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Seventeen-Year Observation on Urinary Cadmium and β_2-Microglobulin in Inhabitants After Cessation of Cadmium-Exposure in Japan

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Abstract The purpose of this study was to clarify the change and relationship of urinary cadmium (Cd) and β_2-microglobulin (β_2-MG) concentrations of inhabitants in Cd-polluted areas after soil restoration. The urinary Cd and β_2-MG concentrations of 25 males and 28 females did not show a significant change, 22 years after the Cd-polluted soil was restored. Once exposed to Cd, it was found to remain in the body, 22 years after the Cd -polluted soil was restored. However, this did not influence renal tubular dysfunction in most of the younger generation compared with elders heavily exposed to Cd.

Keywords Urinary-Cadmium · Urinary-β_2-Microglobulin · Biological half life · Soil restoration

Cadmium (Cd), atomic No 48, atomic weight 112 was a mineral found in mines. Cd was a by-product that was released to the environment when zinc was refined from the mines (Wada 1986). Residents in mining areas have been exposed to chronic Cd poisoning for long periods of time, for example the Kakehashi River in Ishikawa Prefecture from the mine upstream to the rice field where the river water was used for irrigation. Examination of the cadmium concentration in rice, paddy soils, and river water performed in 1974, and 23 villages were considered to be contaminated (Nogawa et al. 1978). Hence, people were directly exposed by consuming farming foods as well as fish from the river during about 100 years. This oral exposure resulted in kidney dysfunction, osteomalacia, osteoporosis, liver injury and hypertension (Kasuya 1985; Saito et al. 1993). The most common characteristic of Cd exposure was renal tubular dysfunction. An indicator of renal tubular dysfunction was urinary β_2-microglobulin (β_2-MG), a low molecular weight protein which was used as an indicator and judged by the limit 1,000 μg/g.creatinine (Nogawa et al.1983; Aoshima et al. 1988; Kubota et al. 1985). Due to Cd being released to the Jinzu River (Toyama Prefecture, Japan), inhabitants in the surrounding areas have been exposed for a long time. Itai-itai disease was the severest disease caused by chronic Cd poisoning. The direct translation of the Japanese phrase "Itai-itai" is based on the patient's response "Ouch-ouch" to the many fractures in their bodies by osteomalacia (Aoshima and Kasuya 1993). The ministry of Health in Japan and Welfare recognized Itai-itai disease as the first mining-related illness in April 1968 (Matsunami 2006). There were six Cd-polluted areas in Japan. From northern Japan, Kosaka mine in Kosakamachi, Akita prefecture discharged smoke, Kamioka mine in Toyama prefecture, discharged into Jinzu River, Ogoya mine in Ishikawa prefecture, discharged into Kakehashi River, Ikuno mine in Hyougo prefecture, discharged into Ichikawa River, Annaka zinc refinery company in Gunma prefecture, discharged smoke and Taisyu mine in Nagasaki prefecture discharged into Sasu River (Tsuchiya 1978). The largest Cd polluted area was Jinzu

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River basin, followed by Kakehashi River basin (Nakagawa and Nishijo 1999).

In 1981 the Ishikawa Prefecture Government decided to restore the soil in the most Cd-polluted Kanehira areas around Kakehashi River basin (Ishikawa Prefecture 1975) and performed a physical examination on Kakehashi River basin inhabitants of over 50 years of age (Ishikawa Prefecture South Kaga Public Health 1997). It was evident from the results obtained that the biological half–life of Cd was 10–30 years (Friberg 1985) and Cd concentration increased with age (Aoshima and Kasuya 1993). The results showed that 14.3% men and 18.7% women were over the 1,000 μg/g.creatinine limit of urinary β_2-MG. However, only 6% men and 5% women were over the limit in the control area. This shows a significant difference (Kido 1995). In 1986, 5 years after soil restoration (Kido et al. 2002) were apprehensive about the effects of Cd on inhabitants 50 years and under, who were not examined in 1981 by Ishikawa Prefecture Government. Further tests were performed four times over a 17 year period from 1986 to 2003, on inhabitants of most Cd -polluted area of Kakehashi River basin. The purpose of this study was to clarify the change and relationship of urinary Cd and β_2-MG concentrations of inhabitants in Cd- polluted areas after soil restoration.

