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A Study of the Relationship between Cadmium Concentrations in Urine and Renal Effects of Cadmium

by Koji Nogawa,* Etsuko Kobayashi,* and Ryumon Honda*

The relationship between urinary cadmium concentration expressed as μg/g creatinine and renal effects of cadmium exposure was studied in 542 inhabitants over 20 years of age who lived in the Jinzu River basin which is polluted by cadmium. Cadmium concentration in urine was employed as the index of cadmium exposure. Total protein with glucose, β_2-microglobulin, retinol binding protein, and proline served as indices of renal effects. The prevalence rate of high β_2-microglobulin excretion was the highest among these indices both in men and women.

Prevalence rates of indices of renal effects increased proportionally with increasing cadmium concentrations in urine and probit linear regression lines could be calculated between them. The urinary cadmium concentrations corresponding to 1% prevalence rates of indices of the renal effects were calculated by the regression line. For β_2-microglobulin, 3.2 μg Cd/g creatinine and 5.2 μg Cd/g creatinine were obtained in men and women, respectively. This method may be useful for evaluating the risk of renal damage of exposed inhabitants.

Introduction

Some studies in Japan on dose-response relationships of renal effects of cadmium used cadmium concentrations in rice for estimating exposure (*1–3*). According to these reports, dose-response relationships between cadmium concentrations in rice and renal effects of cadmium were clearly observed.

Cadmium concentrations in biological materials, such as urine and blood, are also used for estimating exposure. Some reports from experimental animals and from humans have shown a relationship between urinary cadmium excretions and total body burden before renal damage has occurred (*4–6*). There is also a relationship between village average rice cadmium concentrations and village average urinary cadmium concentrations in Japan (*3, 7*).

In a previous report, a close relationship was found between cadmium concentration in urine and renal effect (*3, 8*). Prevalence rates of indices of renal effects increased proportionally with increasing cadmium concentrations in urine. However, the persons used in the previous study were selected ones. In order to get accurate relationships in exposed areas, all inhabitants in these areas must be studied. The aim of the present study was to investigate the relationship between cadmium concentration in urine and abnormal urinary findings by examining all inhabitants in the target area and to find out whether maximum allowable urinary cadmium concentration among the inhabitants in this area can be calculated.

Selection of Target Group

Nine hamlets ("buraku") which are located in the center of the Jinzu River basin were designated as target areas. These hamlets were heavily polluted by cadmium, and itai-itai disease has occurred in this area.

All inhabitants over 20 years of age in the hamlets were selected as the target group (Table 1). Sixty inhabitants, 20–39 years of age, in two hamlets were excluded in this study. Unfortunately we had no time to collect and analyze the samples from these inhabitants. We thought this number of the inhabitants had no significant influence on the results of the calculations.

* Department of Hygiene, Kanazawa Medical University, Uchinada-machi, Ishikawa-ken, Japan.

Table 1. Target groups and number of persons participating in the study.

Sex	Age	Size of target group	Number of participants	Participation rate, %
Male	20-29	63	39	61.9
	30-39	53	39	73.6
	40-49	64	60	93.8
	50-59	54	53	98.1
	60-69	34	34	100.0
	70-	25	21	84.0
	Total	293	246	84.0
Female	20-29	80	59	73.8
	30-39	45	37	82.2
	40-49	75	74	98.7
	50-59	56	52	92.9
	60-69	43	43	100.0
	70-	36	31	86.1
	Total	335	296	88.4
Male and female	Grand total	628	542	86.3

Selection of Indicator Variables for Study

Cadmium-exposed inhabitants, itai-itai patients, and suspected patients show high urinary excretions of total protein, glucose, β_2-microglobulin (β_2-MG), retinol binding protein (RBP), lysozyme, and amino acids (*3, 9, 10*). Among these substances prevalence rates of tubular proteinuria (β_2-MG and RBP), proteinuria with glucosuria and aminoaciduria (proline) were very low, usually less than 1%, in nonexposed areas (*10, 11*). On the contrary, prevalence rates of these variables were high in exposed areas (*3, 9, 10*). Therefore, it is reasonable to say that these variables are specific indices of renal damage caused by cadmium. We selected β_2-MG, RBP, total protein with glucose, and proline in urine as the indicators of cadmium-induced renal effects for this study.

