INTRODUCTION

Cadmium (Cd) is an environmental pollutant ranked as one of the most toxic substances (Satarug et al., 2010). Human industrial activities have markedly increased its distribution in the global environment. The general population is exposed to this toxic pollutant, primarily through food (Klassen et al., 2009); therefore, in the present study, we examined specimens from cases of itai-itai disease (IID), the most severe form of chronic cadmium poisoning, to evaluate the relationship between them. Methods We analyzed kidney and bone specimens of 61 IID cases and the data regarding Cd concentration in kidney and bone. Tubulopathy was graded on the basis of a three-step scale (mild, moderate, and severe) using the following three items: the degree of proximal tubular refluxion, thickness of renal cortex, and weight of the kidney. Osteomalacia was evaluated using the relative osteoid volume (ROV). Results There were 15 cases of mild, 19 cases of moderate, and 27 cases of severe tubulopathy. The average ROV was 24.9 ± 2.0%. ROV tended to increase as tubulopathy advanced in severity, and ROV was significantly higher in cases with severe tubulopathy than those with mild or moderate tubulopathy. ROV had a negative correlation with Cd concentration in the kidney but no correlation with that in the bone. Conclusions Our results suggest that the development of osteomalacia was related to the development of tubulopathy.

Key words: Renal tubulopathy, Osteomalacia, Environmental cadmium, Itai-itai disease

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ical relevance between tubulopathy and osteomalacia in human IID cases.

Our institution, the University of Toyama, is located in the region of IID occurrence and maintains a large collection of autopsy specimens from IID patients. To evaluate the relationship between tubulopathy and osteomalacia in IID patients, we examined these autopsy specimens from 61 patients and investigated the correlations between tubulopathy, osteomalacia, and Cd concentrations. The results suggest that osteomalacia progresses in correlation with the progression of tubulopathy.

MATERIALS AND METHODS

Materials
We examined 89 autopsied IID cases. The clinical diagnoses were based on the opinion of the Ministry of Health and Welfare with regard to IID in Toyama Prefecture (Ministry of Health and Welfare, 1972). A diagnosis of IID was made when a patient met the following three criteria (Kasuya et al., 1989): lived in an area polluted with Cd; had symptoms of osteomalacia that appeared in adults (particularly after menopause) and were not congenital; and exhibited renal tubulopathy. Of the 89 IID cases, we excluded 7 cases from the analysis for kidney issues and 21 cases from the analysis for bone issues. Therefore, we examined a total of 61 IID cases. Reasons for the exclusions are described below. The demographic features of IID cases are listed in Table 1. We also selected 27 non-Cd-polluted cases as the control group for comparison of Cd concentration in kidney and bone. The demographic features of these cases are listed in Table 1.

Analysis of kidney
We excluded 7 cases with no specimen of the kidney from analysis. Tubulopathy was scored on the basis of the following three items: weight of kidney, degree of proximal tubular defluxion, and thickness of the renal cortex. The scoring system is shown in Table 2. We set the reference values of scores in such a way that the number of cases in each score could be roughly equivalent. We obtained the information of kidney weight from autopsy records, and adopted average weight of right and left kidney. Pathological analysis was performed by two pathologists (H.B and K.T). We showed histological images of kidney in IID and normal cases in Fig. 1 (a) and (b).

Analysis of bone
Osteomalacia was evaluated by the relative osteoid volume (ROV; osteoid area in visual field/total bone area in visual field × 100). ROV was measured on spec-

Table 1. Demographic features of IID cases

<table>
<thead>
<tr>
<th></th>
<th>IID</th>
<th>Control for Cd concentration</th>
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<tbody>
<tr>
<td>N</td>
<td>61</td>
<td>27</td>
</tr>
<tr>
<td>Median age (range)</td>
<td>82 (62-95)</td>
<td>71 (46-87)</td>
</tr>
<tr>
<td>Sex Female/male</td>
<td>61/0</td>
<td>27/0</td>
</tr>
<tr>
<td>Average BMI (range)</td>
<td>16.7 (12.8-23.0)</td>
<td>-</td>
</tr>
<tr>
<td>period when autopsies were performed</td>
<td>1980 - 2008</td>
<td>1955 - 1990</td>
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</tbody>
</table>

Cd concentration
To increase accuracy, Cd concentrations in organs were measured at the following two institutions: 1) the Department of Pharmaceutical Science, International University of Health and Welfare and 2) the Department of Occupational and Environmental Medicine, Graduate School of Medicine and School of Medicine, Chiba University. We adopted the average Cd titers from the two institutions. As described by Kuzuhara et al. (1985), each tissue (fresh and unfixed, 1-10 g wet weight) was mineralized by mixing with 12 N nitric acid and subsequently heating the mixture to dryness. The residue was taken up in 1 N nitric acid and subjected to instrumental analyses for Cd using a graphite furnace atomic absorption spectrometer at 228.8 nm (Hitachi Z-8200, Hitachinaka, Japan).

