Fluoride and strontium accumulation in bone does not correlate with osteoid tissue in dialysis patients

Martine E. Cohen-Solal1, Françoise Augry1, Yves Mauras2, Caroline Morieux1, Pierre Allain2 and Marie-Christine de Vernejoul1

1INSERM U 349, Hôpital Lariboisière, Paris and 2Laboratoire de Pharmacologie et Toxicologie, Angers, France

Abstract

Background. Osteomalacia is now a rare disease in dialysis patients in developed countries since the withdrawal of aluminium overload. The involvement of fluoride and strontium in the pathogenesis of the disease has been suggested. The aim of this study was to investigate a possible association between osteomalacia in dialysis patients and the fluoride or strontium contents of bone.

Methods. Of 271 bone biopsies from chronic haemodialysis patients referred to our centre, we studied the nine biopsies from patients with osteomalacia. They were compared with 23 biopsies from patients with hyperparathyroidism and 24 biopsies from patients with adynamic bone disease. Histomorphometric static and dynamic indices were measured. Bone fluoride and strontium contents were measured in biopsies from haemodialysis patients, and were compared with those of control patients.

Results. In the nine patients with osteomalacia, we found an absence of double labelled surfaces and increased osteoid thickness. Mild aluminium overload was observed in two of the nine patients. The bone strontium content of the entire dialysis population studied was not significantly different from control values (0.023 ± 0.001 vs 0.019 ± 0.002% mol/mol, P = 0.15). However, bone strontium level was slightly but significantly increased in patients with osteomalacia (0.030 ± 0.005%), compared with both controls (0.019 ± 0.002%, P < 0.05) and the other bone diseases (0.021 ± 0.002%, P < 0.05). Bone fluoride content was significantly higher in the entire dialysis population than in the controls (0.33 ± 0.04 vs 0.13 ± 0.018% (g/g ash weight), P = 0.04). It was increased in osteomalacic patients compared with controls and with patients having hyperparathyroidism or adynamic bone disease. There was no correlation between formation indices (OV/BV, OS/BS, Ob.S/BS) and bone fluoride or strontium content.

Conclusions. We found a prevalence of osteomalacia of 3.3% in our biopsy series for chronic dialysis patients. However, although bone strontium and fluoride contents were slightly increased, no causal relationship with these individual metals and osteomalacia could be firmly established in this small number of patients. The hypothesis of strontium- or fluoride-induced osteomalacia in renal patients merits further investigation.

Keywords: dialysis; fluoride; osteoid; osteomalacia; strontium

Introduction

The course of renal osteodystrophy has changed with the use of calcium carbonate and vitamin D derivatives in the management of patients with severe renal failure. Therefore, the prevalence of the types of renal bone disease has been modified during the last decades. Histological data from dialysis patients has shown that hyperparathyroidism remains the most frequent disease, although its severity has decreased [1]. The occurrence of osteomalacia has been reduced by the exclusion of various sources of aluminium, leading to a prevalence of 4 to 5% [1]. However, although rare, this disease is still observed and is responsible for clinical manifestations such as bone pain and fractures.

Osteomalacia has multiple causes, including vitamin D deficiency, acidosis, uraemic toxins and the accumulation of aluminium in bone. Aluminium intoxication was previously the most frequent cause of osteomalacia in the dialysis population, but the involvement of several other metals in the pathogenesis of the disease has also been suggested. Many elements accumulate in the bones of patients with renal failure that is related to decreased renal elimination [2], some of which might be responsible for mineralization defects.
For example, fluoride and strontium are associated with a failure of mineralization when accumulated in high concentrations in animal models [3,4]. However, the involvement of each of these metals remains unclear in dialysis patients.

The aim of the present study was to determine the fluoride and strontium content of bone biopsies referred to our centre for the evaluation of dialysis osteodystrophy and to assess the possible association of each of these elements with mineralization defects.

