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Bone disease in pediatric patients undergoing dialysis with CAPD or CCPD

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Bone disease in pediatric patients undergoing dialysis with CAPD or CCPD. The histologic features of renal osteodystrophy and the prevalence of bone aluminum deposition in children receiving regular dialysis have not been described. Forty-four pediatric patients undergoing continuous ambulatory (CAPD) or cycling (CCPD) peritoneal dialysis had bone biopsies and deferoxamine (DFO) infusion tests; all were receiving oral calcitriol. Osteitis fibrosa (OF) was found in 39%, mild lesions (M) in 25%, normal histology (NH) in 16%, aplastic lesions (AP) in 11%, and osteomalacia (OM) in 9%. Bone surface aluminum (SA) was present by histochemical staining in 10 out of 20 given aluminum-containing phosphate-binding agents and in 0 of 24 treated with calcium carbonate; $\chi^2 = 15.5$, $P < 0.0001$. Serum biochemistries and DFO infusion tests failed to predict bone histology, but plasma aluminum levels were markedly elevated and bone aluminum content was highest in patients with OM. Bone formation rate (BFR) correlated with serum parathyroid hormone (PTH), $r = 0.55$, $P < 0.001$; BFR was inversely related to bone aluminum content ($r = -0.42$, $P < 0.01$), even in patients with OF ($r = -0.66$, $P < 0.05$). All patients with SA > 30% had normal or reduced BFR when compared to those with SA < 30%; $\chi^2 = 12.2$, $P < 0.005$. Based on SA > 30%, six patients were classified as aluminum-related bone disease: three OM, one AP, and two NH. Two-thirds of pediatric patients undergiong CAPD/CCPD have persistent hyperparathyroidism despite treatment with calcitriol, but aluminum can adversely affect BFR when SA exceeds 30% regardless of histologic lesion or serum PTH level.

Studies using quantitative histology of bone have provided valuable information about the pathogenesis and treatment of renal osteodystrophy in adult patients receiving long-term dialysis [1–3]. Only limited information is available, however, regarding the histologic features of bone disease in children undergoing maintenance dialysis. Previous reports contain small numbers of patients treated by hemodialysis or by intermittent peritoneal dialysis [4, 5], and they have often included children with both moderate and advanced chronic renal failure [4, 6, 7]. In addition, the histologic features of renal osteodystrophy in pediatric patients undergoing continuous peritoneal dialysis have not been described despite substantial increases in the use of this treatment in uremic children [8].

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Osteitis fibrosa is the most common histologic lesion of renal osteodystrophy both in adults and in children [1–3, 5, 6, 9, 10], and treatment with either 25-hydroxyvitamin D or 1,25 dihydroxyvitamin D may control secondary hyperparathyroidism in such patients [5, 10–12]. However, one-third to one-half of pediatric patients with chronic renal failure have osteomalacia despite normal serum levels of 25-hydroxyvitamin D [6]. Aluminum deposition in bone has been implicated in the pathogenesis of renal osteomalacia [13], but previous studies of bone disease in uremic children have not systematically assessed the prevalence of aluminum deposition [4–7, 9, 10]. With the development of effective therapies for aluminum-related bone disease [14], it is important to determine the relative contributions of secondary hyperparathyroidism and bone aluminum accumulation to the pathogenesis of renal osteodystrophy in children undergoing maintenance dialysis. The present study was undertaken to characterize the histopathology of bone in pediatric patients treated by continuous ambulatory (CAPD) or continuous cycling (CCPD) peritoneal dialysis and to determine the value of non-invasive biochemical measurements as predictors of specific skeletal lesions.

Methods

Study protocol

All pediatric patients treated by CAPD/CCPD at the UCLA Center for the Health Sciences who consented to undergo iliac crest bone biopsy and a deferoxamine infusion test were entered into the study. The investigational protocol was approved by the UCLA Human Subjects Protection Committee, and informed consent was obtained from all patients and/or their parents. Forty-four subjects, 22 females and 22 males, among 55 to 60 patients receiving CAPD/CCPD were evaluated; 13 were treated by CAPD and 31 by CCPD. The mean age of patients was 11.8 ± 5.8 years, and the duration of peritoneal dialysis was 22.2 ± 14.9 months with a range of five to 59 months. Twenty-four patients had congenital renal disease, and twenty had acquired renal disease. Four subjects had undergone subtotal parathyroidectomy three to five years before entry into the study.