Materials and Methods

In 1981 the Ishikawa Prefecture Government restored the soil in the most Cd-polluted area in the Kakehashi River basin. Five years after soil restoration in 1986, investigations were carried out to determine the influence of Cd on the inhabitant health, and three further tests were carried out in 1991, 1999 and 2003, over 17 years. The subjects were 53 inhabitants who received physical examinations four times during 17 years. The 53 inhabitants (50 years and under in 1986) included 25 men and 28 women. The mean age of the subjects was 40.2 years old (men: 38.5, women: 42.1) in 1986.

The research process was spread into two parts; (1) Urinary test (2) Questionnaire. The urinary test involved measuring concentration of urinary creatinine, Cd and β_2-MG in early morning urine. The concentration of urinary indicators was then corrected by creatinine. Urinary β_2-MG, Cd and creatinine were measured by radioimmunoassay (RIA), flameless atomic absorption spectrophotometry (Honda et al. 1989) and Jaffé methods respectively. The questionnaire involved obtaining basic data from subjects by gender, age, period of residence and Brinkman index.

Urinary Cd and β_2-MG concentrations were converted into a logarithm scale. The results were statistically analyzed using the one-way layout ANOVA, *t*-test, Pearson correlation coefficient, and multiple regression analysis. The significant difference was $p \leq 0.05$ and the software used for analysis was SPSS12.OJ for Windows.

Subject participation in this research was voluntary. An agreement was signed regarding this matter. The examination performed in 1999 and 2003 were accepted by the Kanazawa Medical University Bioethical committee. To protect privacy of the individual, only numbers (no names) were used during examination.

Results and Discussion

Table 1 shows that there was no significant difference in urinary Cd concentration (μg/g.creatinine) for men and women during a 17 year period, even though both of them showed decreasing tendency. It can be seen from Table 2 that there was significant difference ($p = 0.000$) in men during 1991–1999 and 1999–2003. However, in women there was no significant difference during a 17 year period. It can be seen from Table 3 that there was a significant difference in 1986 ($p = 0.000$), 1991 ($p = 0.002$), 1999 ($p = 0.003$) and 2003 ($p = 0.000$), when comparing the urinary Cd concentration between men and women. On the other hand, urinary β_2-MG concentration shows a significant difference between men and women in 1986 ($p = 0.000$), 1999 ($p = 0.022$) and 2003 ($p = 0.003$), but there was no significant difference in 1991 ($p = 0.174$). In both cases it can be clearly seen that the geometric mean was higher in females than males, except for mean urinary

Table 1 Change in urinary Cd concentration (μg/g.creatinine) in males and females (one- way layout ANOVA)

Year	Males (N = 25)		Female (N = 28)	
	GM	GSD	GM	GSD
1986	3.02	2.14	5.50	2.19
1991	2.75	2.00	5.50	2.30
1999	2.19	1.66	3.80	2.09
2003	1.95	1.74	4.27	2.00

GM geometric mean, *GSD* geometric standard deviation

Table 2 Change in urinary β_2-MG concentration (μg/g.creatinine) in males and females (one-way layout ANOVA)

	Males (N=25)			Females (N=28)	
Year	GM	GSD		GM	GSD
1986	30.20	8.32		83.18	4.07
1991	89.13	2.51	p=0.000	128.83	2.95
1999	12.88	6.46		51.29	10.23
2003	57.54	2.24	p=0.000	131.83	3.02

GM: Geometric Mean GSD: Geometric Standard Deviation p: p-value

Table 3 Comparison with urinary Cd and β_2-MG concentration (μg/g.creatinine) in 25 males and 28 females (*t*-test)