**Subjects and methods**

Bone biopsies (n = 271) from chronic haemodialysis patients were referred to our laboratory from French haemodialysis centres between 1988 and 1996. This time period was chosen because aluminium intoxication has been disappearing from French centres since 1988, due to the decreased use of aluminium-containing phosphate-binders. We selected biopsies from patients who were exclusively dialysed in French centres, and discarded those who had been dialysed in North Africa even for a short period of time in the past. Most of our biopsies were referred from dialysis centres in the greater Paris area and from central France. We first selected the biopsies with patterns of osteomalacia based on the following criteria: osteoid thickness > 12 μm and bone formation rate (BFR) < 0.03 μm/day. We found nine histological osteomalacia cases among 271 biopsies (3.3%). In these patients, the mean age was 56 ± 4 years and the mean duration of dialysis was 8.8 ± 1.2 years. We also randomly selected two groups of patients among the 262 remaining who had had a bone biopsy during the same period. These two groups were composed of patients with biopsies having the following histological criteria.

Hyperparathyroidism: BFR > 0.08 μm/day; marrow fibrosis > 0.5%. There were 23 patients with a mean age of 61 ± 3 years and mean dialysis duration of 5.7 ± 0.9 years. Adynamic bone disease: BFR < 0.03 μm/day; osteoid thickness < 12 μm. There were 24 patients with a mean age of 52 ± 3 years and mean dialysis duration of 6.4 ± 1.4 years.

**Histomorphometry**

All patients received 1 g tetracycline orally on the 14th and 13th days, and then the 3rd and 2nd days, before biopsy for measurement of dynamic parameters. Transiliac bone biopsies were performed with a Bordier trephine. Bone specimens were fixed in methanol and embedded undecalcified in methylmethacrylate. Sections were cut with a polycut SM 2500S microtome (Leica, Rueil-Malmaison, France) as follows: 5 μm-thick sections stained with toluidine blue and aluminon for the measurements of static parameters; and 10 μm-thick unstained sections for the measurements of dynamic parameters. Quantitative parameters were measured using a semi-automatic image analyser (Biocom, Les Ulis, France) coupled with a Leitz microscope. The following trabecular parameters were measured and expressed according to the standardized nomenclature:

- **BV/TV**: bone volume as a percentage of bone volume, tissue volume referent;
- **OV/BV**: osteoid volume as percentage of bone volume, trabecular bone volume referent;
- **O.Th**: osteoid thickness (mean thickness of osteoid seam expressed in μm);
- **OS/BS**: osteoid surface area as a percentage of surface covered with osteoid, trabecular bone surface referent;
- **Ob.S/BS**: osteoblast surface as a percentage of bone surface covered with osteoblast, trabecular bone surface referent;
- **Oc.S/BS**: osteoclast surface as a percentage of bone surface covered with osteoclast, trabecular bone surface referent;
- **N.Oc/T.Ar**: osteoclast number expressed per mm² of tissue sections; and
- **Aluminium** was measured on undecalcified sections using Aluminon™ staining (Merck, Fontenay/bois, France), and was expressed as ALS/BS (percentage of aluminium surface covered with aluminium, trabecular bone surface referent).

The dynamic parameters of trabecular bone were as follows:

- **sLS/BS**: single-labelled surface area as a percentage of trabecular surface covered with one single label, trabecular bone surface referent;
- **dLS/BS**: double-labelled surface area as a percentage of trabecular surface covered with double label, trabecular bone surface referent;
- **MS/BS**: total length of labelled surfaces (sLS/BS + dLS/BS);
- **MAR**: mineral apposition rate expressed in μm/day;
- **BFR**: calculated as (sLS/BS + dLS/BS) × MAR, expressed as μm/day.


