การสัมผัสแคดเมียมปริมาณสูงในประชากรแม่สอดมีความสัมพันธ์กับความชุกของโรคโลหิตจาง

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บทคัดย่อ

ประชากรที่อาศัยในอำเภอแม่สอด จังหวัดตาก ประเทศไทย ได้รับแคดเมียมซึ่งเป็นโลหะพิษปริมาณสูงจากดินและข้าวในพื้นที่ งานวิจัยนี้ได้ทดสอบสมมติฐานที่ว่า การสัมผัสแคดเมียมแบบเรื้อรังเป็นปัจจัยเสี่ยงที่เพิ่มความชุกของโรคโลหิตจาง ดังนั้นประชากรชาวอำเภอแม่สอดจำนวน 700 คน (ชาย 261 หญิง 439) ที่มีประวัติว่ามีปริมาณแคดเมียมในปัสสาวะสูง ได้ถูกเลือกมาตรวจวัดปริมาณแคดเมียมในเลือดและปัสสาวะ พร้อมกับปริมาณสารชีวภาพ, β_2-microglobulin (β_2-MG), N-acetyl-β-D-glucosaminidase (NAG) เพื่อใช้บ่งชี้ความผิดปกติของท่อไต, ซีรั่ม cystatin C เพื่อบ่งชี้ความผิดปกติของกรวยไต และระดับฮีโมโกลบิน (Hb) เพื่อบ่งชี้ภาวะโรคโลหิตจาง (Hb <13 กรัมต่อเดซิลิตร ในชาย และ <12 กรัมต่อเดซิลิตร ในหญิง) ผลการศึกษาพบว่าค่าเฉลี่ยของแคดเมียมในเลือด (6.71±1.84 ไมโครกรัมต่อลิตร ในชาย, 4.94±2.02 ไมโครกรัมต่อลิตร ในหญิง) มีค่าสูงกว่าค่าแคดเมียมในเลือดที่มีรายงานมาก่อนของประชากรไทยปกติ (0.45-2.11ไมโครกรัมต่อลิตร) และระดับแคดเมียมในปัสสาวะมีค่าสูงกว่าค่าที่กำหนดเป็นค่าปลอดภัยโดยองค์การอนามัยโลก 3 เท่า ผลการตรวจระดับ ฮีโมโกลบิน พบว่า มีประชากรชายที่มีภาวะโรคโลหิตจางจำนวน 60 คน หญิง 102 คน ซึ่งกลุ่มประชากรที่มีภาวะโรคโลหิตจางมีระดับแคดเมียมในปัสสาวะสูงกว่ากลุ่มที่ไม่เป็นโรคโลหิตจาง และพบว่ากลุ่มประชากรหญิงที่มี β_2-MG ≥1,000 ไมโครกรัมต่อกรัมครีตินิน มีโอกาสเป็นโรคโลหิตจาง 2.77 เท่าของกลุ่มประชากรหญิงที่มี β_2-MG <400 ไมโครกรัมต่อกรัมครีตินิน ในขณะที่ชายมีโอกาสเพียง 1.61 เท่า ดังนั้นสรุปผลได้ว่า ความชุกของโรคโลหิตจางในประชากรกลุ่มนี้มีมากกว่าคนไทยปกติโดยทั่วไป และมีความสัมพันธ์กับปริมาณแคดเมียมที่สูงในร่างกาย การติดตามเฝ้าระวังสุขภาพของประชากรกลุ่มนี้จึงจำเป็นอย่างยิ่งเพื่อป้องกันการเกิดความรุนแรงของโรคโลหิตจางและการเกิดพิษจากแคดเมียม

คำสำคัญ: แคดเมียม, แม่สอด, โรคโลหิตจาง

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High Cadmium Exposure in Mae Sot's Population is Associated with High Anemia Prevalence