At the time of bone biopsy, 20 patients were receiving aluminum hydroxide as a phosphate-binding agent; the duration of therapy with aluminum hydroxide was 10 to 55 months, and

the daily dose ranged from 70 to 280 mg/kg. Calcium carbonate was given as a phosphate-binding agent in 24 patients; 11 of the 24 never received aluminum hydroxide whereas 13 had previously been treated with aluminum hydroxide. These 13 patients received calcium carbonate for 16.8 ± 6.7 months prior to study; therefore, they were included in the calcium carbonate group. The daily dose of calcium carbonate ranged from 62 to 230 mg/kg [15]. All patients received oral calcitriol at dosages sufficient to maintain serum calcium levels between 10.5 and 11.0 mg/dl [16]. The concentration of aluminum in various lots of peritoneal dialysate was monitored throughout the study; these were consistently below 5 μg/liter.

The deferoxamine infusion test was done as described elsewhere [17]. Deferoxamine (DFO), 40 mg/kg, was given by slow intravenous infusion over a two hour period. After the start of the DFO infusion, the peritoneal cavity was emptied for either six or 10 hours in patients treated by CAPD and CCPD, respectively. Regular dialysate exchanges were then resumed. Plasma samples for aluminum determinations were obtained before and 24 hours after the start of the infusion of DFO.

Bone biopsies were taken from the iliac crest using a modified Bordier trephine at a site two cm posterior and two cm inferior to the anterior iliac spine with the patient supine [18]. All biopsies were done within four weeks of the DFO infusion test. Prior to biopsy, all patients were given tetracycline, 10 mg/kg/day, on two occasions separated by an interval of 17 days to achieve tetracycline labeling of bone. For children under eight years of age, each course of tetracycline was limited to two days and the total dose did not exceed 40 mg/kg [19]. For children older than eight years of age, three days of tetracycline were given for each label. A separate sample of trabecular bone was obtained using a Jamshidi needle for determinations of total bone aluminum content. Serum samples for the measurement of calcium, phosphorus, alkaline phosphatase and immunoreactive parathyroid hormone (iPTH) levels were obtained at the time of bone biopsy.

Bone histology

Biopsy specimens were prepared for quantitative histological assessment as previously described [20]. Five μm sections of non-decalcified bone were stained by the modified Goldner technique for light microscopic examination and by the aurine tricarboxylic acid method for the histochemical detection of bone surface aluminum [20, 21]. Ten μm sections were mounted unstained in 10% glycerol and examined by fluorescence microscopy for the evaluation of tetracycline labels [20]. All measurements of length, width and area were done using a digitizer interfaced with a microcomputer, and the results represent two dimensional values. Image projection to the surface of the digitizer tablet was achieved using a projection prism and drawing tube attached to a Leitz Dialux microscope with epifluorescent illuminator (Leitz, Wetzlar, FRG) [20].

The following histologic variables were determined for trabecular bone: 1) total bone area (%)—the area of trabecular bone, including both mineralized bone and unmineralized bone matrix (osteoid), expressed as a percentage of the total tissue area; 2) osteoid area (%)—the area of unmineralized bone matrix expressed as a percentage of the total bone area; 3) osteoid surface (%)—the percentage of trabecular bone surfaces covered by osteoid seams; 4) resorption surface (%)—the percentage of trabecular bone surfaces that exhibited characteristic scalloped margins along bony trabeculae; 5) osteoid seam width (μm)—the mean width of surface osteoid seams and obtained by dividing the measured osteoid area, in mm^2, by the length, in mm, of trabecular surface covered by osteoid; 6) double tetracycline-labeled surface (%)—the percentage of trabecular bone surfaces that exhibited double bands of tetracycline fluorescence; and 7) double labeled osteoid surface (%)—the percentage of osteoid surfaces that assumed a double tetracycline label [20]. Histochemical measurements of the bone aluminum content were expressed as the percentage of trabecular surfaces that stained positive for aluminum [20, 21].

The rates of mineralized bone apposition were determined from measurements of the width of separation of double tetracycline labels divided by the time interval between the administration of the two labels; the results are expressed in μm/day [20]. No correction was made for obliquity of the plane of tissue section. Bone formation rates were calculated as described previously, and these values represent the area of mineralized bone formed per day [20]. Results are expressed per unit area of existing trabecular bone (BFR_b) and per unit area of tissue (BFR_t), both in $\mu m^2/mm^2/day$.