**Bone fluoride and strontium contents**

None of the dialysis patients were receiving fluoride or strontium as a medication. Bone fluoride, strontium and calcium contents were measured in all biopsies in 100 μm-thick sections. Sections were dehydrated, calcinated and the ashes were weighed and then dissolved in nitric acid. Calcium and strontium were measured in the ashes using an inductively coupled plasma optical emission spectrophotometry (ICP-OES) method as previously described by Mauras et al. [7]. Results are expressed as the ratio strontium: (strontium + calcium) (% mol/mol). Strontium normal values from 23 control patients with normal renal function are 0.019 ± 0.002% [Y. Mauras, unpublished data]. Fluoride content was determined as previously described [8]. Calcinated sections were incubated with perchlorid acid, and fluoride content was measured using a specific electrode. Results of fluoride bone content were expressed as percentages of ash weight (% g/g ash weight). The bone fluoride contents of 14 control subjects with normal renal function who participated in a trial [8] were 0.13 ± 0.01% of ash weight.

**Statistical analysis**

Results are expressed as mean ± SEM. Comparisons between groups were performed with ANOVAs, followed by Fisher PLSD tests. Correlations were tested by single regression analyses (Statistica Software, Abacus, USA).
Results

Mean age was not significantly different between the dialysis patients with osteomalacia, hyperparathyroidism and adynamic bone disease. Histomorphometric parameters of bone turnover are displayed in Table 1. In the nine patients with osteomalacia, cellular activity was maintained, and three patients (33%) also had secondary hyperparathyroidism, with an osteoclast number above the upper limit of the normal range. Their mean osteoblast surfaces and osteoclast numbers were respectively 41 and 82% lower than those of patients with hyperparathyroidism. Mineral apposition rate in osteomalacia was zero because there were no double-labelled surfaces. In contrast, the extent of single labelled surfaces was as high as in those patients with hyperparathyroidism, but the labelled surfaces were diffuse. The increased osteoid thickness and absence of double labelled surfaces confirm the mineralization defect without failure of osteoid apposition. Aluminium overload, as shown by aluminon staining, was moderate in patients with osteomalacia since aluminium-covered surfaces were above 25% in two patients and absent in six of the nine patients. In contrast, aluminium overload was higher in patients with adynamic bone disease, with aluminium surfaces above 25% in 16 out of the 24 patients. These data are different from previous reports by the Amiens group where aluminium was absent in patients with adynamic bone disease. In this study, patients were dialysed in the Paris area and central France and some of them were still receiving low doses of aluminium phosphate-binders.

Bone strontium content of the entire dialysis population was not significantly different from control values of patients with normal renal function (0.023 ± 0.001% vs 0.019 ± 0.002%, *P* = 0.15). However, bone strontium level was slightly but significantly increased in patients with osteomalacia (0.030 ± 0.005%), compared with either controls (0.019 ± 0.002%, *P* < 0.05), hyperparathyroidism patients (0.021 ± 0.001%, *P* < 0.05) or adynamic bone disease patients (0.021 ± 0.002%, *P* < 0.05), or the latter two combined (0.021 ± 0.002%, *n* = 47, *P* < 0.05 (Table 1, Figure 1).

On the other hand, bone fluoride content was significantly higher in the entire dialysis population than in controls (0.33 ± 0.04% vs 0.13 ± 0.018%, *P* = 0.04). Bone fluoride levels were increased in osteomalacic patients (0.57 ± 0.1%) compared with normal controls (0.13 ± 0.01%, *P* < 0.05), with patients with hyperparathyroidism (0.34 ± 0.09%, *P* < 0.05), those with adynamic bone disease (0.22 ± 0.03%, *P* < 0.05) or the latter two combined (0.28 ± 0.04%, *n* = 47, *P* < 0.05 (Table 1, Figure 1). The highest values were found in patients with osteomalacia with six out of nine having values above 0.5%.

There were no correlations between bone formation indices (OV/BV, OS/BS, Ob.S/BS) and bone fluoride or strontium contents. Osteoid thickness was neither correlated with strontium content (*P* = 0.30), nor with fluoride content (*P* = 0.31) (Figure 2). There were no correlations between bone fluoride and strontium contents either in the entire population or in osteomalacic patients.