Statistical analysis
Statistical analyses were performed using SPSS Statistics version 19.0 software (SPSS Inc., Chicago, IL, USA). Bonferroni’s method for multiple comparisons was used to evaluate statistical differences in the mean ROV and Cd concentration among the three groups with different grades of tubulopathy. Spearman’s correlation test was

imens of the iliac bone stained as per the Yoshiki method, which is a staining method commonly used to observe osteoid (Yoshiki et al., 1983). We excluded 16 cases with no specimen of the iliac bone, and 5 cases with extremely little trabecular bone from this analysis because ROV can not be measured accurately in such cases. We defined cases with total bone volume (total bone area in visual field/total area of visual field × 100) less than 10% as inadequate cases. We analyzed the visual field at × 400 magnification and excluded endosteum, which has a different ossification system, from that of trabecular bone. Imaging analysis was performed using WinRoof version 7.0 software (Mitani corporation, Tokyo, Japan). We showed histological images of bone in IID and normal cases in Fig. 1 (c) and (d).
used to evaluate correlations between ROV and Cd concentrations. The differences were considered statistically significant at $p < 0.05$.

**RESULTS**

**Analysis of kidney**

The average weight of kidney was $56.9 \pm 2.5$ g. The median, maximum, and minimum weights were 52, 150, and 25 g, respectively. Of 61 IID cases, mild proximal tubular defluxion were observed in 5 (8%), moderate defluxion in 30 (49%), and severe defluxion in 26 patients (43%). The average length of renal cortex was 3,360 ± 150 μm. According to our grading system, therefore, there were 15 cases of mild tubulopathy, 19 of moderate tubulopathy, and 27 of severe tubulopathy. The severity of tubulopathy was not related with age ($P = 0.88$ for trend).

**Analysis of bone**

The average ROV was $24.9 \pm 2.0\%$. The median, maximum, and minimum of ROV were 20.4%, 67.0%, and 0.9%, respectively. ROV did not correlate with age ($r = -0.202$, $P = 0.119$).

**Cd concentration**

In IID cases, the average Cd concentration in kidney was $35.7 \pm 2.5$ μg/g. The median, maximum, and minimum concentrations were 29.5, 128, and 9.5 μg/g, respectively. The average Cd concentration in bone was $1.9 \pm 0.08$ μg/g. The median, maximum, and minimum concentrations were 1.7, 5.9, and 0.5 μg/g, respectively.

In control cases, the average Cd concentration in kidney was $92.9 \pm 10.1$ μg/g. The median, maximum, and minimum concentrations were 87.7, 205.5, and 5.7 μg/g, respectively. The average Cd concentration in bone was $0.6 \pm 0.07$ μg/g. The median, maximum, and minimum
concentrations were 0.5, 1.3, and 0.1 μg/g, respectively.

Cd concentration in kidney was significantly lower in IID cases than in control cases ($P < 0.01$), and that in bone was significantly higher in IID cases than in control case ($P < 0.01$).

**ROV vs. tubulopathy**

The relationship between the severity of tubulopathy and ROV is shown in Fig. 2. Stepwise decrease in ROV with tubulopathy grade progression was observed. In particular, ROV was significantly higher in cases with severe tubulopathy than those with mild and moderate tubulopathy.

**ROV vs. Cd concentration**

ROV did not correlate with Cd concentration in the bone (Fig. 4 (a)). In contrast, it correlated with Cd concentration in the kidney (Fig. 4 (b)).

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**Table 2. Grading system for tubulopathy**

<table>
<thead>
<tr>
<th>Grade</th>
<th>Score</th>
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<tbody>
<tr>
<td>Mild</td>
<td>3-5</td>
</tr>
<tr>
<td>Moderate</td>
<td>6-7</td>
</tr>
<tr>
<td>Severe</td>
<td>8-9</td>
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**Index**

<table>
<thead>
<tr>
<th>Index Score</th>
<th>Score</th>
</tr>
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<tbody>
<tr>
<td>Area of proximal tubular defluxion/ Total area</td>
<td>$&lt;20%$</td>
</tr>
<tr>
<td>Thickness of renal cortex</td>
<td>$4,000 , \mu m \leq$</td>
</tr>
<tr>
<td>Weight of kidney</td>
<td>$80 , g \leq$</td>
</tr>
</tbody>
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**Fig. 2.** The relation between ROV and severity of tubulopathy. ROV was expressed as mean ± standard deviation. $P$-value was obtained using Bonferroni’s method for multiple comparisons.

