Biol Trace Elem Res (2008) 124:164–172
DOI 10.1007/s12011-008-8125-8

Changes in Renal Tubular and Glomerular Functions and Biological Acid–Base Balance after Soil Replacement in Cd-Polluted Rice Paddies Calculated with a General Linear Mixed Model

Etsuko Kobayashi · Yasushi Suwazono · Ryumon Honda · Mirei Dochi · Muneko Nishijo · Teruhiko Kido · Hideaki Nakagawa

Received: 25 February 2008 / Accepted: 17 March 2008 /
Published online: 25 June 2008

Abstract Using a general linear mixed model, we conducted a 10-year follow-up investigation of 29 persons requiring observation in the cadmium (Cd)-polluted Kakehashi River basin to determine serial changes in biological parameters after removal of Cd-polluted soil present in rice paddies. In particular, we investigated changes in urinary Cd, urinary total protein, biological acid–base balance based on arterial blood pH, carbon dioxide tension (Pa_{CO2}), base excess (BE), serum chloride ion (SCl^{-}), and renal glomerular function based on serum creatinine (SCr) and creatinine clearance (C_{Cr}). In both sexes, urinary Cd concentrations decreased and total protein concentrations increased with increasing number of years elapsed, with the partial regression coefficients statistically significant in the women. Partial regression coefficients showed positive values for SCl^{-}, with statistical significance in both sexes. The value for Pa_{CO2} was significantly negative in men. Given the serial changes in arterial blood pH, Pa_{CO2}, BE, SCl^{-}, it is suggested that the biological acid–base balance will progress to metabolic acidosis with hyperchloremia. Moreover, glomerular dysfunction as indicated by an increase in SCr and a decrease in C_{Cr}

E. Kobayashi (✉) · Y. Suwazono · M. Dochi
Department of Occupational and Environmental Medicine (A2), Graduate School of Medicine, Chiba University, 1-8-1 Inohana, Chuohku, Chiba 260-8670, Japan
e-mail: ekoba@faculty.chiba-u.jp

R. Honda
Department of Nursing, Kanazawa Medical University, 1-1 Daigaku, Uchinada, Ishikawa 920-0293, Japan

M. Nishijo · H. Nakagawa
Department of Public Health, Kanazawa Medical University, 1-1 Daigaku, Uchinada, Ishikawa 920-0293, Japan

T. Kido
Department of Community Health Nursing, Kanazawa University School of Health Sciences, 5-11-80, Kodatsuno, Kanazawa 920-0942, Japan

will continue to progress even after soil replacement. Interventions such as soil replacement appear to be too late to prevent progressive renal failure in Cd-exposed populations.

Keywords Cadmium · Soil replacement · Follow-up study · General linear mixed model · Renal tubular injuries · Renal glomerular functions · Biological acid–base balance

Introduction

In Japan, it has been determined that one to two thirds of the lifetime cadmium (Cd) intake is derived from the staple food, rice [1]. To reduce Cd concentrations in homegrown rice, the most effective treatment is to remove the Cd-polluted soil present in rice paddies and to replace it with non-polluted soil. In 1977, in the scattered Cd-polluted districts in Japan (35 districts in 19 prefectures), rice paddies measuring a total of 4,297.2 ha were determined to require such treatment. To investigate the influence of such soil replacement on renal tubular function, three researchers undertook investigations using indices of renal tubular dysfunction such as β_2-microglobulin (β_2-mg) [2–4]. However, they performed the comparison with the excretion values only at every two time points of the observation in their studies. Recently, using a general linear mixed model, we reported the serial changes of renal tubular function throughout the observational period in 50 subjects with Cd-induced renal dysfunction who were identified as persons requiring observation by the Ishikawa Prefectural Health Authority in the Cd-polluted Kakehashi River basin [5]. The general linear mixed model is an appropriate statistical method to perform a follow-up study with missing data [6, 7].

On the other hand, few studies have investigated the influence of such soil replacement on renal glomerular function, biological acid–base balance, and other indices. Our research group observed only the association between serum creatinine concentration (SCr) and arterial blood pH values for 9–14 years in 21 subjects (6 men, 15 women) requiring observation in the same region [8]. Therefore, by using a general linear mixed model, we quantitatively investigated serial changes in urinary Cd as an indicator of Cd exposure, urinary total protein as an index of renal tubular injury, biological acid–base balance based on arterial blood pH, carbon dioxide tension (Pa_{CO2}), base excess value (BE), serum chloride ion concentration (SCl^-), and renal glomerular function based on SCr and creatinine clearance value (C_{Cr}) in the persons requiring observation in the Kakehashi River basin.