Discussion

The low prevalence of osteomalacia in our biopsy series (3.3%) is similar to those reported by other centres. Some authors [1] reported a prevalence of 3–5% in studies in which biopsies were performed for diagnostic purposes in dialysis patients. Bone biopsies were referred to our centre for the evaluation of renal osteodystrophy, mainly because of the presence of bone pain, or for the assessment of aluminium

Table 1. Histomorphometric data according to the bone diseases

<table>
<thead>
<tr>
<th></th>
<th>Osteomalacia</th>
<th>Hyperparathyroidism</th>
<th>Adynamic bone disease</th>
<th>Normal values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>n</em> = 9</td>
<td><em>n</em> = 23</td>
<td><em>n</em> = 24</td>
<td></td>
</tr>
<tr>
<td>BV/TV (%)</td>
<td>26.1 ± 3.8</td>
<td>24.6 ± 2.2</td>
<td>17.7 ± 1.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>18.5 ± 3.5</td>
</tr>
<tr>
<td>O.V/BV (%)</td>
<td>24.1 ± 4.1</td>
<td>11.9 ± 1.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.5 ± 0.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2 ± 1</td>
</tr>
<tr>
<td>O.Th (µm)</td>
<td>23.5 ± 3.1</td>
<td>11.5 ± 0.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.5 ± 0.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.5 ± 0.7</td>
</tr>
<tr>
<td>OS/BS (%)</td>
<td>75.9 ± 5.8</td>
<td>62.1 ± 3.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>39.1 ± 2.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>17.9 ± 1.2</td>
</tr>
<tr>
<td>Oc.S/BS (%)</td>
<td>7.1 ± 3.2</td>
<td>17.3 ± 0.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.5 ± 0.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.4 ± 1.9</td>
</tr>
<tr>
<td>Oc.S/BS (%)</td>
<td>0.6 ± 0.21</td>
<td>4.4 ± 0.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.34 ± 0.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.69 ± 0.73</td>
</tr>
<tr>
<td>N.Oc.T.Ar (mm&lt;sup&gt;–2&lt;/sup&gt;)</td>
<td>0.58 ± 0.16</td>
<td>3.06 ± 1.92&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.24 ± 0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.79 ± 0.37</td>
</tr>
<tr>
<td>MAR (µm/day)</td>
<td>0</td>
<td>0.75 ± 0.10</td>
<td>0.13 ± 0.06</td>
<td>0.63 ± 0.17</td>
</tr>
<tr>
<td>sLS/BS (%)</td>
<td>18.2 ± 4.4</td>
<td>18.4 ± 1.7</td>
<td>7.1 ± 4.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>–</td>
</tr>
<tr>
<td>dLS/BS (%)</td>
<td>0&lt;sup&gt;+&lt;/sup&gt;</td>
<td>13.4 ± 2.4</td>
<td>0.24 ± 0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.2 ± 1.2</td>
</tr>
<tr>
<td>MS/BS (%)</td>
<td>18.2 ± 4.4</td>
<td>31.9 ± 3.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.2 ± 1.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.2 ± 1.3</td>
</tr>
<tr>
<td>BFR (µm/day)</td>
<td>0</td>
<td>0.263 ± 0.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.016 ± 0.006&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.089 ± 0.022</td>
</tr>
<tr>
<td>ALS/BS (%)</td>
<td>20.5 ± 9.6</td>
<td>10.5 ± 5.1</td>
<td>52.8 ± 7.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0</td>
</tr>
<tr>
<td>Fluoride content (% ash weight)</td>
<td>0.57 ± 0.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.34 ± 0.09&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>0.22 ± 0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.13 ± 0.01</td>
</tr>
<tr>
<td>Strontium content (% mol/mol)</td>
<td>0.030 ± 0.005&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.021 ± 0.001&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.021 ± 0.002&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.019 ± 0.002&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Histomorphometric parameters were measured on undecalciﬁed bone sections. Bone fluoride and strontium contents were measured as described in the Subjects and methods section. Data are expressed as mean ± SEM.