Year	Gender	Urinary Cd (μg/g.creatinine)			Urinary β_2-MG (μg/g.creatinine)		
		GM	GSD	*p*-value	GM	GSD	*p*-value
1986	*M*	3.05	2.16	$p = 0.000$	29.90	8.36	$p = 0.000$
	F	5.52	2.19		83.12	4.09	
1991	*M*	2.79	2.01	$p = 0.002$	88.19	2.47	$p = 0.174$
	F	5.47	2.27		128.94	2.94	
1999	*M*	2.17	1.66	$p = 0.003$	12.84	6.49	$p = 0.022$
	F	3.79	2.10		50.90	10.15	
2003	*M*	2.00	1.73	p = 0.000	57.50	2.20	$p = 0.003$
	F	4.30	2.00		133.05	3.01	

GM geometric mean, *GSD* geometric standard deviation

Table 4 The correlation coefficient between urinary Cd and β2-MG concentration in females and males (Pearson correlation method)

	1986	1991	1999	2003
Males (N = 25)	$r = 0.202$	$r = 0.183$	$r = 0.349$	$r = 0.155$
	$p = 0.167$	$p = 0.191$	$p = 0.044$	$p = 0.229$
Females (N = 28)	$r = 0.435$	$r = 0.241$	$r = 0.093$	$r = 0.351$
	$p = 0.010$	$p = 0.108$	$p = 0.320$	$p = 0.033$

r Pearson correlation coefficient, *p* p-value

β_2-MG concentration in 1991. Table 4 shows that in 25 males there was a significant correlation with urinary Cd and β_2-MG concentrations in 1999 ($p = 0.044$). On the other hand, in 28 females there was a significant correlation between urinary Cd and β_2-MG concentration in 1986 ($p = 0.010$) and 2003 ($p = 0.033$). Urinary Cd and β_2-MG concentrations showed significant relationship in 2003. Figure 1 shows that only two subjects were found to exceed the critical urinary β_2-MG limit (1,000 μg/g.creatine) in 2003. Table 5 shows that there was no significant regression between urinary β_2-MG and urinary Cd, age, period of residence, Brinkman index in 25 males and 28 females, except for significant association in females between urinary β_2-MG and age ($p = 0.036$) as well as Brinkman index ($p = 0.040$) in 2003.

As shown in Table 1 there was no significant difference in urinary Cd concentration in 25 males and 28 females, 22 years after cessation of Cd exposure (soil restoration in 1981, first investigation 5 years after in 1986 until 2003). This shows that once exposed to Cd it remains in the body for a long time. This agrees with the biological half life of Cd which was 10–30 years (Nordberg et al. 1985). This shows that once exposed to Cd, irreversible injury (renal tubular dysfunction) was noted to occur when urinary β_2-MG excretion exceeded 1,000 μg/g.creatinine (Cai et al. 2001; Iwata et al. 1993; Kido et al. 1988). On the other hand, for non-contaminated area inhabitants in Japan, males had a geometric mean urinary Cd concentration of 1.8 μg/g.creatinine and females 2.4 μg/g.creatinine (Suwazono 2000).

As shown in Table 2 in males there was no significant difference in urinary β_2-MG concentration over 17 years. However, between 1991 and 1999, 1999 and 2003 ($p = 0.000$) there was a significant decrease and increase respectively. There was no plausible reason for this "V" shaped curve. But the geometric means of urinary β_2-MG

Table 5 Multiple regression analysis of urinary β_2-MG and Age, urinary Cd, period of residence, Brinkman index in males and females

	1986		1991		1999		2003	
	β	*p*-value	β	*p*-value	β	*p*-value	β	*p*-value
Males (N = 25)								
Age	−0.142	0.682	−0.037	0.914	−0.174	0.501	−0.073	0.804
Cd	0.174	0.593	−0.114	0.775	0.268	0.284	0.038	0.907
Period of residence	0.145	0.651	0.241	0.426	0.137	0.632	0.254	0.425
Brinkman index	0.125	0.674	0.444	0.132	0.401	0.119	0.118	0.689
R^2	0.055		0.140		0.232		0.056	
Females (N = 28)								
Age	0.111	0.684	0.247	0.410	0.301	0.177	0.426	0.036
Cd	0.294	0.327	−0.056	0.858	−0.181	0.386	0.015	0.941
Period of residence	0.112	0.625	9.225	0.340	0.397	0.053	0.244	0.208
Brinkman index	−0.037	0.847	−0.083	0.676	−0.211	0.237	−0.346	0.040
R^2	0.435		0.134		0.322		0.435	