Materials and Methods

In the Kakehashi River basin, large-scale screening checkups of residents were conducted in 1974–1975 and 1981–1983. Subjects requiring observation were designated based on the results of these checkups. The maximum number of subjects was 126 in 1988, and the minimum was 6 in 1982. Soil replacement was undertaken on 459.7 ha (28 areas) during the 12-year period from 1977 to 1988. Of the subjects requiring observation in seven hamlets in which soil replacement had been completed approximately 1 year previously, there were 29 individuals (10 men, 19 women) who were known to ingest household rice and received serially the health examinations. Age (mean age and range) at the completion of polluted soil replacement was 71.0 years (72–80 years) and 69.3 years (59–82 years) in the men and women, respectively. Six subjects (3 men, 3 women) had died by the end of the study.

Twenty-four-hour and 2-h urine specimens were collected to measure urinary Cd and total protein concentrations in the former and to calculate C_{Cr} in the latter. Urinary Cd concentration was measured by graphite-furnace atomic absorption spectrometry after wet ashing in $HNO_3/H_2SO_4/HClO_4$ and extraction with ammonium pyrrolidine dithiocarbamate-metyl isobutyl ketone. Urinary total protein was determined by the modified method of Kingsbury–Clark. Urinary creatinine and SCr were determined by the Jaffe reaction method [9]. Blood samples were collected in the morning under fasting conditions. Arterial blood was obtained from the radial artery after the subject had spent approximately 10 min in the supine position, and arterial blood gases (pH, Pa_{CO2}) were determined immediately using Corning model 165. SCl^- was measured with ion-selective membrane electrodes.

As participation in health examinations is voluntary, data from some subjects were not available for the year in which soil replacement was completed. However, we were able to confirm retrospectively that values for the previous 4–5 years had been approximately the same, and so adopted the geometric mean values from a total of 5 years. The soil replacement in four hamlets had been completed in 1 year and in three hamlets in 2 years. Because in three hamlets the solid replacement reached 81.8%, 91.7%, and 80.7% in 1 year, respectively, this year was regarded as the respective years of completion of soil replacement.

The urinary Cd and total protein concentrations were corrected for creatinine values. All biological parameter values were analyzed after common logarithmic transformation.

The data of the analyzed period were calculated according to sex using a general linear mixed model and expressed as serial estimated values [10]. To avoid multicollinearity among the number of observation years and the years of age at the time of the annual measurements, the age of each subject at completion of the replacement of polluted soil was included in the model as a covariate for the adjustment of age effect. SPSS 12.0J (SPSS Japan Inc., Japan) was used for statistical analysis of the data.

Results

Changes in the biological parameter values were investigated according to age group (50–69, ≥70 years) in men and women. Results of the women alone are shown in Fig. 1. The year of completion of soil replacement is noted as year 0, and the subsequent years as years 1–10. Urinary Cd concentration in men showed an increasingly higher excretion level with increasing age. Within the respective age groups of female subjects, Cd excretion appeared to decrease as more time elapsed from the completion of soil replacement. Excretion levels of total protein were increased in both sexes in the increasing age groups. Within the respective age groups, total protein appeared to increase with increasing number of years elapsed in both sexes. The changes in other parameters were not clear, but SCl^- appeared to increase as more time elapsed from the completion of the soil replacement in both sexes and age groups.

Table 1 shows the regression coefficients for biological parameter values according to sex calculated from a general linear mixed model. In both sexes, urinary Cd concentrations decreased and total protein concentrations increased with increasing number of years elapsed, with the partial regression coefficients of the number of years elapsed statistically significant in the women. Partial regression coefficients of the number of years elapsed showed positive values for SCl^-, with statistical significance in both sexes. The value for Pa_{CO2} was significantly negative in men. Figure 2 shows the serial changes in urinary substances calculated from a general linear mixed model in the mean age (70 years) by sex.