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ABSTRACT

Residents of the Mae Sot district, Tak province in Thailand are exposed to high levels of cadmium (Cd), a toxic metal that is contaminated in the soil and rice in this region. We hypothesized that chronic Cd exposure was a risk factor for enhancement of anemia prevalence. Blood (B) and urinary (U) -Cd levels in addition to biomarkers of renal tubular dysfunction [U-β_2-microglobulin (β_2-MG) and N-acetyl-β-D-glucosaminidase (NAG)], glomerular dysfunction (serum cystatin C) and anemia (hemoglobin <13 g/dl in men, <12 g/dl in women) in 700 subjects (261 men, 439 women) with a history of elevated U-Cd were assayed. The results showed that average levels of B-Cd (6.71±1.84μg/l in men, 4.94±2.02μg/l in women) was higher than reported Cd levels from a normal Thai population (0.45-2.11μg/l). U-Cd levels were 3 times higher than the safe level of 2 μg/gCr, established by the WHO. Anemia was found in 60 men and 102 women. In anemic subjects, U-Cd was significantly higher than non-anemic subjects. In addition, concentrations of β_2-MG showed a stronger relation to an increase of anemia prevalence than the NAG and cystatin C. Women with β_2-MG levels ≥1,000 μg/gCr were 2.77 times more likely to be anemia than women with β_2-MG levels <400 μg/gCr, whereas the probability in men was 1.61. Therefore, we concluded that prevalence of anemia among the subjects was higher than Thai general population and associated with the elevated cadmium levels. Clinical interventions should be implemented in the Mae Sot community to prevent severe anemia and cadmium intoxication.

Keywords: cadmium, anemia, Mae Sot, proximal tubular dysfunction, glomerular dysfunction

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INTRODUCTION

Cadmium is a toxic metal which is mainly contaminated to environment via zinc mine and industrial processes[1]. Because of its long half-life in human body[2], cadmium could induce renal dysfunction[3], osteoporosis[4], osteomalacia[5], hypertension[6] and anemia[7].

Recently, high exposure of contaminated cadmium in Mae Sot district, Tak Province, Thailand, has been reported[6,8,10-12]. There were approximately 9,668 inhabitants with high risk of cadmium toxicities whose age was more than 15 years residing in the area and health surveys were annually conducted by the Mae Sot General Hospital and the Bureau of Occupational and Environmental Diseases, Ministry of Public Health since 2001. It was found that 7.2% of the inhabitants had urinary cadmium more than 5 µg/g creatinine (Cr) which was 2.5 times of the WHO safe level of 2 µg/gCr[9]. Renal dysfunction[10,12], hypertenstion[6] and an increase of bone resorption[15] were reported and shown to be related to cadmium exposure. However, a relation between cadmium exposure of Mae Sot population and anemia had not yet been studied.

An increase of anemia prevalence among high cadmium exposure population has been investigated[7,13-14] but the results were controversial. Moriguchi et al.[13] and Tsukahara et al.[14] reported that high levels of cadmium in population exposed to environmental cadmium had no relation to anemia or iron deficiency but Horigushi et al.[7] reported that Itai-Itai patients with high exposure to cadmium showed severe anemia accompanying with proximal tubular dysfunction.

Among Mae Sot population, proximal tubular dysfunction was found to be dominant[12] and some of them showed sign of glomerular dysfunction[11]. Therefore, we hypothesized that chronic cadmium exposure was a risk factor for enhancement of anemia prevalence. An association between anemia and proximal tubular and glomerular dysfunctions were determined along with a determination of correlation between anemia and high levels of cadmium in blood and urine samples of 700 Mae Sot people collected in October-December, 2007.

MATERIALS AND METHODS

Study area and sample population

Seven hundred subjects aged >20 years in Mae Sot district, Tak province, Thailand whose urinary cadmium was over 5 µg/gCr determined in the 2004 survey conducted by the Department of Community and Social Medicine of the Mae Sot General Hospital[10] were selected for this study.

The study protocol was approved by the Institutional Review Board and Ethical Committee of the Kanazawa Medical University, Japan and the Ethical Committee of the Faculty of Medicine, Chiang Mai University, Thailand. All subjects were advised of the aims and methods of the project before entering the survey. All consented to provide random morning urinary samples and 5-10 ml of venipuncture blood. After biological sample collection, demographic, health and nutrition information on each subject was collected by interview using a questionnaire.

Collection of urine and blood samples

Morning urine samples were collected in polyethylene bottles after the subjects underwent physical examination and anthropometric measurements. The samples were immediately tested qualitatively for pH, protein, glucose, occult blood, urobilinogen and ketone body using paper indicator strips (Ames test, Bayer, Germany).