Normal values for bone histology and tetracycline-based measurements of bone formation were obtained from iliac crest bone biopsies done in ten children, ages 2.5 to 17 years, (mean 11.9 ± 5.0 yr), who had undergone orthopedic surgery for conditions not associated with metabolic bone disease (**Results**, Tables 1 and 2). None had received corticosteroids, anticonvulsant medications, or vitamin D preparations, and none were diabetic. All were physically active according to their ages, and none had a history of renal disease.

On the basis of the results obtained in normal children, patients were classified according to the following criteria: osteitis fibrosa—osteoid area <12%, presence of fibrosis and BFR_t >97 $\mu m^2/mm^2/day$; mild lesion—osteoid area <12%, no tissue fibrosis and BFR_t >613 $\mu m^2/mm^2/day$; normal histology—no fibrosis, osteoid volume <12%, and BFR_t >97 but <613 $\mu m^2/mm^2/day$; aplastic bone—osteoid area <12%, no fibrosis and BFR_t <97 $\mu m^2/mm^2/day$; osteomalacia—osteoid area >12%, no fibrosis and BFR_t <97 $\mu m^2/mm^2/day$; and mixed lesion—osteoid area >12%, fibrosis present, and BFR_t <97 $\mu m^2/mm^2/day$.

Biochemistries

Calcium, phosphorus and alkaline phosphatase levels in serum were determined using a Technicon Autoanalyzer II. Serum iPTH was measured by radioimmunoassay using an antiserum (Ch 9) that reacts with the intact and carboxy-terminal regions of the PTH molecule [22]. Serum alkaline phosphatase values were corrected for age and sex according to the data of Cherian and Hill [23], and the results for individual patients were expressed as the percentage of the median value for normals as previously reported [16]. Plasma aluminum levels were measured by flameless atomic absorption spectrophotometry using a graphite furnace [24], and chemical determinations of the aluminum content of bone were done after ashing and extraction with EDTA for 24 hours as previously described [24].

Table 1. Histomorphometry and aluminum content of bone in children on CAPD/CCPD[a]

	Histologic groups					Normal values	
	Osteitis fibrosa [OF] $N = 17$	Mild [M] $N = 11$	Normal histology [NH] $N = 7$	Aplastic [AP] $N = 5$	Osteomalacic [OM] $N = 4$	Mean ± SD $N = 10$	Range
Bone volume %	31.8 ± 8.8	29.4 ± 5.7	26.6 ± 5.7	27.9 ± 11.5	30.7 ± 10.1	21.3 ± 3.2	14.6–25.7
Osteoid area %	6.0 ± 4.1	6.4 ± 3.4	4.9 ± 2.7	3.1 ± 3.4	20.3 ± 9.8[bcde]	2.6 ± 1.5	1.1–5.8
Osteoid surface %	43.1 ± 17.7	41.4 ± 14.8	36.6 ± 17.6[f]	18.9 ± 15.6	63.2 ± 11.3[de]	18.1 ± 6.1	10.2–29.9
Osteoid seam width *μm*	10.1 ± 3.8	9.4 ± 2.6	8.5 ± 2.3	8.2 ± 3.9	24.9 ± 15.3[bcde]	7.1 ± 2.3	4.1–11.3
Resorption surface %	7.0 ± 3.1[ghij]	4.1 ± 1.7	2.9 ± 1.4	2.3 ± 1.2	1.6 ± 1.1	2.3 ± 0.9	1.1–3.5
Surface stainable Al %	3.2 ± 8.9	2.2 ± 5.2	12.8 ± 23.4	12.1 ± 27.2	58.5 ± 41.4[bcde]	0	—
N[k]	2/17	2/11	2/7	1/5	3/4		
Bone Al *mg/kg/dry wt*	38.5 ± 29.6	13.5 ± 21.9	51.7 ± 90.7	70.6 ± 81.9	123.7 ± 109.7[bcl]	2.4 ± 1.2	0–4
Parathyroidectomy N	2	1	—	—	1		

[a] Probabilities determined by analysis of variance except as indicated
[b] $P < 0.01$ OM vs. OF
[c] $P < 0.01$ OM vs. M
[d] $P < 0.01$ OM vs. NH
[e] $P < 0.01$ OM vs. AP
[f] $P < 0.05$ NH vs. AP
[g] $P < 0.01$ OF vs. M
[h] $P < 0.01$ OF vs. NH
[i] $P < 0.01$ OF vs. AP
[j] $P < 0.01$ OF vs. OM
[k] Refers to number of cases with a positive surface stainable aluminum
[l] $P < 0.05$ OM vs. NH

