ranged from 0.25 to 21.53 in male and 0.25 to 24.00 $\mu\text{g/gm}$ creatinine in female participants. Twenty male and 22 female participants had urinary Cd levels below the LOQ. GM concentration of microalbuminuria was 21.55 mg/L (SD 45.03, with ranges of 0.45-281.64 mg/L) in males and 17.95 mg/L (SD 39.94 with ranges of 0.45-281.64 mg/L) in females. GM concentration of alpha-1 microglobulin was 25.23 mg/gm creatinine (SD 27.11, with ranges of 1.87-258.94 mg/gm creatinine) in males and 24.51 mg/gm creatinine (SD 36.42 with ranges of 2.09-425.79 mg/gm creatinine) in females.

Table 1 Characteristics of the study population.

Gender	Characteristics/Parameters	Mean±SD (min-max)
Males (n=309)	Age (years)	58±11 (35-91)
	Body mass index (kg/m ²)	24±4 (15-38)
	Years of residence (years)	54±16 (3-91)
	Urinary-Cd (µg/gm creatinine)	3.67±3.08 (0.25-21.53)
	microalbuminuria (mg/L)	21.55±45.03 (0.45-281.64)
	alpha-1microglobulin (mg/g creatinine)	25.23±27.11 (1.87-258.94)
Females (n=508)	Age (years)	56±11 (35-84)
	Body mass index (kg/m ²)	24±4 (14-38)
	Years of residence (years)	53±14 (3-83)
	Urinary-Cd (µg/gm creatinine)	4.83±3.82 (0.25-24.00)
	microalbuminuria (mg/L)	17.95±39.94 (0.45-281.64)
	alpha-1 microglobulin (mg/gm creatinine)	24.51±36.42 (2.09-425.79)

Urinary Cd, microalbuminuria and urinary alpha-1 microglobulin among study subjects

Standard reference urinary cadmium values of HBM I and HBM II were exceeded among the male participants by 134 (44%) and 75 (24%) males respectively, whereas among the female participants, by 213(42%) and 188 (37%) females respectively (Table 2). The normal cut-off microalbuminuria values (<20 mg/L) were exceeded among the male participants by 62 (20%) males and the female participants by 92 (18%) females. While the normal cut-off alpha-1 microglobulin values (<15 mg/gm creatinine) were exceeded

among the male participants by 180 (58%) males and the female participants by 235 (46%) females. Subjects were classified into three groups with low-exposure (urinary-Cd level below 2 µg/gm creatinine), middle-exposure (urinary-Cd level between 2-5 µg/gm creatinine) and high-exposure (urinary-Cd level >5 µg/gm creatinine) (Table 2). Increasing urinary alpha-1 microglobulin levels were detected in both male and female populations exposed to lower cadmium levels (<2 µg/gm creatinine). While increasing microalbuminuria levels were cross-sectionally associated with increased urinary cadmium levels in both populations.

Table 2 Urinary Cd levels by the degree of microalbuminuria and urinary alpha-1 microglobulin among study subjects.

Gender	Urinary Cd levels ($\mu\text{g/g}$ creatinine)	N (%)	N (%) with microalbuminuria >20 mg/L	N (%) with alpha-1 microglobulin >15 mg/gm creatinine
Males	<2	100 (32%)	13 (13%)	49 (49%)
	2-5	134 (44%)	28 (21%)	81 (60%)
	>5	75 (24%)	21(28%)	50 (67%)
Females	<2	107 (21%)	7 (7%)	43 (40%)
	2-5	213 (42%)	37 (17%)	86 (40%)
	>5	188 (37%)	48 (26%)	106 (56%)

Correlation and linear regression analysis of the determinant parameters

Correlation analysis between urinary-Cd and renal biochemical makers are summarized in Table 3. The urinary cadmium level was positively correlated with age (males: 0.184 $p<0.001$ & females: 0.226, $p<0.001$), and years of residence (males: 0.228 $p<0.001$ & females: 0.243, $p<0.001$), microalbuminuria (males: 0.274, $p<0.001$ & females: 0.174, $p<0.001$) and alpha-1 microglobulin (males: 0.133, $p<0.05$ & females: 0.175, $p<0.001$) concentrations. Furthermore,

the urinary cadmium levels revealed a significant inverse correlation with BMI (males: -0.161 $p<0.05$ & females: -0.207, $p<0.001$).