Materials and Methods

Morning urine specimens were collected from each participant in paper cups and transferred to polyethylene bottles previously washed in HNO_3. The samples were frozen as soon as possible and kept frozen during storage until analyses were performed. Total protein was determined by a modification of the method of Kingsbury-Clark (*12*). Glucose was analyzed by using *o*-toluidine–boric acid (*13*). Analysis of β_2-MG, and RBP was performed by using a single immuno-diffusion method. (Plates used for β_2-MG analysis were made by Seikagakukogyo Co. and for RBP analysis by Hoechst Co.) Proline was determined by the method of Fukushima (*14*). The cadmium concentration was analyzed by flameless atomic absorption spectrophotometry after wet ashing in $HNO_3/H_2SO_4/HClO_4$ and extraction with ammonium pyrrolidine dithiocarbamate–methyl isobutyl ketone (APDC/MIBK).

In the present study proteinuria was defined as excretion of more than 50 mg/l. 50, and glucosuria was defined as more than 200 mg/l. Tubular proteinuria was defined detecting more than 5 mg/l. and 4 mg/l. for β_2-MG and RBP, respectively. Aminoaciduria was defined as more than 10 mg/g creatinine of proline. Under these definitions of abnormal levels, the prevalence rate of each indicator variable used for this study was less than 1% in nonexposed areas (*10, 11*). Due to insufficient urine volumes, all of the planned analyses were not performed, which is obvious from the number of persons for which data are given in Tables 2 and 3.

Urinary cadmium concentrations, expressed as μg/g creatinine, were used in this study. Previous reports indicated that there was a close relationship between renal effects of cadmium and urinary cadmium concentration expressed as μg/g creatinine. However, no relationship was found between renal effects and cadmium concentrations in urine expressed as μg/l. without concentration adjustment (*3, 8*).

Results

In Figures 1 and 2, urinary β_2-MG concentrations are plotted as a function of cadmium concentrations in urine. There is a tendency which indicates that the prevalence rate of high β_2-MG excretion increased with increasing cadmium concentrations in urine. The 246 men examined were divided into seven groups according to their urinary cadmium concentrations. The 296 women were also divided into eight groups in the same way. Percentages of tubular proteinuria, aminoaciduria, and proteinuria with glucosuria in each group were calculated as shown in Tables 2 and 3. These tables indicate that the prevalence rates of the abnormal urinary findings were closely related to cadmium concentrations in urine. These values were plotted against cadmium concentrations in urine and curves obtained indicated sigmoid lines, as shown in Figure 3. Among the substances, β_2-MG shows the highest prevalence rate for both men and women. The lowest prevalence rate for men is proline and for women is proteinuria with glucosuria. By using the median urinary cadmium concentration in each group as dose levels, probit analysis was performed

Table 2. Prevalence rates of abnormal urinary findings in relation to urinary cadmium concentrations among the female inhabitants over 20 years of age.

	Cadmium in urine, μg/g creatinine															
	0–4.9 (median = 3.6)		5.0–9.9 (median = 7.6)		10.0–14.9 (median = 12.5)		15.0–19.9 (median = 17.2)		20.0–24.9 (median = 23.1)		25.0–29.9 (median = 27.3,[a] 27.2[b])		30.0–39.9 (median = 34.2)		>40.0 (median = 47.8)	
	N[c]	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
Tubular proteinuria																
β_2-Microglobulin[d]	26	3.9	36	2.8	36	22.2	37	27.0	45	51.1	30	70.0	39	79.5	47	85.1
Retinol binding protein[e]	26	0	36	0	36	8.3	37	10.8	45	33.3	30	46.7	39	53.8	47	70.2
Aminoaciduria (Proline)[f]	26	0	36	2.8	36	11.1	37	10.8	44	22.7	29	31.0	37	35.1	47	51.1
Proteinuria[g] with glucosuria[h]	26	0	36	0	36	2.8	37	5.4	44	20.5	29	24.1	37	24.3	47	34.0
Age (mean ± S.D.)	27.7 ± 6.0		31.0 ± 11.0		43.2 ± 15.6		44.8 ± 15.4		51.5 ± 15.5[i] 51.2 ± 15.6[j]		56.4 ± 11.6[a] 56.9 ± 11.5[b]		57.7 ± 13.0[k] 58.5 ± 12.9[l]		55.8 ± 10.6	

[a] $N = 30$.
[b] $N = 29$.
[c] Number of persons examined.
[d] About 5 mg/l. and over.
[e] About 4 mg/l. and over.
[f] 10 mg/g creatinine and over.
[g] 50 mg/l. and over.
[h] 200 mg/l. and over.
[i] $N = 45$.
[j] $N = 44$.
[k] $N = 39$.
[l] $N = 37$.