Each urine sample was divided into three (3–5 ml) aliquots. In samples with pH <5, the pH of one aliquot was adjusted to pH 6–8 by 0.5 N sodium hydroxide, to prevent degradation of β_2-microglobulin. Ten milliliters of venipuncture blood was collected in two separated tubes with and without heparin. All aliquots were then frozen and stored at -20˚C for further analysis.

Determination of blood and urinary cadmium, and urinary creatinine

Blood and urinary cadmium concentrations were quantified using a flameless atomic-absorption spectrometer (Shimadzu Model AAS-6300, Japan), with palladium chloride in 5% nitric acid solution as a modifier. Method validation of the analytical techniques were performed and verified by certified standard reference materials (The National Institute of Standards, Washington, DC). The urinary creatinine concentration was measured by a method based on the Jaffe reaction.

Determination of renal markers

The concentration of urinary β_2-microglobulin (β_2-MG) was determined by enzyme immunoassay (GLAZYME β_2-microglobulin-EIA test kit, Sanyo Chemical Industries, Ltd., Japan) and the concentration of urinary N-acetyl-β-D-glucosaminidase (NAG) was determined by a colorimetric assay (NAG test kit, Shionogi Pharmaceuticals, Japan). Serum cystatin C level (Cystatin C) was determined by latex particle enhanced turbidimetric immunoassay PET kit (Dako, Glostrup, Denmark). Cut-off values of the urinary markers for an indication of renal dysfunction were marked as 1,000 μg/gCr for β_2-MG, 8 unit/gCr for NAG and 1.4 mg/l for cystatin C[12,16-17].

Determination of Haemoglobin and Haematocrit

Haemoglobin (Hb) in whole blood was based on a modified cyanmethemoglobin assay using an automate machine (Coulter HmX, Konelab) at the Mae Sot General Hospital. The laboratory was evaluated and certified by Thai Medical Technology Council. Hematocrit (Hct) was also measured and other blood indices were determined according to the described equations[18]. Anemia was identified as Hb level <13 g/dl in men and <12 g/dl in women[19].

Data analysis

Mean of age, BMI, cystatin C, Hb, Hct and RBC were shown as arithmetic means whereas B-Cd, U-Cd, β_2-MG, and NAG were logarithmic transformed geometric means to correct for departure from normal distribution using Kolmogorov-Smirnov Goodness of Fit test. Independent student t-test was used to determine the differentiation between the subject groups of anemia/non-anemia, renal dysfunction and cadmium exposure. Spearman rho analysis and Fisher Exact Test were used to

determine correlation and distribution of anemia prevalence according to urinary cadmium levels, respectively. Odd ratio was calculated by binary logistic regression model.

RESULTS

The 700 studied subjects were from 13 villages in Mae Sot district, Tak province. They were 261 men (37.28%) and 439 women (62.71%). Body mass index of both genders were less than the normal level of 25 kg/m^2 (Table 1). Average values of urinary and blood cadmium of the subjects were found to be 5 times higher than the concentrations found in general Thai population in both genders indicated high cadmium exposure of the Mae Sot inhabitants.

The concentrations of renal tubular dysfunction markers, β_2-MG and NAG, were significantly high in both genders of the subjects compared to the reference values (Table 1). Urinary cadmium levels were 3 times higher than the safe level of 2 μg/gCr, established by the WHO. These results indicated that proximal tubular of the kidney of Mae Sot people in average were not in normal function.

Anemia was found in 60 men and 102 women. Even though the mean value of Hb of all subjects was higher than the cut-off value of anemia (Table 1) but the urinary cadmium concentrations of the anemic subjects were significantly ($p=0.013$) higher than non-anemic subjects (Table 2). In addition, β_2-MG, NAG and cystatin C of the anemic subjects were also significantly higher than non-anemic subjects in both genders ($p<0.001$).

The concentrations of the Hb and Hct of total subjects showed significantly negative correlated with age, U-Cd, β_2-MG, NAG and cystatin C, and positively correlated with BMI and B-Cd (Table 3). However, in men only urinary cadmium that was not correlated to Hb and Hct levels and in women only blood cadmium that was not correlated to Hb and Hct levels.

Prevalence of irreversible proximal tubular dysfunction (β_2MG $\geq$1000 μg/gCr), proximal tubular cell damage (NAG $\geq$8 unit/gCr), glomerular dysfunction (cystatin C $\geq$1.4 mg/l), and anemia of the subjects in four groups of the subjects with urinary cadmium concentrations < 2, 2-5, 5-10 and 10 μgCd/gCr were shown in Table 4. The prevalence was increased when cadmium concentrations increased. In addition, anemia prevalence of women with urinary cadmium >10 μg/gCr was 32.50% higher than other groups.