The regression analysis yielded that alpha-1 microglobulin and microalbuminuria significantly increased with increasing urinary cadmium levels (Table 4). Moreover, two demographic parameters (age & years of residence) significantly increased with increasing of two renal biomarkers. Interestingly, the BMI was significantly associated with alpha-1 microglobulin levels in an inverse manner (Table 4).

Table 3 Pearson correlation coefficient analysis between urinary-Cd and parameters.

Parameters	Correlations	Male	Female
Age (years)	r Significant (2-tailed)	0.184** <0.001	0.226** <0.001
BMI (kg/m ²)	r Significant (2-tailed)	-0.161* 0.004	-0.207** <0.001
Years of residence (years)	r Significant (2-tailed)	0.228** <0.001	0.243** <0.001
microalbuminuria (mg/L)	r Significant (2-tailed)	0.274** <0.001	0.174** <0.001
alpha-1 microglobulin (mg/gm creatinine)	r Significant (2-tailed)	0.133* 0.020	0.175** <0.001

*Correlation is significant at 0.05 level **Correlation is significant at 0.01 level.

Table 4 Linear regression analysis of study parameters and renal markers.

Gender	Independent variables	alpha-1microglobulin (mg/g creatinine)		microalbuminuria (mg/l)	
		β	p value	β	p value
Males	Urinary-Cd ($\mu\text{g/g}$ creatinine)	1.185 (adjusted $r^2=0.015$)	<0.05	4.007 (adjusted $r^2=0.072$)	<0.001
	Age (years)	0.663 (adjusted $r^2=0.069$)	<0.001	0.933 (adjusted $r^2=0.049$)	<0.001
	BMI (kg/m^2)	-1.176 (adjusted $r^2=0.026$)	<0.001	-0.049 (adjusted $r^2=-0.003$)	0.941
	Years of residence (years)	0.430 (adjusted $r^2=0.062$)	<0.001	0.607 (adjusted $r^2=0.044$)	<0.001
Females	Urinary-Cd ($\mu\text{g/g}$ creatinine)	1.664 (adjusted $r^2=0.029$)	<0.001	1.816 (adjusted $r^2=0.028$)	<0.001
	Age (years)	1.032 (adjusted $r^2=0.091$)	<0.001	0.628 (adjusted $r^2=0.027$)	<0.001
	BMI (kg/m^2)	-1.547 (adjusted $r^2=0.025$)	<0.001	-0.354 (adjusted $r^2=-0.001$)	0.445
	Years of residence (years)	0.638 (adjusted $r^2=0.062$)	<0.001	0.382 (adjusted $r^2=0.017$)	<0.05

Discussion

Cadmium (Cd) is one of serious health and environment problems in several countries. Due to the toxicant is non-biodegradable with a long biological half-life approximately 10 to 30 years.^{3,18} Generally, the biomonitoring of Cd exposure in human can be assessed in urine for long-term exposure and in blood for a short period of time or recently exposed.¹⁹ The survey of Cd-contamination in Thailand has been reported by several authors.^{6-10, 14} The highest mean level of urinary cadmium was recorded in the subjects from northern (Mae Sot, Tak Province) and followed by the subjects from northeastern and central part of the country.⁵ Swaddiwudhipong and colleagues was surveyed 7,697 subjects in Mae Sot District, Tak Province.⁹ They reported that 4.9% of subjects had Cd levels between 5 to 10 $\mu\text{g/g}$ creatinine and 2.5% of subjects showed a Cd levels more than 10 $\mu\text{g/g}$ creatinine.⁹

In present study, we conducted a cross-sectional study among general population of Tak Province which is historically exposed to cadmium. Two biomarkers were used to assess the expression of renal function, namely alpha-1 microglobulin and microalbuminuria. The alpha-1 microglobulin is a low molecular weight protein that can pass through the glomerulus.¹⁶ Increasing of the alpha-1 microglobulin in urine indicate early renal tubular dysfunction.^{15, 16, 18} In term of microalbuminuria is described as the urinary albumin excretion of 20 to 200 mg/L.¹⁷ The urinary albumin excretion below 20 mg/L define as normoalbuminuria while the value exceed 200 mg/L define as macroalbuminuria indicating an end-stage renal impairment within 10 to 20 years.¹⁷ Based on this property, alpha-1 microglobulin and microalbuminuria have been studied as a renal biomarker in various diseases such