Table 3. Prevalence rates of abnormal urinary findings in relation to urinary cadmium concentrations among the male inhabitants over 20 years of age.

	Cadmium in urine, μg/g creatinine													
	0–4.9 (median = 4.0)		5.0–9.9 (median = 7.7)		10.0–14.9 (median = 12.6[b] 12.5[c])		15.0–19.9 (median = 17.0)		20.0–24.9 (median = 22.7)		25.0–29.9 (median = 27.7)		>30.0 (median = 41.1[d] 39.8[e])	
	N[a]	%	N	%	N	%	N	%	N	%	N	%	N	%
Tubular proteinuria														
β_2-Microglobulin	29	0	48	16.7	45	46.7	25	76.0	49	69.4	21	95.2	29	93.1
Retinol binding protein	29	0	48	8.3	45	20.0	25	36.0	49	51.0	21	71.4	29	82.8
Aminoaciduria (proline)	29	0	48	0	44	2.3	25	8.0	48	10.4	21	19.0	28	39.3
Proteinuria with glucosuria	29	0	48	2.1	44	4.5	25	12.0	48	22.9	21	19.0	28	57.1
Age (mean ± S.D.)	33.9 ± 10.8		41.2 ± 15.8		43.8 ± 14.1[b] 43.5 ± 14.1[c]		50.4 ± 10.2		54.4 ± 12.1[f] 54.6 ± 12.2[g]		58.0 ± 12.6		57.2 ± 11.8[d] 57.4 ± 12.0[e]	

[a] Number of persons examined.
[b] $N = 45$.
[c] $N = 44$.
[d] $N = 29$.
[e] $N = 28$.
[f] $N = 49$.
[g] $N = 48$.

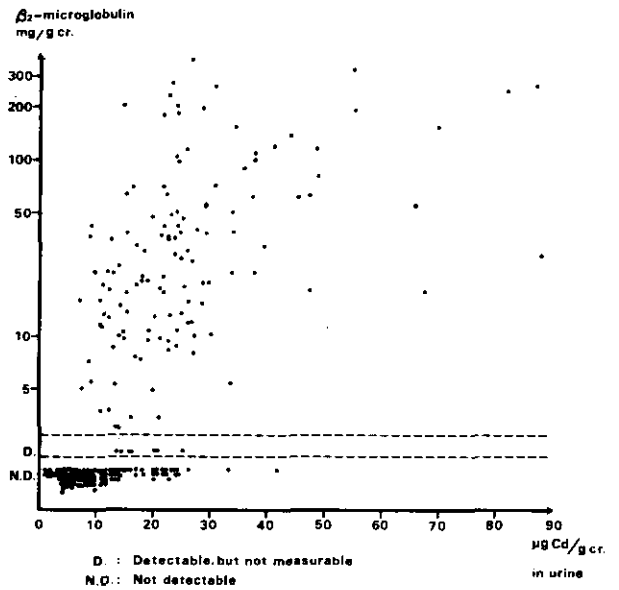

FIGURE 1. Comparison of urinary excretion of β_2-microglobulin and cadmium (male).

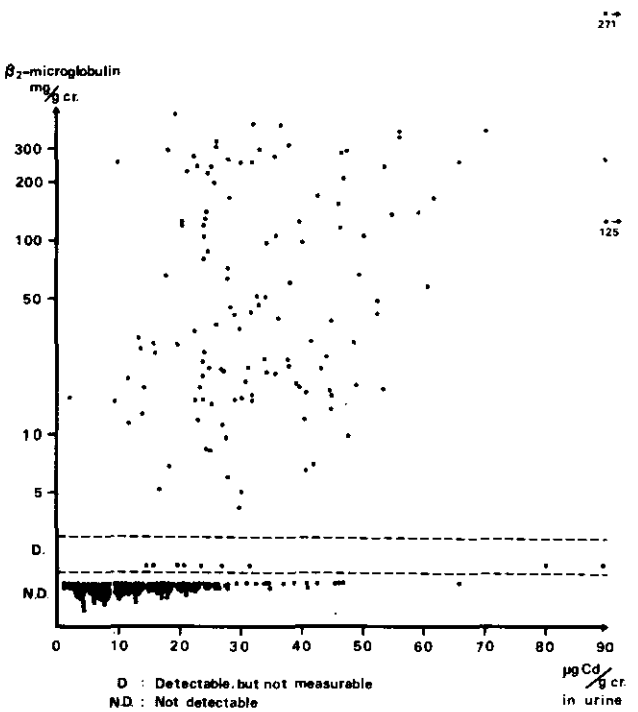

FIGURE 2. Comparison of urinary excretion of β_2-microglobulin and cadmium (female).