Table 2. Tetracycline-based bone dynamics in children on CAPD/CCPD[a]

	Histologic groups					Normal values	
	Osteitis fibrosa [OF] $N = 17$	Mild [M] $N = 11$	Normal histology [NH] $N = 7$	Aplastic [AP] $N = 5$	Osteomalacic [OM] $N = 4$	Mean ± SD $N = 10$	Range
Double tetracycline labelled surface % *bone surface*	22.5 ± 7.5[bc]	14.5 ± 2.3[d]	4.8 ± 2.3	0.4 ± 0.6[efg]	0.1 ± 0.2[hij]	4.0 ± 3.0	0.3–8.5
Osteoblastic osteoid % *osteoid*	60.3 ± 27.1[bc]	40.2 ± 23.3[d]	17.3 ± 14.9	3.7 ± 6.3[ef]	0.2 ± 0.3[hik]	24.5 ± 18.9	1.8–58.3
Mineral apposition rate *μm/day*	1.3 ± 0.2	1.4 ± 0.3	1.2 ± 0.4	0.6 ± 0.9[efl]	0.2 ± 0.4[him]	1.2 ± 0.2	1.0–1.5
Bone formation[q] rate—BFR_b $\mu m^2/mm^2/day$	4718 ± 1519[c]	4310 ± 1680[d]	1340 ± 890	140 ± 193	38 ± 75[hi]	1197 ± 762	484–2782
Bone formation[q] rate—BFR_t $\mu m^2/mm^2/day$	1502 ± 626[c]	1246 ± 510[d]	319 ± 155	35 ± 49[efn]	9 ± 18[hij]	255 ± 171	97–613
Mineralization[o] lag time *days*	16 ± 10[c]	27 ± 34[d]	83 ± 79	61 ± 24	26 ± 15[p]	36 ± 36	5–104

[a] Probabilities determined by analysis of variance except as indicated
[b] $P < 0.01$ OF vs. M
[c] $P < 0.01$ OF vs. NH
[d] $P < 0.01$ M vs. NH
[e] $P < 0.01$ AP vs. OF
[f] $P < 0.01$ AP vs. M
[g] $P < 0.01$ AP vs. NH, non-paired t-test
[h] $P < 0.01$ OM vs. OF
[i] $P < 0.01$ OM vs. M
[j] $P < 0.01$ OM vs. NH, non-paired t-test
[k] $P < 0.05$ OM vs. NH, non-paired t-test
[l] $P < 0.01$ AP vs. NH
[m] $P < 0.01$ OM vs. NH
[n] $P < 0.01$ AP vs. NH, non-paired t-test
[o] analysis done excluding OM
[p] does not include 3 patients with values approaching infinity
[q] statistical differences reflect criteria for classification (**Methods**)

Statistical analysis

All results are expressed as the mean ± standard deviation. Statistical analysis of the data was done using the t-test for unpaired samples, one way analysis of variance with contrasts, linear regression analysis, and chi-square analysis [25]. The rank-sum test was used for comparisons between groups with unequal within-groups variances [25].

Table 3. Serum biochemistries and calcitriol dosages according to the type of bone disease in children on CAPD/CCPD

	Osteitis fibrosa [OF] $N = 17$	Mild [M] $N = 11$	Normal histology [NH] $N = 7$	Aplastic [AP] $N = 5$	Osteomalacic [OM] $N = 4$
Age *yrs*	12.8 ± 5.5	10.3 ± 6.2	11.9 ± 7.0	11.1 ± 7.7	12.1 ± 4.2
CAPD/CCPD *months*	28 ± 14	25 ± 17	14 ± 7	12 ± 7	21 ± 11
Calcium *mg/dl*	9.6 ± 0.7	10.0 ± 1.0	9.9 ± 0.6	10.2 ± 0.9	10.6 ± 1.2
Phosphorus *mg/dl*	6.4 ± 1.2	6.0 ± 1.4	5.2 ± 1.5	7.2 ± 1.9	5.9 ± 2.5
Alk. phosphatase					
Uncorrected *IU/liter*	424 ± 357[ab]	174 ± 84	174 ± 144	95 ± 12	308 ± 88
Corrected *%*	274 ± 170[ab]	108 ± 53	99 ± 58	62 ± 49	265 ± 285[cd]
iPTH *μlEq/ml*	493 ± 505	231 ± 149	224 ± 238	148 ± 116	248 ± 77
Baseline Al *μg/liter*	30 ± 18	31 ± 23	18 ± 15	60 ± 52	470 ± 848[cfgh]
AL increment after DFO *μg/liter*	148 ± 128	85 ± 123	119 ± 185	345 ± 490	495 ± 456
Calcitriol dose *ng/kg*	33 ± 27[abei]	17 ± 9	11 ± 9	9 ± 7	10 ± 5