as diabetes mellitus, hypertension and heavy metal intoxication.^{1, 3, 16, 18}

Of the 817 subjects surveyed, the total geometric mean level of urinary cadmium from males did exceed regulatory limits of the Commission of Human Biological Monitoring.¹⁵ Amongst the total number of 309 males, 209 (68%) exceeded the HBM I Cd value of 2 $\mu\text{g/g}$ creatinine, whereas 401 females exceeded HBM I (79%). Moreover, the geometric mean urinary Cd level in both subjects (males: 3.67 ± 3.08 $\mu\text{g/g}$ creatinine & females: 4.83 ± 3.82 $\mu\text{g/g}$ creatinine) were higher than the levels found in previous study.⁹

Increasing of urinary cadmium with age, years of residence, microalbuminuria and urinary alpha-1 microglobulin were similar observed in both male and female participants. A significant inverse correlation was found between the urinary cadmium levels and BMI. Previous studies have demonstrated that the levels of urinary cadmium decreased with increasing of BMI. However, their correlation is still inadequate.^{20, 21}

In the model testing for the association between the urinary cadmium levels and two renal biomarkers, yielded that alpha-1-microglobulin and microalbuminuria were significantly increased with increasing of urinary cadmium levels. Interestingly, the prevalence of increased alpha-1 microglobulin was higher than the prevalence of increased microalbuminuria in subjects with urinary cadmium concentrations below 2 $\mu\text{g/g}$ creatinine. According to HBM I level, the concentrations below the lower HBM I level are not considered to be a risk for the general population.¹⁵ This result was consistent with the studies conducted by

Järup et al.²² They suggested that the development of renal tubular damage associated with low-level cadmium exposures.²² While the microalbuminuria levels appeared to be significant association with the moderately (>2-5 µg/gm creatinine) to high (>5 µg/gm creatinine) levels of urinary cadmium.

Conclusion

The findings of this study indicate that most 610 subjects did exceed the standard of the Commission of Human Biological Monitoring of the HMB I level. The urinary cadmium level showed a significant correlation with age, years of residence, microalbuminuria and urinary alpha-1 microglobulin in both male and female. Moreover, this study indicated that the cadmium exposure at levels below 2.0 µg/gm creatinine may produce measurable changes in renal biomarker of urinary alpha-1 microglobulin. Therefore, we suggested that the urinary alpha-1 microglobulin can be used for monitoring the early stages of Cd exposure.

Acknowledgments

This work was financially supported by the Department of Medical Sciences, Ministry of Public Health (Thailand). The authors wish to thank Miss Punthip Teeyapant, Ms. Panisa Getngern and Toxicology center staffs for their useful suggestions.

Conflict of interest

The authors declare that there is no conflict of interest.