β Standardized partial regression coefficient, R^2 coefficient of determination

concentration during 17 years were within the normal range. This can be explained by the physiological changes of the human body. Urinary β_2-MG was a sensitive indicator of renal tubular dysfunction (Nogawa et al. 1983; Aoshima et al. 1988; Kubota et al. 1985). Previous studies have shown that there was a significant relationship between urinary Cd and β_2-MG (Kido et al. 1988; Honda et al. 1982). It can be seen from table 3 that females have a higher geometric mean urinary Cd and β_2-MG concentration relative to males (Kido et al. 2001).This study showed that urinary Cd still remained in the body 22 years after cessation of exposure. Moreover, urinary β_2-MG remained in the body for the same period of time as Cd (Tables 1, 2). Therefore, there was a significant correlation between urinary Cd and β_2-MG concentration in males in 1999 ($p = 0.004$) and in females in 1986 ($p = 0.010$) and 2003 ($p = 0.033$; Table 4). In multiple regression analysis with urinary β_2-MG, urinary Cd, age, period of residence and Brinkman index were contributing factors for Cd exposed subjects. The results in Table 5 show that in 2003 there was a significant relationship between urinary β_2-MG and age ($p = 0.036$) and also Brinkman index ($p = 0.040$) in females. Even though Cd remained in the body for 22 years after cessation of Cd exposure it did not influence renal tubular dysfunction. This was because when the first investigation was conducted in 1986, 5 years after cessation of Cd exposure, the ages of all 53 subjects were under 50 years. At this time their exposure to Cd was less than that of inhabitants 50 years and over. Only four subjects were found to exceed the critical urinary β_2-MG limit (1,000 μg/g.creatinine) during 17 years, as shown in Fig. 1.

Recently, not many long term epidemiological studies have been performed internationally. So it was imperative that long term epidemiological studies be carried out such as our investigation. This study thoroughly examined urinary Cd and β_2-MG in inhabitants after cessation of Cd exposure. In Cd-polluted areas of Japan, this was the first report that shows inhabitants having no adverse health effects. Countermeasure of replacement of Cd-polluted soil might contribute to younger inhabitants rather than the elder people in the former Cd-polluted areas. Even though the 17 year observation data showed no significance in relationships between urinary Cd and β_2-MG due to the aforementioned reasons, further investigations will be carried out to confirm these present results.

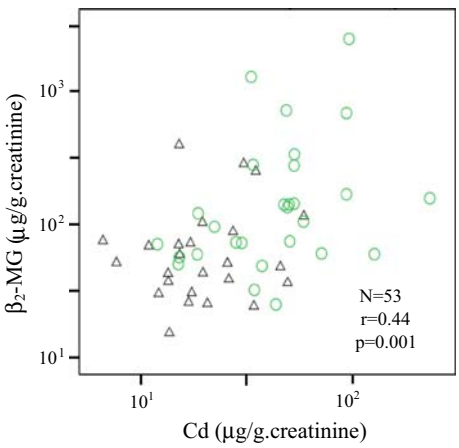

Fig. 1 Scatter plot of correlation between urinary Cd and β_2-MG concentration for 28 females (*open circle*) and 25 males (*open triangle*) in 2003 (Pearson correlation method; *r*: Pearson correlation coefficient)

This manuscript can be concluded by the following points:

1. The change of urinary Cd and β_2-MG concentrations in 25 males and 28 females was not significant during 17 years after the Cd-polluted soil was restored even though they showed decreasing tendency.
2. The comparison with urinary Cd and β_2-MG concentrations between men and women showed a significant difference during a 17 year period (1986, 1991, 1999 and 2003), except for urinary β_2-MG concentrations in 1991. The geometric mean was higher in females than males.
3. Once exposed to Cd, it was found to remain in the body, 22 years after the Cd-polluted soil was restored. However, this did not influence renal tubular dysfunction generally.

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