The relations between an increase of anemia prevalence and age, U-Cd, β_2-MG, NAG and cystatin C were shown in Table 5. Age was significantly related to anemia prevalence in men (expected odds p-value <0.001) whereas urinary cadmium and renal dysfunction markers were not. The expected odds ratio of women with β_2-MG $\geq$1,000 μg/gCr was 2.77 with statistically significant ($p<0.001$). The odds ratios of women with NAG $\geq$8 unit/gCr and cystatin C $\geq$1.4 mg/l were 3.28 and 2.86, respectively ($p <0.05$). The analyzed data showed that women with β_2-MG levels $\geq$1,000 μg/gCr were 2.77 times more likely to be anemia than women with β_2-MG levels <400 μg/gCr, whereas the probability in men was 1.61.

DISCUSSION

A relationship between cadmium exposure and anemia was previously reported[7,20]. In animal models chronic cadmium exposure induced anemia by enhanced iron deficiency, and decreased erythropoietin production. These pathologies appeared together with proximal tubular dysfunction but conserved glomerular morphology[20]. Similarly, an anemia in Itai-Itai patient showed relation to severe proximal tubular dysfunction[7]. Glomerular dysfunction showed no relation to anemia or iron-deficiency among these patients. These results elucidated association between anemia and proximal tubular dysfunction in heavy exposed population but not glomerular dysfunction. However, among non-polluted area population whose urinary cadmium is below 2 μg/gCr, the relationship between anemia and cadmium exposure or proximal tubular dysfunction was not observed[13,14].

Anemia prevalence among unexposed cadmium Thai urban population were 18.50% in men and 13.00% in women[21], which was lower than anemia prevalence in our studied inhabitants (23.00% in men and 23.20% in women.

A dose-response relationship between cadmium exposure and renal dysfunction was shown in Table 4 indicating that cadmium induced both renal proximal tubular and glomerular dysfunctions. β_2-MG and NAG showed significant relation to urinary cadmium in both genders in this study in accordance with previous finding of Teeyakasem et al. 2007[12]. β_2-MG was a sensitive marker of proximal tubular dysfunction[22] and urinary β_2-MG at the level $\geq$1,000 μg/gCr was used to indicate an irreversible proximal tubular dysfunction among cadmium exposure population[23]

Glomerular dysfunction was indicated by serum cystatin C $\geq$1.4 mg/l[17]. The prevalence of an increased cystatin C indicated the glomerular dysfunction development in accordance to the report of Limpatanachote et al. 2007[24]. Glomerular dysfunction prevalence among our subjects was more frequent than the subjects reported by Chittinandana et al. 2006[25] in the Thai population who live in non-polluted area. Additionally, the glomerular dysfunction prevalence in Chinese residing in the cadmium polluted area whose urinary cadmium is over 5 μg/gCr was only 9.35%[4].

The expected odd ratio of the people with NAG $\geq$8 unit/g Cr had wider 95% C.I. than the people with β_2-MG $\geq$1,000 μg/gCr (NAG $\geq$8 unit/gCr odds range: 1.05-10.26 vs β_2-MG $\geq$1,000 μg/gCr odds: 1.56-4.92). This result elucidated that β_2-MG can help predict anemia prevalence more precisely than the NAG.

Dysfunction of proximal tubular cell occurred in the early stage of cadmium intoxication whereas the necrotic injury was shown in the later stage. In animal models anemia and a decrease of erythropoietin production were demonstrated only when proximal tubular cells injury appeared[26]. The evidence obtained from this study supported that proximal tubular dysfunction related to an increase of anemia prevalence.

Proximal tubular cell contains numerous mitochondria indicating many

physiological functions of the cell such as reabsorbing water, electrolytes and low-molecular-weight substances and hormone synthesis[27]. Anemia could induce kidney hypoxia even under normal blood flow conditions[28], disturbing normal physiological function of proximal tubular cell. Then, more severe anemia by lack of erythropoietin production and bone metabolic dysfunction by disturbance of calciotropic hormone production were developed[9]. Therefore, anemia should be corrected among cadmium exposed inhabitant to prevent anemia complications and prevent severe kidney dysfunction.