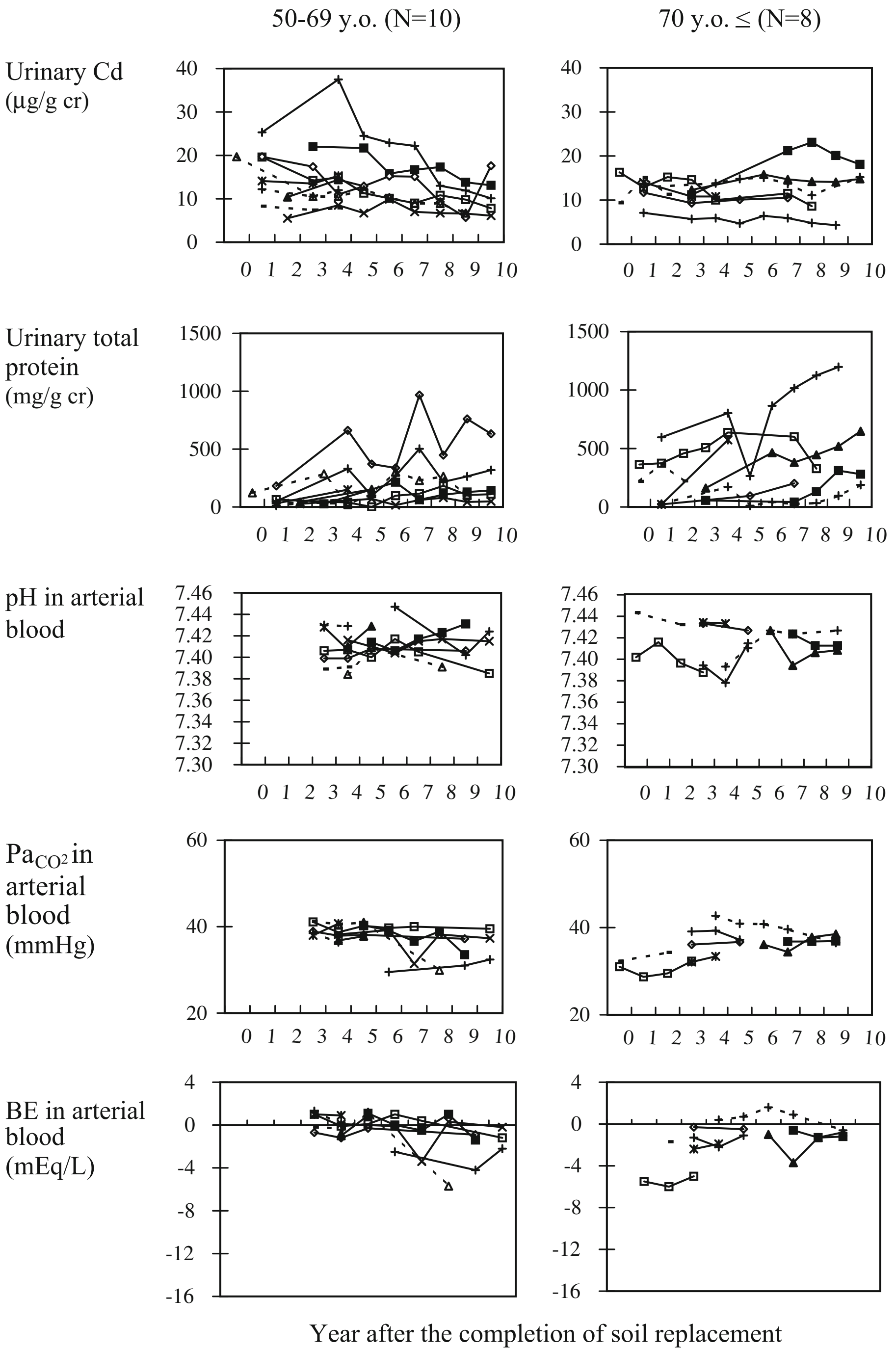

Fig. 1 Serial changes in biological parameter values after the completion of soil replacement according to age group in women