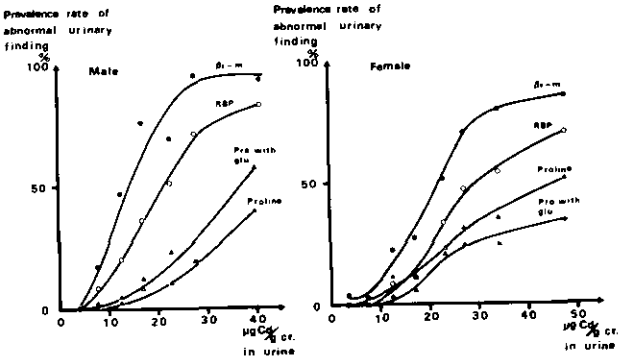

FIGURE 3. Prevalence rates of abnormal urinary findings in relation to urinary cadmium concentrations.

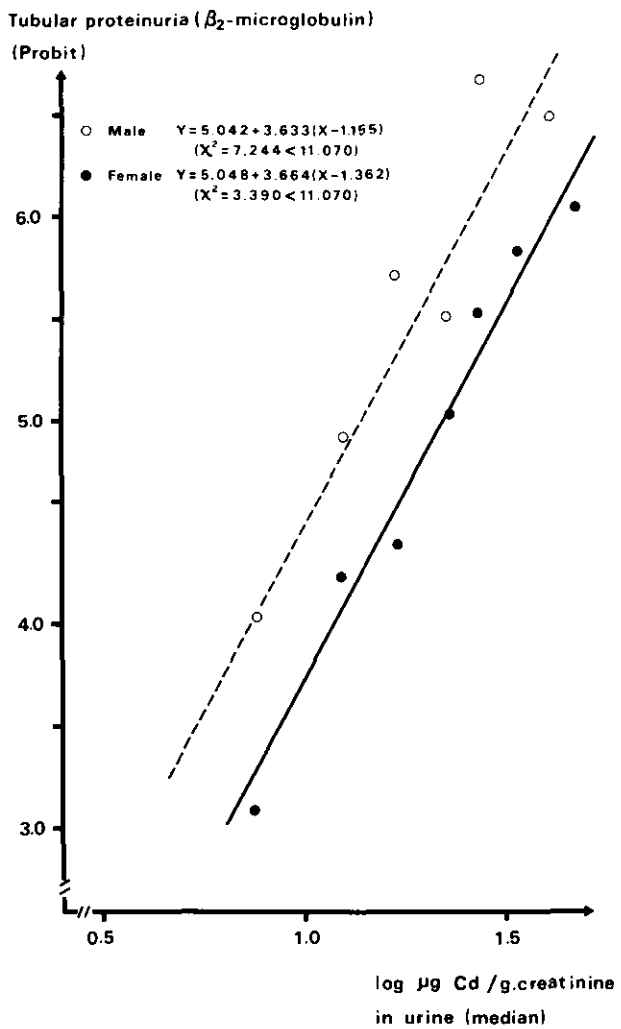

FIGURE 4. Probit linear regression line between urinary cadmium concentration and prevalence of tubular proteinuria (β_2-microglobulin).