Even renal dysfunction and cadmium exposure were proposed as a cause of anemia but studies of Japanese and Thais also showed that anemia could increase cadmium absorption[13,29,30]. Cadmium and iron are transported to blood circulation via Divalent Metal Transporter 1 (DMT1) and iron deficiency increased expression of these transportation proteins resulted in an increase cadmium absorption from the gastrointestinal tract[29]. Mean values of the urinary cadmium in anemia subjects were significantly higher than non-anemia subjects which supported the assumption that anemia is associated with cadmium accumulation.

On the other hand, The relationship between glomerular dysfunction and anemia is an unexpected result because glomerular dysfunction is not a dominant pathology of cadmium intoxication and it showed no relation to anemia in the previous study[7]. However, the relation between anemia and glomerular dysfunction was similar to those of chronic kidney disease patients who showed anemia when renal glomerular dysfunction became severe[31,32].

Data from questionnaire showed that rice was a staple food of the study population and it was proposed as a main source of cadmium exposure in these inhabitants[7-8,10-12,15]. An interesting finding was the smoking women had urinary cadmium levels lower than non-smoking women (data not shown) which led us to assume that the Mae Sot women would have been exposing to contaminated cadmium via eating local rice grown in the area, not from the cigarette smoke. However, the amount of cadmium contaminated in rice was not investigated in this study.

Anemia could be shown by thalassemia, kidney disease, chronic underlying disease, malnutrition and iron deficiency[7,14,20]. Decreasing of erythropoietin synthesis was proposed as a cause of anemia in cadmium exposed inhabitants[27]. Therefore, these factors should be addressed in the next survey to clarify the mechanism of cadmium exposure induced anemia among these inhabitants.

In conclusion, a relation between cadmium exposure and anemia was shown as well as the proximal tubular dysfunction and anemia. Glomerular dysfunction biomarker also positively related to an increase of anemia. Therefore, clinical interventions should be implemented in the Mae Sot community to prevent severe anemia and cadmium intoxication.

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Table 1 The average, standard deviation, minimum and maximum values of age, body mass index, blood cadmium, urinary cadmium and renal dysfunction markers of 700 people living in Mae Sot district, Tak province who had been identified as high exposure to cadmium population

	Men (n=261)			Women (n=439)			Total (n=700)			Reference levels
	Mean	S.D.	Min - Max	Mean	S.D.	Min - Max	Mean	S.D.	Min - Max	
Age(years)[a]	55.48	14.01	23 - 89	53.04	12.69	21 - 87	53.95	13.24	21 - 89	
BMI	21.07	3.13	12.88 - 32.40	22.51	4.19	10.96 - 38.01	21.97	3.89	10.96 - 38.01	<25
B-Cd (µg/l)[b]	6.71	1.84	1.05 - 30.90	4.94	2.02	0.31 - 33.11	5.54	1.99	0.31 - 33.11	0.98 (0.45-2.11)[33]
U-Cd (µg/g Cr)[b]	6.35	1.88	0.83 - 41.69	6.97	1.93	0.83 - 42.66	6.73	1.91	0.83 - 42.66	0.87(0.25-1.98)[33]
β_2-MG (µg/g Cr)[b]	367.96	9.45	6.46 - 74,131.02	182.60	6.05	5.50 - 107,151.93	237.14	7.43	5.50 - 107,151.93	156 $\pm$ 3.10[34)]
NAG (unit/g Cr)[b]	5.30	1.88	0.51 - 48.98	5.66	1.85	0.52 - 245.47	5.52	1.86	0.51 - 245.47	4.40 $\pm$ 1.90[34]
Cystatin C (mg/l)[a]	1.17	1.36	0.63 - 4.47	1.05	1.35	0.60 - 3.89	1.09	1.36	0.60 - 4.47	<1.4[35]
Hb (g/dl)[a]	13.97	1.86	6.70 - 18.20	12.84	1.48	3.30 - 16.60	13.26	1.72	3.30 - 18.20	Men >13, women >12[36]
Hct (%)[a]	42.79	5.21	22.90 - 53.80	39.41	4.27	9.60 - 51.40	40.67	4.92	9.60 - 53.80	Men >39, Women >36[36]
RBC (10^6 cells/µl)[a]	4.90	0.74	2.39 - 7.33	4.63	0.61	1.08 - 6.80	4.73	0.67	1.08 - 7.33	4.5-6.0[19]