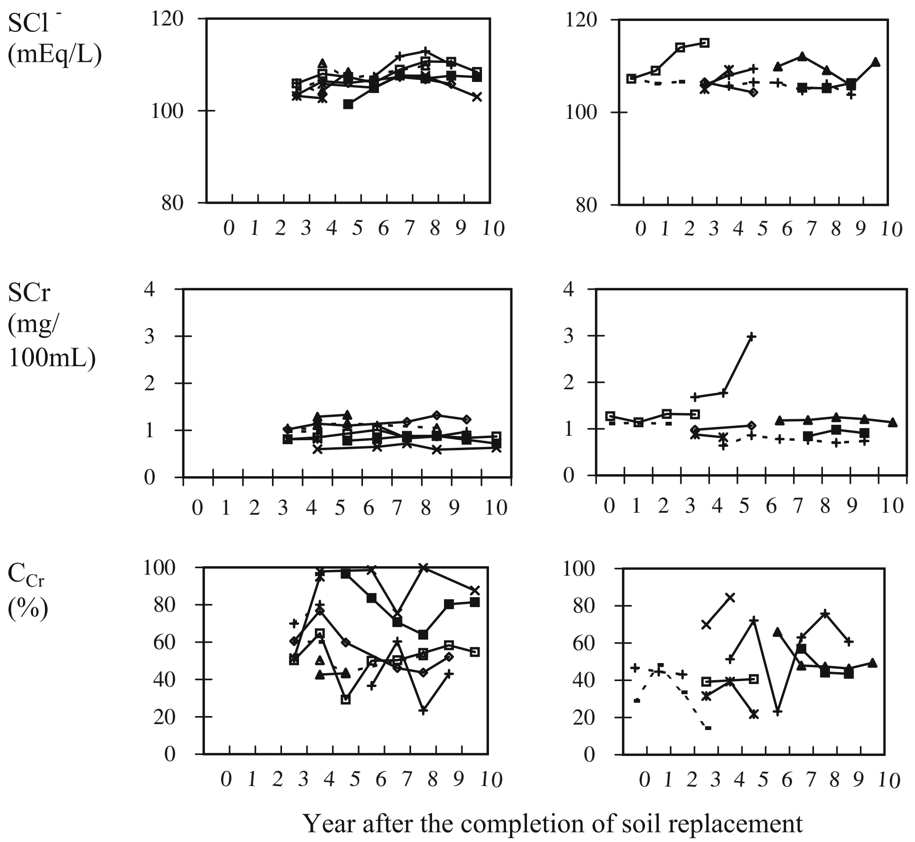

Fig. 1 (continued)

Discussion

In this study, to quantitatively determine changes in the biological parameter values after replacement of polluted soil, seven hamlets in which soil replacement had been completed approximately 1 year previously were selected. However, whether or not these seven hamlets are truly representative of all the Cd-polluted hamlets in the Kakehashi River basin remains an important issue. In 1974, rice was collected from all farmers in the polluted 23 hamlets where large-scale screening checkups of residents were conducted, and 35,451 rice bags were stored in warehouses. According to the number of rice bags collected from each polluted hamlet, random samples were extracted before being assayed for Cd. Mean Cd concentration in rice (minimum, maximum) in all hamlets (23 hamlets) was 0.38 (0.11, 0.67) ppm, while the value in the seven hamlets was 0.43 (0.17, 0.62) ppm. Namely, it was considered that the target seven hamlets are representative of the entire polluted area and that investigations of them would provide valuable information. Additionally, after soil replacement, rice Cd concentrations in every hamlet decreased in almost all cases to less than 0.1 ppm.

In our previous work that focused on the serial changes of renal tubular function, we investigated 50 subjects requiring observation in the seven hamlets [5]. The subjects who

Table 1 Regression Coefficients for Biological Parameter Values According to Sex Calculated from a General Linear Mixed Model

	Covariable	Males		Females	
		β	*P*	*β*	*P*
Urinary Cd (μg/g cr)	Constant	−0.06036	0.938	1.42036	0.004
	Age	0.01302	0.248	−0.00335	0.587
	Year	−0.00394	0.399	−0.02004	0.000
Urinary total protein (mg/g cr)	Constant	−2.98762	0.060	0.06625	0.952
	Age	0.06665	0.008	0.02418	0.136
	Year	0.03009	0.109	0.06247	0.000
pH	Constant	0.87063	0.000	0.87119	0.000
	Age	−0.00002	0.691	−0.00002	0.474
	Year	0.00005	0.502	−0.00002	0.710
Pa_{CO2} (mmHg)	Constant	1.56499	0.000	1.77048	0.000
	Age	0.00073	0.690	−0.00270	0.078
	Year	−0.00288	0.023	−0.00343	0.101
BE (mEq/l)	Constant	1.27355	0.000	1.51823	0.000
	Age	0.00055	0.542	−0.00314	0.042
	Year	−0.00147	0.285	−0.00395	0.081
SCl^- (mEq/l)	Constant	2.03812	0.000	1.98780	0.000
	Age	−0.00048	0.238	0.00049	0.174
	Year	0.00249	0.000	0.00126	0.021
SCr (mg/100 ml)	Constant	−0.34463	0.230	−0.43964	0.172
	Age	0.00599	0.141	0.00619	0.178
	Year	0.00388	0.184	0.00249	0.438
C_{Cr} (%)	Constant	2.33600	0.000	2.71129	0.000
	Age	−0.00944	0.088	−0.01394	0.009
	Year	0.00513	0.555	−0.00510	0.510