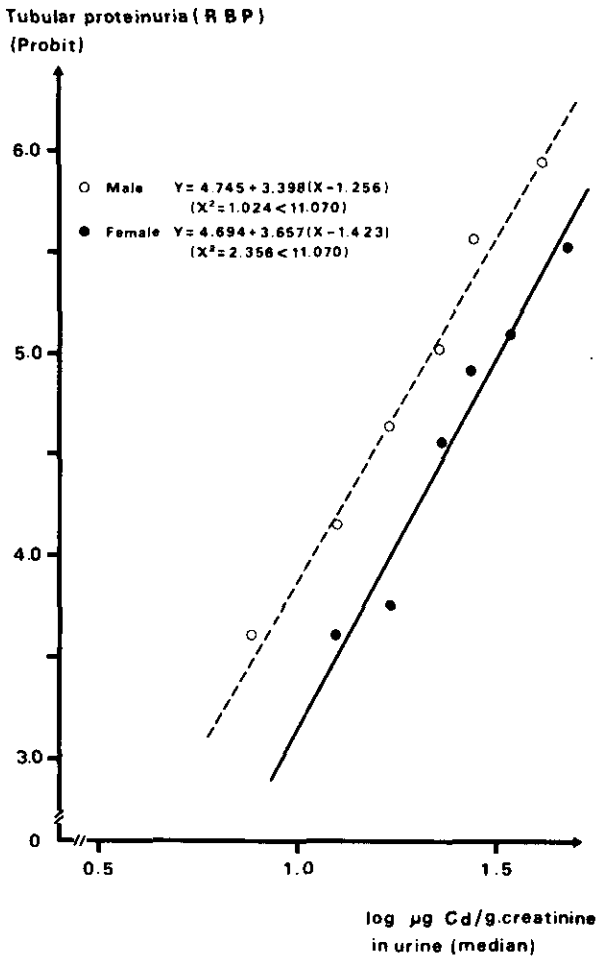

FIGURE 5. Probit linear regression line between urinary cadmium concentration and prevalence of tubular proteinuria (RBP).

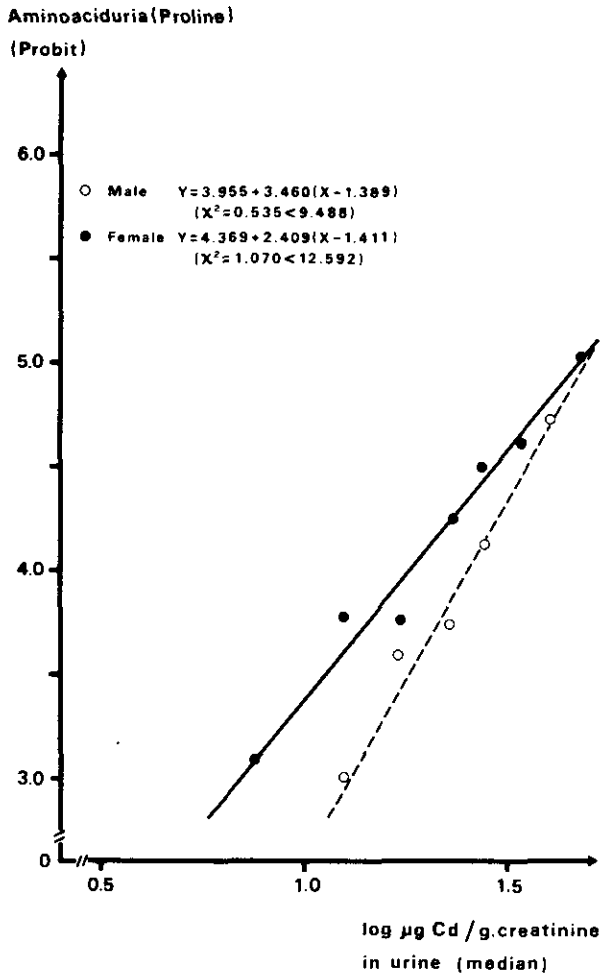

FIGURE 6. Probit linear regression line between urinary cadmium concentration and prevalence of aminoaciduria (proline).

Table 4. Probit linear regression lines for urinary cadmium concentrations and prevalences of abnormal urinary findings, and urinary cadmium concentration corresponding to 1% prevalence rate for each abnormal urinary finding.

Sex	Urinary finding	Probit linear regression line[a]	Urinary cadmium concentration, μg/g creatinine[b]
Male	Tubular proteinuria		
	β_2-Microglobulin	$Y = 5.042 + 3.633\ (X - 1.155)$	3.2
	Retinol binding protein	$Y = 4.745 + 3.398\ (X - 1.256)$	4.4
	Aminoaciduria (proline)	$Y = 3.955 + 3.460\ (X - 1.389)$	10.4
	Proteinuria with glucosuria	$Y = 4.183 + 3.190\ (X - 1.342)$	7.4
Female	Tubular proteinuria		
	β_2-Microglobulin	$Y = 5.048 + 3.664\ (X - 1.362)$	5.2
	Retinol binding protein	$Y = 4.694 + 3.657\ (X - 1.423)$	7.4
	Aminoaciduria (proline)	$Y = 4.369 + 2.409\ (X - 1.411)$	5.1
	Proteinuria with glucosuria	$Y = 4.158 + 2.503\ (X - 1.458)$	7.4

[a] Y = prevalence of abnormal urinary finding (probit); X: log μg Cd/g creatinine in urine.
[b] Urinary cadmium concentration which corresponds to 1% prevalence rate for each abnormal urinary finding.