B-Cd = blood cadmium, U-Cd = urinary cadmium, Cr = creatinine, β_2-MG = urinary β_2-microglobulin, NAG = urinary N-acetyl-β-D-glucosaminidase, Cystatin C = serum cystatin C, Hb = hemoglobin, Hct = hematocrit, RBC = red blood cell
[a]arithmetic value; [b]geometric value

Table 2 Comparison of blood and urinary cadmium concentrations and the levels of renal dysfunction markers of the anemia and non-anemia subjects of this study

	Men					Women					Total				
	Anemia (n=60)		Non-anemia (n=201)		t-test p-value	Anemia (n=102)		Non-anemia (n=337)		t-test p-value	Anemia (n=162)		Non-anemia (n=538)		t-test p-value
	Mean	S.D.	Mean	S.D.		Mean	S.D.	Mean	S.D.		Mean	S.D.	Mean	S.D.	
B-Cd (μg/l)	6.86	1.84	6.67	1.84	0.751	4.65	2.00	5.03	2.03	0.318	5.37	1.99	5.59	1.99	0.511
U-Cd (μg/gCr)	7.33	2.06	6.08	1.82	0.071	7.63	1.98	6.78	1.91	0.114	7.52	2.01	6.51	1.88	0.013
β_2-MG (μg/gCr)	1,616.59	11.52	236.54	7.28	<0.001	358.26	8.21	148.90	5.19	<0.001	625.99	10.44	177.02	6.01	<0.001
NAG (unit/gCr)	7.94	1.88	4.69	1.79	<0.001	7.25	1.94	5.26	1.79	<0.001	7.50	1.92	5.04	1.79	<0.001
Cystatin C (mg/l)	1.48	1.46	1.09	1.28	<0.001	1.18	1.44	1.02	1.31	<0.001	1.28	1.47	1.04	1.30	<0.001

Anemia identified by hemoglobin <13 g/dl in men and <12 g/dl in women. P-value < 0.05 demonstrated significant different between anemia and non-anemia subjects

Table 3 Correlation between hemoglobin (Hb) & hematocrit (Hct), and age, body mass index (BMI), blood and urinary cadmium concentrations, renal markers in all of the study subjects

	Men (n=261)		Women (n=439)		Total (n=700)	
	Hb	Hct	Hb	Hct	Hb	Hct
Age(year)	-0.498***	-0.481***	-0.193***	-0.195***	-0.264***	-0.264***
BMI(km/m^2)	0.323***	0.303***	0.102*	0.108*	0.100**	0.093*
B-Cd (μg/l)	-0.026	-0.019	0.106*	0.135*	0.136***	0.157***
U-Cd (μg/gCr)	-0.168**	-0.180**	-0.085	-0.076	-0.132***	-0.128***
β_2-MG (μg/gCr)	-0.437***	-0.442***	-0.151**	-0.163***	-0.204***	-0.210***
NAG (unit/gCr)	-0.387***	-0.412***	-0.191***	-0.201***	-0.279***	-0.290***
Cystatin C (mg/l)	-0.477***	-0.473***	-0.198***	-0.195***	-0.209***	-0.210***

The correlations were expressed as Spearman rho coefficient values. *p <0.05, **p <0.01, ***p <0.001

Table 4 Prevalence of irreversible proximal tubular dysfunction (β_2MG ≥1000 μg/gCr), proximal tubular cell damage (NAG ≥8 unit/gCr), glomerular dysfunction (cystatin C ≥1.4 mg/l), and anemia of the subjects classified by urinary cadmium concentrations