[a] $P < 0.01$ OF vs. M
[b] $P < 0.01$ OF vs. NH
[c] $P < 0.01$ OM vs. NH
[d] $P < 0.01$ OM vs. AP
[e] $P < 0.01$ OM vs. OF
[f] $P < 0.05$ OM vs. M
[g] $P < 0.01$ OM vs. NH
[h] $P < 0.01$ OM vs. AP
[i] $P < 0.01$ OF vs. AP

Results

Of the 44 patients studied, 17 had osteitis fibrosa, 11 had mild lesions, seven had normal findings on bone biopsy, five had aplastic lesions, and four had osteomalacia (Tables 1 and 2). None had mixed renal osteodystrophy. Patients in each group were similar with respect to age, duration of peritoneal dialysis and end-stage renal disease, the number with congenital and acquired renal diseases, and the number that had undergone previous renal transplantation or parathyroidectomy (Tables 1 and 3).

Serum calcium, phosphorus and immunoreactive parathyroid hormone (iPTH) levels did not differ among groups, although the mean values for serum iPTH were two to three times higher in patients with osteitis fibrosa than in those with other histologic lesions (Table 3). Serum alkaline phosphatase values, both uncorrected and corrected, were highest in patients with osteitis fibrosa and osteomalacia, but the results did not differ between the two groups. In patients with mild and aplastic lesions, alkaline phosphatase levels were not different from the values determined in non-uremic subjects or patients with normal bone formation and histology (Table 3). The daily dose of calcitriol was higher in patients with osteitis fibrosa than in each of the other four groups (Table 3).

In patients with osteitis fibrosa, resorption surface was greater than in each of the other four groups, and the rate of bone formation was approximately 2.5 times the upper limit of normal (Tables 1 and 2). Osteoid area was between 10% and 12% in two biopsies, but tetracycline-based measurements did not confirm a defect in mineralization in either patient. In two other patients with osteitis fibrosa, the histochemical stain for aluminum was positive, but the values were below 30%. In both cases, the rate of bone formation was increased and the mineralization lag time was normal.

In the eleven patients with mild lesions, percent resorption surface was lower than in patients with osteitis fibrosa, and the values did not differ from those determined in non-uremic children or patients with normal bone biopsy findings (Table 1). Osteoid surface and osteoid area were not different in patients with mild and fibrotic lesions, and bone formation was elevated to a similar degree in each group (Tables 1 and 2). Two cases with mild renal osteodystrophy had histochemical evidence of aluminum deposition in bone with values of 5.9% and 15.9%; bone aluminum contents were 69 and 42 mg/kg dry weight, respectively, and these were the highest levels among patients with mild lesions.

Bone formation was within the normal range and the histologic findings were predominantly normal in seven patients (Tables 1 and 2). Two of these patients had deposits of aluminum at 59.3% and 30.3% of trabecular surfaces, and the corresponding bone aluminum levels were 231 and 61 mg/kg dry weight.

Five patients had aplastic lesions, but the values for osteoid area, osteoid seam width, and percent resorption surface did not differ from those in non-uremic subjects or dialysis patients with normal bone biopsy findings (Table 1). In contrast, the percent osteoid surface and the extent of double tetracycline-labeled surface were lower than in the seven dialysis patients with normal bone histology and bone formation (Tables 1 and 2). No double tetracycline labels were seen in three patients, and bone formation could not be measured. One patient with the aplastic lesion had 60.7% surface stainable aluminum and a chemical bone aluminum level of 165 mg/kg dry weight. The other four cases had no surface stainable aluminum, and bone aluminum levels were below 30 mg/kg dry weight.