Age age at the beginning of the observation, *Year* year after the completion of soil replacement, *β* partial regression coefficient, *P* statistical probability

participated in the health examinations were fewer than the persons who had collected 24-h urine specimens in their homes. This is because it was burdensome for some of the subjects to have to go to the site of the health examinations to provide an arterial blood sample for determination of pH, Pa_{CO2} and BE, and 2-h urine specimens for calculating C_{Cr}. In this way, only 29 of the original 50 subjects made themselves available for our present study. In our previous report [5], Cd concentrations decreased in both sexes with increasing number of years elapsing after soil replacement, with the partial regression coefficient of the number of years elapsed statistically significant in the women. From the regression formula in the women (β=−0.02227), it was calculated that the urinary Cd reduction rate was 5% per year. In this study, similar results were obtained, and it was similarly calculated that the urinary Cd reduction rate was 5% per year in women (β=−0.02004). Partial regression coefficients of the number of years elapsed in total protein excretion as an indicator of renal tubular injury were statistically significant in both sexes (β=0.04269 in men and 0.06231 in women, respectively) in the previous study. However, in the present study, the value was statistically significant only in the women, with β values being 0.03009 in the men and 0.06247 in women. Because few differences in the serial changes of Cd or total protein excretions were identified at any time during the

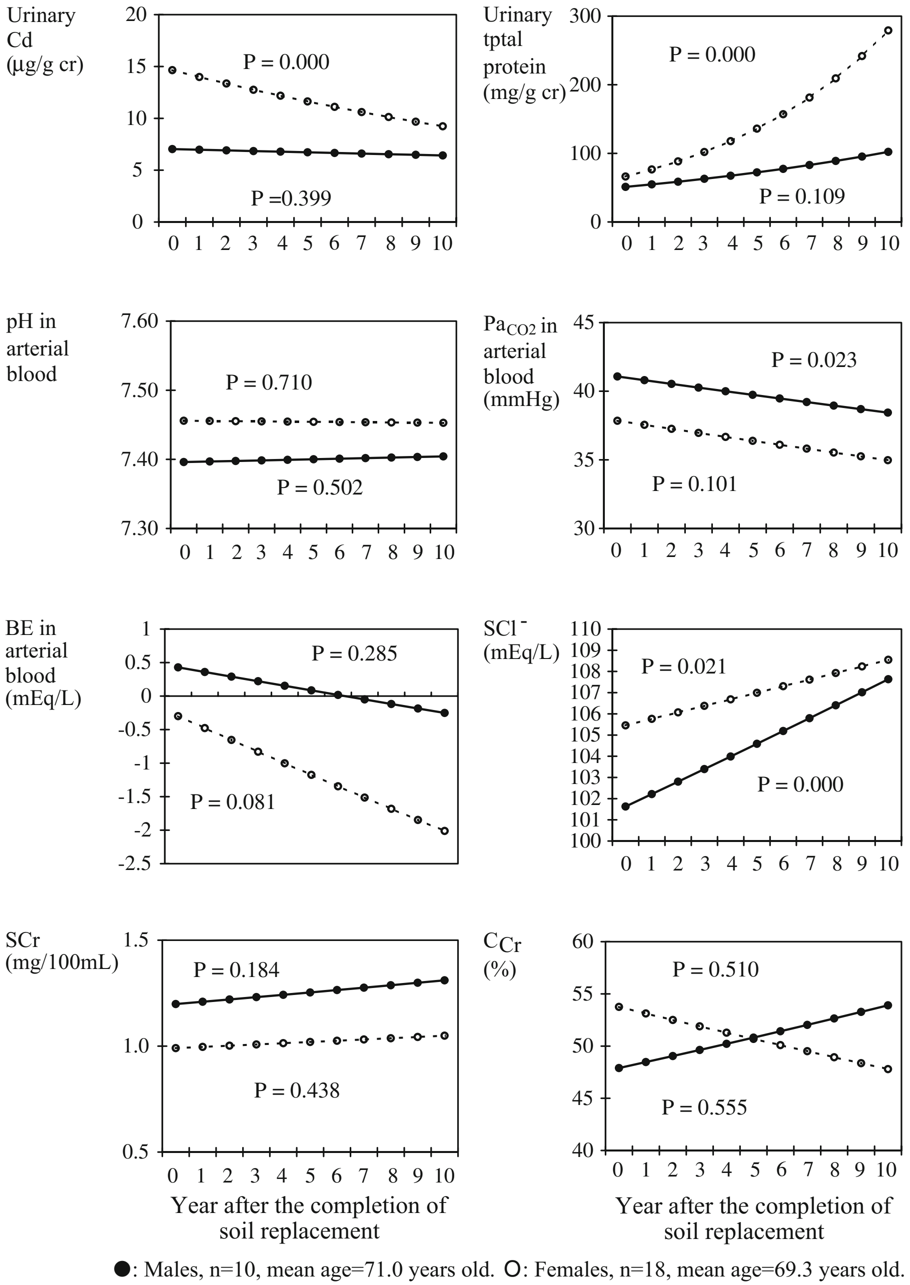

Fig. 2 Serial changes in biological parameter values in 70 years old according to sex calculated from estimated values in a general linear mixed model

observational period in the two studies, we concluded that the 29 persons focused on in this study can be considered representative of the 50 subjects in the previous study.

In the previous and present studies, the target subjects were known to ingest household rice from the questionnaire survey conducted in 1981–1983. Contents of the questionnaire concerning household rice are "Did you previously ingest household rice (including the rice produced in the Kakehashi River basin)?" and "Do you ingest household rice (including the rice produced in the Kakehashi River basin)?" Because soil replacement was undertaken from 1977 to 1988, we selected the subjects who answered "yes" to the latter. In Kakehashi River basin, a duplicate sample of the meals consumed was collected, and it was demonstrated that the daily mean rice intake was 333.5 g [11].