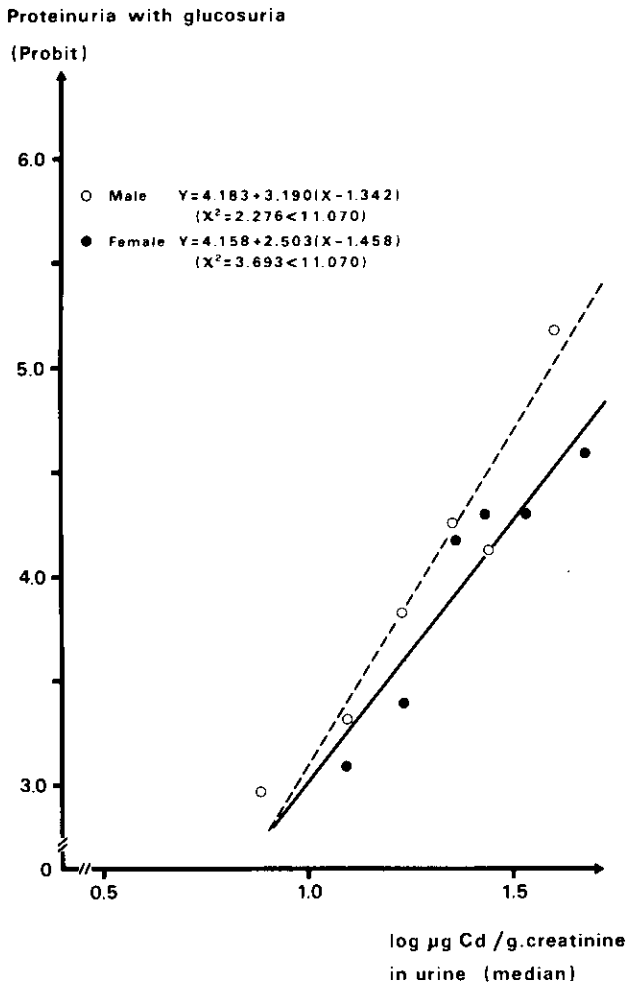

FIGURE 7. Probit linear regression line between urinary cadmium concentration and prevalence of proteinuria with glucosuria.

(*15, 16*). Probit regression lines between urinary cadmium concentrations and prevalence rates of abnormal urinary findings were obtained, as shown in Figures 4–7. Urinary cadmium concentrations which correspond to 1% prevalence rate for each abnormal urinary finding were calculated by using the probit regression lines and the results are shown in Table 4. The urinary cadmium concentration corresponding to 1% prevalence rate for β_2-MG was 3.2 μg/g creatinine in men and 5.2 μg/g creatinine in women. For proline, values of 10.4 μg/g creatinine in men and 5.1 μg/g creatinine in women were obtained.

Discussion

When trying to investigate dose-response relationship of cadmium, two kinds of dose are used, an external dose and an internal dose. As an external dose, cadmium in foodstuff, water, and air are generally employed. Some epidemiological studies on dose-response relationships of cadmium in the general environment of Japan showed a close relationship between village average cadmium concentrations in rice and abnormal urinary findings (*1–3*). Kawano, for example, reported that the so-called threshold limit value in the inhabitants more than 50 years of age was 0.148–0.246 μg Cd/g in rice and indicated that there was only a small margin between present intake levels and intake levels that may cause a significant prevalence of renal tubular damage (*17*). It is, however, very difficult to measure the total dose by using external dose. The biological half-time of cadmium is so long that there might have been changes in exposure of the environment over long periods of time. It is also difficult to measure the individual exposure from the external dose.

As an internal dose, cadmium in urine, blood, and organs are used. If an internal dose which can be used as the index of long-term dose is found, such an index has advantages over using an external dose.