References

- [1] Gallagher CM, Meliker JR. Blood and urine cadmium, blood pressure, and hypertension: a systematic review and meta-analysis. *Environ Health Perspect* 2010; 118(12): 1676–84.
- [2] Tellez-Plaza M, Jones M, Dominguez Lucas A, Guallar E, Navas-Acien A. Cadmium exposure and clinical cardiovascular disease: a systematic review. *Curr Atheroscler Rep* 2013; 15: 356.
- [3] Anetor JJ, Uche CZ, Ayita EB, Adedapo SK, Adeleye JO, Anetor GO, et al. Cadmium level, glycemic control, and indices of renal function in treated type II diabetics: implications for polluted environments. *Front public Health* 2016; 4: 114.
- [4] Pinot F, Kreps S, Bachelet M, Hainaut P, Bakonyi M, Polla B. Cadmium in the environment: sources, mechanisms of biotoxicity, and biomarkers. *Rev Environ Health* 2000; 15: 299–323.
- [5] Apinan R, Satarug S, Ruengweerayut R, Tassaneeyakul W, Na-bangchang K. Cadmium exposure in Thai populations from central, northern and northeastern Thailand and the effects of food consumption on cadmium levels. *Southeast Asian J Trop Med Public Health* 2009; 40: 177–86.
- [6] Chunhabundit R. Cadmium exposure and potential health risk from foods in contaminated area, Thailand. *Toxicol Res* 2016; 32(1): 65–72.
- [7] Boonprasert K, Kongjam P, Limpatanachote P, Ruengweerayut R, Na-Bangchang K. Urinary and blood cadmium levels in relation to types of food and water intake and smoking status in a Thai population residing in cadmium-contaminated areas in Mae Sot. *Southeast Asian J Trop Med Public Health* 2011; 42(6): 1521–30.
- [8] Sriprachote A, Kanyawongha P, Ochiai K, Match T. Current situation of cadmium-polluted paddy soil, rice and soybean in the Mae Sot District, Tak Province, Thailand. *Soil Sci Plant Nutr* 2012; 58(3): 349–59.
- [9] Swaddiwudhipong W, Limpatanachote P, Mahasakpan P, Krintratun S, Padungtod C. Cadmium-exposed population in Mae Sot District, Tak Province: 1. Prevalence of high urinary cadmium levels in the adults. *J Med Assoc Thai* 2007; 90(1): 143–8.
- [10] Pollution Control Department. Cadmium contamination in Mae Tao Creek, Mae Sot District, Tak Province. Bangkok, Thailand: Ministry of Natural Resources and Environment; 2004.
- [11] Honda R, Swaddiwudhipong W, Nishijo M, Mahasakpan P, Teeyakasem W, Ruangyuttikarn W, et al. Cadmium induced renal dysfunction among residents of rice farming area downstream from a zinc-mineralized belt in Thailand. *Toxicol Lett* 2010; 198(1): 26–32.
- [12] Swaddiwudhipong W, Limpatanachote P, Nishijo M, Honda R, Mahasakpan P, Krintratun S. Cadmium-exposed population in Mae Sot district, Tak province: 3. Associations between urinary cadmium and renal dysfunction, hypertension, diabetes, and urinary stones. *J Med Assoc Thai* 2010; 93(2): 231–8.
- [13] Zhang Y, Wang P, Liang X, Tan CS, Tan J, Wang J, et al. Associations between urinary excretion of cadmium and renal biomarkers in nonsmoking females: A cross-sectional study in rural areas of south China. *Int J Environ Res Public Health* 2015; 12(10): 11988–2001.
- [14] Satarug S, Swaddiwudhipong W, Ruangyuttikarn W, Nishijo M, Ruiz P. Modeling cadmium exposures in low- and high-exposure areas in Thailand. *Environ Health Perspect* 2013; 121(5): 531–6.
- [15] Jakubowski M, Trzcinka-Ochocka M. Biological monitoring of exposure: trends and key developments. *J Occup Health* 2005; 47(1): 22–48.
- [16] Hong C-Y, Hughes K, Chia K-S, Ng V, Ling S-L. Urinary α1-microglobulin as a marker of nephropathy in type 2 diabetic asian subjects in Singapore. *Diabetes Care* 2003; 26(2): 338–42.
- [17] Chavan VU, Sayyed A, Durgawale PP, Sontakke A V, Nilakhe SD. Practical aspects of calculation, expression and interpretation of urine albumin measurement. *Natl J Integr Res Med* 2011; 2: 29–34.

- [18] Pennemans V, De Winter LM, Munters E, Nawrot TS, Van Kerkhove E, Rigo J-M, et al. The association between urinary kidney injury molecule 1 and urinary cadmium in elderly during long-term, low-dose cadmium exposure: a pilot study. *Environ Health* 2011; 10:77.
- [19] Adams S V, Newcomb PA. Cadmium blood and urine concentrations as measures of exposure: NHANES 1999-2010. *J Expo Sci Environ Epidemiol* 2014; 24(2): 163–70.
- [20.] Padilla MA, Elobeid M, Ruden DM, Allison DB. An examination of the association of selected toxic metals with total and central obesity indices: NHANES 99-02. *Int J Environ Res Public Health* 2010; 7(9): 3332–47.
- [21] Tinkov AA, Filippini T, Ajsuvakova OP, Aaseth J, Gluhcheva YG, Ivanova JM, et al. The role of cadmium in obesity and diabetes. *Sci Total Environ* 2017; 601–602: 741–55.
- [22] Järup L, Hellström L, Alfvén T, Carlsson MD, Grubb A, Persson B, et al. Low level exposure to cadmium and early kidney damage: the OSCAR study. *Occup Environ Med* 2000; 57(10): 668–72.