**Fig. 3.** The relationship between Cd concentration in kidney and severity of tubulopathy. Cd concentration was expressed as mean ± standard deviation. $P$-value was obtained using Bonferroni’s method for multiple comparisons.

**Tubulopathy vs. Cd concentration**

The relation between the severity of tubulopathy and Cd concentration is shown in Fig. 3. Cd concentration in the kidney tended to decrease as tubulopathy advanced in severity. In particular, it was significantly lower in cases with severe tubulopathy than in those with moderate or mild tubulopathy.
DISCUSSION

Previous reports have described the histopathological changes that occur in kidney and bone of IID cases and have revealed that tubulopathy and osteomalacia are due to Cd exposure (Yasuda et al., 1995; Yamashita et al., 1996). However, most of these studies focused on either kidney or bone, and there are few pathological studies on the relation between tubulopathy and osteomalacia in IID. This is the first study to examine the histopathological relationship between the two pathoses in a large number of IID cases. Our study showed two points regarding tubulopathy and osteomalacia in IID. First, the development of osteomalacia was related to the development of tubulopathy. Second, the development of osteomalacia had no correlation with Cd concentration in bone, but had a significant correlation with Cd concentration in the kidney. Although similar findings have been reported by several epidemiological studies (Aoshima et al., 1988; Doyle., 1979; Wallin et al., 2013), the significance of our results is to find the link between the two pathoses from the histopathological perspective.

As mentioned above, indirect and direct pathways contributing to the onset of Cd-induced osteomalacia have been proposed. The indirect pathway, which has been accepted as the main explanation, supposes that Cd causes acquired Fanconi syndrome (Takebayashi et al., 2000; Blainey et al., 1980). In other words, osteomalacia is caused by the urinary leak of essential metabolites like phosphate and calcium, which is related to tubulopathy that causes defects in renal tubular reabsorption. On the other hand, the direct pathway supposes that Cd directly impairs bone metabolism and osteocytes and alters osteoblast gene expression (Bhattacharyya et al., 1988; Brama et al., 2012; Arbon et al., 2012). Although our results do not rule out the direct pathway, it is believed to be evidence supporting and reconfirming the theory that renal osteomalacia occurs in IID patients.

It is known that the Cd level in kidney is lower in autopsy samples in an area with heavy Cd exposure than in those in non-polluted areas, and this is thought to be because most of Cd that attacked kidney may have leaked out (Hayashi et al., 2012). Our analysis also showed that Cd concentration tended to decrease as the Cd-induced kidney injury advanced in severity. We previously reported the same tendency also in the liver of IID patients (Baba et al., 2013). Our findings strongly support the conventional idea. That is, chronic Cd exposure causes high accumulation of Cd and subsequent organ disorder; however, once organ disorder occurs, Cd is believed to flow out of the organ as the disorder advances in severity.

Because there is no established grading system for tub-

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**Fig. 4.** The relationship between ROV and Cd concentration.
(a) ROV was not correlated with Cd concentration in bone ($r^* = 0.237, P = 0.055$).
(b) ROV was significantly correlated with the Cd concentration in kidney ($r = -0.370, P = 0.002$). $r^*$ = correlation coefficient.
ulopathy, our study is based on our original grading system. Therefore, it is necessary to examine the adequacy of this system. It is known that Cd primarily causes tubulopathy in proximal tubules, which results in the thinning of renal cortex and decrease in kidney weight (Takebayashi et al., 2000; Yasuda et al., 1995). We use the three items mentioned above as the elements of our grading system. Although both the weight of the kidney and thickness of the renal cortex are indexes for atrophy of the kidney, we use the former as an index to evaluate the whole kidney and the latter as an index to evaluate the worst part. Therefore, our system reflects histopathological evaluation of tubulopathy and damage to both the entire kidney and the most severe part. This enables us to multilaterally evaluate the development of tubulopathy.

In conclusion, osteomalacia progresses in correlation with the progression of tubulopathy in IID. It suggests that Cd causes osteomalacia through renal tubular injury.

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REFERENCES