Osteomalacia was confirmed in four patients; all exhibited marked increases in osteoid area and osteoid seam width with diminished tetracycline uptake into bone (Tables 1 and 2). Bone formation was measurable in only one of the four patients, and this value was substantially below the lower limit of normal; in

Table 4. Bone formation and bone surface stainable aluminum in 44 patients receiving CAPD/CCPD

	Surface stainable aluminum[a]			
	<30%	30–50%	>50%	Totals
Bone formation rate				
Low	5	0	4	9 (20.5%)
Normal[b]	5	1	1	7 (15.9%)
High	28	0	0	28 (63.6%)
Totals	38 (86.4%)	1 (2.3%)	5 (11.4%)	44 (100%)

Chi-square = 19.0; Degrees of freedom = 4; $P < 0.005$.
[a] The percentage of trabecular bone surfaces positive for aluminum
[b] Bone formation per unit area of tissue; normal range: 97–613 $\mu m^2/mm^2/day$

Table 5. Characteristics of 44 pediatric patients on CAPD/CCPD with values for surface stainable aluminum <30% or >30%

	<30% N = 38	>30% N = 6	P value[a]
Age *yrs*	11.4 ± 5.6	14.7 ± 7.2	NS
Duration of CAPD/CCPD *months*	22.3 ± 15.6	20.5 ± 9.3	NS
Serum calcium *mg/dl*	9.8 ± 0.8	10.6 ± 0.9	<0.05
Serum iPTH *μlEq/ml*	352 ± 376	146 ± 122	<0.05
Plasma Al *μg/liter*	28 ± 20	344 ± 685	<0.05
Increment in plasma Al after DFO *μg/liter*	140 ± 119	546 ± 399	<0.05
Bone Al content *mg/kg dry wt*	24 ± 27	157 ± 83	<0.01
Osteoid area %	6.1 ± 6.0	12.3 ± 4.2	<0.05
Resorption surface %	5.0 ± 3.0	2.3 ± 1.1	<0.01
Double tetracycline labeled surface %	15.0 ± 10.2	0.6 ± 1.2	<0.001
Bone formation rate[b] $\mu m^2/mm^2/day$	1083 ± 744	90 ± 137	<0.001
Mineralization lag time *days*	26 ± 30	975 ± 1422[c]	<0.05[d]

[a] Probabilities estimated by the *t*-test for unpaired samples except as indicated
[b] Expressed per unit area of bone tissue
[c] Results do not include 4 patients with values approaching infinity
[d] Probability estimated using the Wilcoxon rank-sum test

the other three, no tetracycline uptake was observed. Three of four patients had histochemical evidence of skeletal aluminum deposition; all values exceeded 50%, and the bone aluminum content was 161 ± 99 mg/kg dry weight. The bone aluminum content was 13 mg/kg dry weight and the histochemical stain for aluminum was negative in the remaining patient with osteomalacia.

Serum iPTH levels in the 44 patients correlated with percent resorption surface, $r = 0.61$, $P < 0.001$. Bone formation was also directly related to the serum level of iPTH when all patients were considered, $r = 0.55$, $P < 0.001$. This relationship was stronger when only patients with mild and fibrotic lesions were evaluated, $r = 0.61$, $P < 0.001$, and it was greatest when the analysis was confined to the 17 patients with osteitis fibrosa, $r = 0.84$, $P < 0.001$. Bone formation was inversely related to the bone aluminum content, whether determined by chemical, $r = -0.42$, $P < 0.01$, or by histochemical methods, $r = -0.46$, $P < 0.01$. Even in patients with osteitis fibrosa, the rate of bone formation was inversely related to the total aluminum content of bone, $r = -0.66$, $P < 0.05$.

There was a direct relationship between the chemical measurement of bone aluminum content and percent surface stainable aluminum, $r = 0.86$, $P < 0.001$, and patients with osteomalacia had the highest levels of aluminum in bone by chemical determinations. Although the histochemical measurements of stainable bone aluminum varied among groups, the distribution of cases with surface stainable aluminum greater than 30% was substantially different (Table 4). None of the 28 patients with bone formation rates above the normal range had bone aluminum levels in excess of 30% by histochemical staining. In contrast, four of the nine patients with subnormal rates of bone formation had more than 30% surface stainable aluminum, $\chi^2 = 14.0$, df = 1, $P < 0.005$, and all patients with values for stainable aluminum above 30% had normal or reduced bone formation, $\chi^2 = 12.2$, df = 4, $P < 0.005$. Serum calcium concentrations, basal plasma aluminum levels and the increment in plasma aluminum after DFO were higher, whereas serum iPTH levels were lower in patients with more than 30% surface stainable aluminum (Table 5). Patients with more than 30% surface stainable aluminum had higher values for osteoid area and mineralization lag time, but resorption surface, tetracycline-labeled surface and bone formation were lower when compared to those with less than 30% surface stainable aluminum (Table 5).