To our knowledge, our series of studies is the first to utilize serial observations to determine the effects of Cd exposure using a general linear mixed model. In this model, parameters take into account individual variables, thereby facilitating more accurate calculation of changes in repeatedly measured data [6, 7]. Hitherto conducted observations of environmental Cd exposure have not relied on this general linear mixed model with the exception of our previous study [5]. As can be appreciated from Fig. 1, the fact that biological parameter data differ according to age group makes it difficult to discern trends in these data. Our studies are thus thought to be the first in which age was taken into consideration.

Arterial blood pH level in men (normal range, 7.39–7.42) was in the range of acidosis throughout the observation period, and little change was noted over the course of the study. Pa_{CO2} in men (normal range, 37.7–42.7 mmHg) was in the normal range throughout the observation period; however, the values decreased significantly with increasing number of years elapsed. Assuming that a similar rate of decrease would be sustained, the Pa_{CO2} level would reach the lower limit of normal 13.8 years from the last year of observation. In women, Pa_{CO2} (normal range, 34.9–40.3 mmHg) had already reached the lower limit of normal by the last year of the study. BE (normal range, −2.1 to +0.1 mEq/l) was calculated to reach a value of −2.2 mEq/l 3 years after the last year of observation in women. SCl^{-} (normal range, 101–109 mEq/l) increased significantly in both sexes and reached the upper limit of normal by the last year of observation. These results suggested that after completion of the soil replacement, biological acid–base balance in the subjects requiring observation would progress to a state of metabolic acidosis with hyperchloremia due to the presence of severe renal tubular dysfunction. Kido et al. [8] observed an association with SCr and arterial blood pH values in 21 persons requiring observation in the same region. Compared to the mean arterial blood pH values at the time of the initial examination (at which time no replacement of polluted soil had been performed), at the time of removal of Cd-polluted soil and at the most recent examination, significant decreases were seen in the increased SCr group. Shinoda and Yuri [12] found hyperchloremic metabolic acidosis in Itai-itai disease patients whose disease represents the most severe stage of chronic environmental Cd intoxication.