The present study was performed to investigate whether cadmium in urine could be used for evaluating renal effects of cadmium. In order to evaluate the accuracy of our chemical analyses, comparisons were performed between our laboratory and Toyama Institute of Health (*18*). Urine samples from itai-itai patients were used for this study. There were good correlation coefficients and regression equations for cadmium (n = 10, r = 0.97), total protein (n = 53, r = 0.94), and glucose (n = 53, r = 0.97). However, β_2-MG and RBP concentrations in urine at our laboratory were higher than those at Toyama Institute, although there were fairly good correlation coefficients for β_2-MG (n = 51, r = 0.77) and RBP (n = 50, r = 0.92) between our laboratory and Toyama Institute. This is probably because of a difference in the standard solutions. We used commercial standard solutions, and the investigations at Toyama Institute used ones prepared in their laboratory.

The target group consisted of the inhabitants over 20 years of age who have lived in heavily polluted areas in the Jinzu River basin. As shown in Table 1, the participation rate was 86%. The inhabitants examined were sufficiently representative of this area. The average cadmium concentration in household rice in 1972 was 0.71 μg/g in this area (out of 176 households, 85 household rice samples were examined).

Kjellström et al. (*19*) reported that there was a significant correlation (r = 0.66, n = 129) in exposed inhabitants between cadmium and β_2-MG in urine.

The present study did not show a significant correlation if the undetectable values were excluded. The detection limit of our analytical method for β_2-MG in urine was about 5 mg/l. and their method (radio-immuno assay) was about 1 μg/l. The average β_2-MG concentration in urine for nonexposed women aged 51–60 was 113 μg/l. (*18*). Therefore, our detection limit is much higher than the average β_2-MG concentration for nonexposed persons. This fact can explain why we could not observe a significant correlation between β_2-MG and cadmium in urine.

The prevalence rate of high β_2-MG excretion was highest in both men and women, even though our analytical method had insufficient sensitivity. Therefore, it is reasonable to say that β_2-MG is the most sensitive indicator of renal effects of cadmium.

In previous studies (*3*, *8*), no relationship was found between the prevalence of renal effects and urinary cadmium concentration expressed as μg/l. without concentration adjustment. In the present study, there was also no relationship, although the results of such calculations are not presented in this report. Concentration adjustment in urine should be done for this kind of study.

When wider ranges of urinary cadmium concentrations were used for the calculation of the regression lines, each empirical values fit the regression lines better than the present ones. However, almost the same regression lines were obtained in both calculations. In the calculation of the regression line for β_2-MG in females, the first point at 3.9% was excluded. The next point was at 2.8%. Therefore it should be better to assume that the response should start from the next point. The same method was used if the first two groups showed the same response rates.

As shown in Tables 2 and 3, the age distributions of each group were not very different. Therefore, differences of age within each group did not have a large effect on the results. The urinary cadmium concentrations corresponding to the 1% prevalence rate of each abnormal urinary finding should be treated carefully. This value is the median cadmium concentration in urine among the inhabitants over 20 years of age. Therefore, comparison of this result with other studies should be performed on the same age groups and with the same exposure situation. Considering the prevalence rates of the indicator variables used for this study in nonpolluted areas, the urinary cadmium concentrations corresponding to the 1% prevalence rate of each indicator variable may be applied as the "maximum allowable urinary cadmium concentration" in this area.

The significance of this value is not clear. It is not yet fully clarified whether cadmium concentration in urine can be used as an index of long-term dose or recent exposure.

Animal experiments showed that after renal damage had occurred, there was no relationship between cadmium in urine and total exposure (*4*, *20*). Therefore, the relationship obtained in this study might not show a causal relationship.

Urinary cadmium concentration is affected by cadmium exposure level and exposure time. It is reasonable to say that urinary excretion curves of cadmium are different depending on the exposure time, even though inhabitants are exposed to the same total amount of cadmium. These two factors were not separated in the present study.

A similar study on the inhabitants who are in the same age groups (and thus have the same exposure time) is necessary to clarify the significance of urinary cadmium concentrations. For practical purposes, the "maximum allowable urinary cadmium concentration" may be useful if other studies confirm the same result. This value could be used for evaluating risk of renal damage in the inhabitants under examination.

In conclusion, the present study indicates that urinary cadmium concentration can be useful for evaluating the renal effects of cadmium among exposed inhabitants. The maximum allowable urinary cadmium concentration can be calculated by using probit regression lines between urinary cadmium concentrations and prevalence rates of abnormal urinary findings.

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