Basal plasma aluminum levels correlated with the chemical measurements of bone aluminum content, $r = 0.59$, $P < 0.001$, and plasma aluminum concentrations were substantially higher in patients with osteomalacia (Table 3). For the entire study population, the increment in plasma aluminum after a single infusion of DFO correlated with the degree of aluminum accumulation in bone as assessed by chemical, $r = 0.73$, $P < 0.001$, and histochemical methods, $r = 0.73$, $P < 0.001$. Despite these findings, the increase in plasma aluminum following DFO infusions did not differ among the five groups (Table 3).

The bone aluminum content was higher in the 20 patients treated with aluminum hydroxide than in the 24 patients given calcium carbonate as a phosphate-binding agent (Table 6), and the finding of surface stainable aluminum was confined to patients who had received aluminum orally, $\chi^2 = 15.5$, df = 1, $P < 0.0001$. The rise in plasma aluminum after DFO infusions was also greater in patients who had ingested aluminum-containing antacids (Table 6). Serum iPTH levels were lower in this group despite serum calcium values that did not differ from those in patients receiving calcium carbonate. Conversely, the uncorrected values for serum alkaline phosphatase were higher in aluminum-treated patients (Table 6). The mean age of patients was similar in each group, but the duration of peritoneal dialysis was greater for those receiving aluminum gels.

Discussion

The distribution of histologic lesions of renal osteodystrophy and the predominance of secondary hyperparathyroidism in the current study is similar to that reported in adults receiving regular dialysis and in some studies of pediatric hemodialysis patients [1–3, 9, 10], but it differs substantially from other observations in uremic children [4, 6]. In the latter reports, osteomalacia and mixed lesions were prevalent findings. The use of calcitriol in the current study may account, in part, for

Table 6. Characteristics of 44 pediatric patients on CAPD/CCPD receiving calcium carbonate or aluminum containing gels, as phosphate-binding agents

	Calcium[a] $N = 24$	Aluminum[b] $N = 20$	P value[c]
Bone aluminum content *mg/kg dry wt*	7.8 ± 8.5	87.8 ± 69.8	<0.001
No. with surface stainable Al	0/24	10/20	<0.0001[d]
Increment in plasma Al after DFO *μg/liter*	44.5 ± 34.9	325 ± 299	<0.01
Plasma Al *μg/liter*	22 ± 15	141 ± 400	<0.005
Serum calcium *mg/dl*	9.8 ± 0.9	10.2 ± 0.9	NS
Serum alkaline phosphatase			
Uncorrected *IU/liter*	186 ± 112	402 ± 358	<0.05
Corrected %	146 ± 129	235 ± 192	NS
Serum iPTH *μlEq/ml*	387 ± 403	186 ± 132	<0.05
Age *yrs*	11.2 ± 6.2	12.5 ± 5.7	NS
Duration of CAPD/CCPD	16.7 ± 14.4	28.5 ± 12.0	<0.01

[a] Calcium carbonate
[b] Aluminum hydroxide/aluminum carbonate
[c] Probabilities estimated using the *t*-test for unpaired samples except as indicated
[d] Probability estimated by chi-square analysis
[e] Probability estimated by rank-sum test

this difference. In addition, previous reports in pediatric patients have not consistently used double tetracycline labeling of bone [6, 7, 9, 10] or histochemical assessments of bone aluminum content [4–10].

Sixty-four percent of patients in the current study demonstrated findings of secondary hyperparathyroidism despite treatment with calcitriol. The predominance of hyperparathyroid bone disease is consistent with data previously reported from this institution [26] and by others [27, 28]. Secondary hyperparathyroidism is difficult to control in children receiving peritoneal dialysis, and doses of calcitriol sufficient to raise serum calcium concentrations into the upper range of normal are often necessary to lower serum iPTH levels and to ameliorate the biochemical and roentgenographic features of osteitis fibrosa [16].