SCr levels (normal range, 1.00–1.39 mEq/100 ml for men, 0.88–1.10 mEq/100 ml for women) were already near the upper limit of normal by the last year of observation in both sexes. SCr levels were predicted to exceed the upper limit of normal within 10 years after the last year of observation. C_{Cr} levels (normal range, 90.1–102.1% for men, 74.9–81.3% for women) were far below normal levels and did not improve throughout the observation period. This means that the renal glomerular dysfunction that had already been recognized upon completion of soil replacement continued to slowly progress after the soil replacement. It has already been established that Cd causes decreased renal glomerular filtration [13–15], while Kido et al. [8] determined that the decrease in glomerular filtration was progressive even after cessation of environmental Cd exposure, culminating in some cases in renal failure.

In the present study, using a general linear mixed model, we conducted a 10-year follow-up investigation of the same subjects. The serial changes calculated from a general linear mixed model documented in arterial blood pH, Pa_{CO2}, BE, and SCl suggest that the biological acid–base balance will progress to metabolic acidosis with hyperchloremia. The glomerular dysfunction as indicated by an increase in SCr and a decrease in C_{Cr} will continue to progress after soil replacement.

Although there are no clinical outcomes of patients, since we performed an epidemiological study, interventions such as soil replacement may be too late to prevent progressive renal failure in this kind of environmental Cd-exposed population.

References

1. Tsuchiya K, Iwao S (1978) Results and evaluation on cadmium intake of Cd-exposed inhabitants in Akita, Ishikawa and Nagasaki Prefectures. Kankyo Hoken Report 44:86–115, (in Japanese)
2. Kido T, Honda R, Tsuritani I, Yamaya H, Ishizaki M, Yamada Y, Nogawa K (1988) Progress of renal dysfunction in inhabitants environmentally exposed to cadmium. Arch Environ Health 43:213–217
3. Iwata K, Saito H, Moriyama M, Nakano A (1993) Renal tubular function after reduction of environmental cadmium exposure: a ten-year follow-up. Arch Environ Health 48:157–163
4. Cai Y, Aoshima K, Katoh T, Teranishi H, Kasuya M (2001) Renal tubular dysfunction in male inhabitants of a cadmium-polluted area in Toyama, Japan—an eleven-year follow-up study. J Epidemiol 11:180–189
5. Kobayashi E, Suwazono Y, Honda R, Dochi M, Nishijo M, Kido T, Nakagawa H (2008) Serial changes in urinary cadmium concentrations and degree of renal tubular injury after soil replacement in cadmium-polluted rice paddies. Toxicol Lett 176:124–130
6. Harville DH (1977) Maximum likelihood approaches to variance component estimation and to related problems. J Am Stat Assoc 72:320–340
7. Laird NM, Ware JH (1982) Random effects models. Ann Stat 17:624–642
8. Kido T, Nogawa K, Ishizaki M, Honda R, Tsuritani I, Yamada Y, Nakagawa H, Nishi M (1990) Long-term observation of serum creatinine and arterial blood pH in persons with cadmium-induced renal dysfunction. Arch Environ Health 45:35–41
9. Bonsnes RW, Taussky HH (1945) On the calorimetric determination of creatinine by the Yaffe reaction. J Biol Chem 158:581–591
10. Ishimura S, Nejim J (2004) Linear mixed model by SPSS and the procedure. Tokyo Tosho, Tokyo, (in Japanese)
11. Ishikawa Prefecture, Department of Health (1976) Report of the health examination on the Inhabitants in the Kakrhashi River basin, pp 79–84 (in Japanese)
12. Shinoda A, Yuri T (1981) Clinical findings of Itai-itai disease and patient observation: the relationship among renal function, biochemical findings, and bone lesions. Kankyo Hoken Report 47:208–211, (in Japanese)
13. Shinoda A, Yuri T, Nakagawa A (1977) Medical conditions of Itai-itai disease patients. Findings of internal medicine. Kankyo Hoken Report 41:41–43, (in Japanese)
14. Lauwerys R, Buchet J, Roels H, Brouwers J, Stanescu D (1974) Epidemiological survey of workers exposed to cadmium. Arch Environ Health 28:145–148
15. Nogawa K, Kobayashi E, Honda R, Ishizaki A, Kawano S, Matsuda H (1980) Renal dysfunctions of inhabitants in a cadmium-polluted area. Environ Res 23:12–23