	Number of subjects (%)				
U-Cd (μg/gCr)	**≤2**	**2-5**	**5-10**	**>10**	**Total**
Men					
β_2-MG (μg/gCr)					
<1000	12 (92.31)	54 (78.26)	82 (70.09)	34 (54.84) *	182 (69.73)
≥1000	1 (7.69)	15 (21.74)	35 (29.91)	28 (45.16)	79 (30.27)
NAG (unit/gCr)					
<8	11 (84.62)	59 (85.51)	98 (83.76)	35 (56.45) ***	203 (77.78)
≥8	2 (15.38)	10 (14.49)	19 (16.24)	27 (43.55)	58 (22.22)
Cystatin C (mg/l)					
<1.4	10 (76.92)	56 (81.16)	99 (84.62)	41 (66.13) **	206 (78.93)
≥1.4	3 (23.08)	13 (18.84)	18 (15.38)	21 (33.87)	55 (21.07)
Anemia group					
Non-anemia	12 (92.31)	52 (75.36)	94 (80.34)	43 (69.35)	201 (77.01)
Anemia	1 (7.69)	17 (24.64)	23 (19.66)	19 (30.65)	60 (22.99)
Total subject	13 (4.98)	69 (26.44)	117 (44.83)	62 (23.75)	261 (100.00)
Women					
β_2-MG (μg/gCr)					
<1000	23 (95.83)	79 (84.95)	172 (85.15)	95 (79.17) *	369 (84.05)
≥1000	1 (4.20)	14 (15.10)	30 (14.90)	25 (20.80)	70 (15.90)
NAG (unit/gCr)					
<8	22 (91.67)	69 (74.19)	158 (78.22)	70 (58.33) ***	319 (72.67)
≥8	2 (8.30)	24 (25.80)	44 (21.80)	50 (41.70)	120 (27.30)
Cystatin C (mg/l)					
<1.4	21 (87.50)	76 (81.72)	181 (89.60)	101 (84.17)	379 (86.33)
≥1.4	3 (12.50)	17 (18.28)	21 (10.40)	19 (15.83)	60 (13.67)
Anemia group					
Non-anemia	19 (79.17)	72 (77.42)	165 (81.68)	81 (67.50) *	337 (76.77)
Anemia	5 (20.83)	21 (22.58)	37 (18.32)	39 (32.50)	102 (23.23)
Total subject	24 (5.47)	93 (21.18)	202 (46.01)	120 (27.33)	439 (100.00)

U-Cd ≤ 2 μg/gCr was considered as a safe reference level; the distribution of the prevalence was determined by Fisher Exact Test. *p <0.05, **p <0.01, ***p <0.001

Table 5 Relation between anemia prevalence and renal tubular dysfunction markers, β_2-MG and NAG, after adjusted by means age and urinary cadmium among the study subjects

	Men			Women			Total		
	Expected Odds	95.0% C.I.		Expected Odds	95.0% C.I.		Expected Odds	95.0% C.I.	
		Lower	Upper		Lower	Upper		Lower	Upper
Age	1.07***	1.04	1.10	1.01	0.99	1.03	1.03***	1.02	1.05
log U-Cd	2.68	0.80	8.91	1.48	0.64	3.41	1.80	0.92	3.54
β_2-MG <400 μg/gCr	1.00			1.00			1.00		
β_2-MG 400-1,000	1.58	0.59	4.19	1.44	0.68	3.09	1.47	0.82	2.63
β_2-MG >1,000	1.61	0.77	3.35	2.77***	1.56	4.92	2.25***	1.45	3.49
Age	1.07***	1.04	1.09	1.02	1.00	1.03	1.03***	1.01	1.04
log U-Cd	2.10	0.63	7.04	1.43	0.63	3.25	1.46	0.74	2.88
NAG <2.5 unit/gCr	1.00			1.00			1.00		
NAG 2.5-8	2.79	0.35	22.31	1.98	0.67	5.86	2.63***	1.51	4.56
NAG >8	4.81	0.55	41.98	3.28*	1.05	10.26	3.71***	2.03	6.79
Age	1.06***	1.03	1.10	1.01	0.99	1.03	1.03**	1.01	1.05
log U-Cd	2.85	0.86	9.44	1.86	0.81	4.25	2.16*	1.10	4.23
Cystatin C <0.9 mg/l	1.00			1.00			1.00		
Cystatin C 0.9-1.4	0.69	0.21	2.21	1.20	0.67	2.16	0.95	0.57	1.59
Cystatin C >1.4	1.59	0.40	6.29	2.86*	1.27	6.44	2.40**	1.23	4.67

The relation was analyzed by binary logistic regression model. Anemia prevalence was set as dependent whereas age, log U-Cd, β_2-MG, and NAG were explanators. β_2-MG <400 μg/gCr , and NAG <2.5 unit/gCr and cystatin C >0.90 mg/l were used as reference values and their expected odds ratio was set as 1. *p <0.05, **p <0.01, ***p <0.001

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