Histochemical evidence of aluminum deposition in bone was found in 23% of the patients, all of whom received aluminum hydroxide. The role of aluminum in the pathogenesis of renal osteodystrophy is a subject of continued interest. Twenty-five to 30% of asymptomatic adult hemodialysis patients were found to have aluminum-related bone disease [29–31], but the definition of this disorder is not yet established and diagnostic criteria vary substantially among investigators [29–32]. Dialysis patients with both osteomalacia and aplastic lesions often have surface stainable aluminum in bone [13], and Andress et al defined aluminum-related bone disease by the presence of subnormal rates of bone formation and more than 25% surface stainable aluminum [31]. Nevertheless, some dialysis patients have aluminum accumulation in bone both by chemical and by histochemical determinations, but lack the histologic features and low bone formation which suggest skeletal aluminum toxicity [2, 3, 13, 31, 33]. Consequently, the pathogenic role of aluminum in renal osteodystrophy has been questioned [34].

The data from the current cross-sectional study of pediatric patients receiving CAPD/CCPD indicate that aluminum adversely affects bone formation when values for histochemical surface stainable aluminum exceed 30%. None of the 28 patients with high rates of bone formation due to mild or advanced secondary hyperparathyroidism had more than 30% surface stainable aluminum; lesser values were occasionally seen, but 24 had a negative histochemical stain for aluminum. Conversely, six of 16 patients with normal or reduced rates of bone formation had >30% surface stainable aluminum, and bone formation varied inversely with total bone aluminum content. This was true both for the study group and for the 17 patients with osteitis fibrosa. Thus, aluminum deposition in bone may reduce mineralized bone formation in patients with persistently high serum levels of PTH and histologic evidence of PTH action on bone.

Based on the finding of surface stainable aluminum >30%, six patients from the current series were classified as aluminum-related bone disease; three had osteomalacia, one had aplastic bone, but two had normal bone histology and bone formation. Although the current results are consistent with previous data which indicate that the serum level of PTH is a major determinant of bone formation in patients receiving regular dialysis [35, 36], they also suggest that aluminum accumulation in bone can modify this relationship. Accordingly, bone formation may be inappropriately low regardless of the histologic findings in individual patients undergoing regular dialysis when there is substantial aluminum deposition in bone.

As noted by others [4, 6, 7, 10], serum biochemical measurements are poor predictors of bone histology in pediatric patients undergoing maintenance dialysis. Patients with osteitis fibrosa and osteomalacia both had increased serum levels of alkaline phosphatase, but there were no differences between groups. High basal plasma aluminum levels suggested substantial bone aluminum accumulation; values were greatest in patients with osteomalacia, but the increase in plasma aluminum after DFO infusions failed to distinguish among histologic subgroups. Of particular interest, serum iPTH levels were not reduced in children with renal osteomalacia. Although serum iPTH levels were somewhat lower in the six patients with >30% surface stainable aluminum, the results overlapped considerably with those for patients with <30% surface stainable aluminum. Thus, pediatric patients may be less susceptible than adults to the putative suppressive effect of aluminum on PTH secretion [37].

Several studies in adult patients undergoing maintenance dialysis suggest that low serum levels of PTH may contribute to the development of aluminum-related bone disease, particularly after parathyroidectomy [38–40]. However, only one of four patients in the current study who had undergone subtotal parathyroidectomy developed aluminum-related bone disease despite the continued ingestion of aluminum-containing gels. Serum iPTH levels also did not differ among the five histologic subgroups, and the values remained moderately elevated in all patients with histochemical evidence of bone aluminum deposition. Although these findings differ from observations using the same radioimmunoassay for PTH in adult patients with renal osteodystrophy [40], the data indicate that reductions in the serum level of PTH are not a prerequisite for the development of aluminum-related bone disease in pediatric patients receiving CAPD/CCPD.

Aluminum-containing, phosphate-binding agents were the apparent source of aluminum loading in the patients studied.

Bone aluminum levels were higher in those receiving aluminum hydroxide or aluminum carbonate, and all patients with a positive histochemical stain for aluminum had received these drugs. The mean serum iPTH levels were lower in patients given aluminum, but individual values did not distinguish aluminum-treated patients from those managed with calcium carbonate. Although use of calcium carbonate as a phosphate-binding agent reduces the potential for enteral aluminum loading in patients undergoing maintenance dialysis [15, 41], the long-term efficacy and safety of this agent has yet to be established [42].

In summary, renal osteodystrophy in pediatric patients undergoing CAPD/CCPD is a heterogeneous disorder not unlike the situation described in adults. Hyperparathyroidism remains the predominant skeletal lesion in dialyzed children despite the use of oral calcitriol. Aluminum accumulation in bone secondary to the use of aluminum containing gels is a prominent finding in most, but not all, cases of osteomalacia, and it may adversely affect bone formation in pediatric patients with other histologic lesions of renal osteodystrophy.

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