<sup>a</sup>Different from hyperparathyroidism, *P* < 0.05; <sup>b</sup>different from osteomalacia, *P* < 0.05; <sup>c</sup>different from controls, *P* < 0.05.
intoxication before parathyroidectomy. Although this low prevalence may be related to the design of the study and does not necessarily reflect the prevalence of the disease in the entire haemodialysis population, these data nevertheless confirm that osteomalacia is now a rare disease. Causes of osteomalacia may be numerous. Vitamin D insufficiency appears to be rare in France but may be observed in other countries. Indeed, a recent study [9] has reported a high prevalence of Looser–Milkman zones in patients dialysed in Algeria who also had low 25 OH vitamin D levels.

A high metal burden has been suggested as a cause of mineralization defects. Aluminium overload, which was a frequent cause of osteomalacia, has tended to decrease in both Europe and the United States since 1985 [1]. In the present biopsy series, the aluminium burden was moderate in patients with osteomalacia or hyperparathyroidism, whereas it was high in patients with adynamic bone disease.

Since 1982, it has been shown that renal failure is a condition that favours metal accumulation in bone as well as in other tissues. Metals are retained because...
of the reduced ability to eliminate them through renal excretion and dialysis. In dialysis patients, several metals such as chromium, zinc, tin and strontium were found to be increased in bone, regardless of the water treatment and the country [2]. In previous studies, aluminium and strontium contents were higher in patients with osteomalacia, but all metal concentrations were correlated with each other, suggesting that the development of mineralization defects may not be attributed to a single metal but may be due to the deposition of several metals. Strontium is incorporated and retained in newly-formed mineralized bone and its concentration is related to cumulative exposure and dosage [10,11]. As with many other metals, strontium mineralization is altered at high doses only. In osteopenic rats, controlled doses of strontium ranelate or strontium carbonate prevent trabecular bone loss with histological osteomalacia (0.92 ± 3), and the bone strontium content of ten osteomalacic patients was three times higher than in control subjects. In our series, bone strontium was only 1.5-fold higher in patients with osteomalacia than in controls or in patients with the other types of renal osteodystrophy. We found no correlation between osteoid accumulation and bone strontium content. Therefore, strontium seems unlikely to be responsible for osteomalacia in the present patient series. However, the small number of patients with osteomalacia in this study does not allow a firm conclusion to be drawn. Moreover, the presence of other metals or trace elements in the protein matrix could interfere with bone mineralization and may be involved in delayed mineralization in dialysis patients [2]. Another possible explanation for the increased strontium content could be that patients with osteomalacia had received higher doses of vitamin D, since calcitriol may increase intestinal strontium absorption [15,16]. Although most of these patients did indeed receive vitamin D derivatives, precise information on their administration has unfortunately not been available for this study.

As fluoride may accumulate in uraemic patients because of their decreased renal excretion, serum fluoride levels were found to be higher in dialysis patients than in healthy controls [17]. The role of fluoride in mineralization defects has been shown in rats consuming low concentration of fluoridated water, with the amount of osteoid being related to serum fluoride levels [4]. There are also a few reports of fluorosis in patients with moderate renal failure who were treated with fluoride for osteoporosis [18,19], but there are no previous reports of bone fluoride levels in dialysis patients. In our series, bone fluoride contents were higher in patients with osteomalacia than in controls, but were similar to levels found in patients with normal renal function who were treated for osteoporosis [8]. In osteoporosis patients treated with sodium fluoride, bone fluoride content increased in a dose-dependent manner and reaches 0.45% after a 4-year treatment, whereas levels varied from 0.60 to 1.33% in patients with fluorosis [20]. Bone fluoride levels were above 0.5%, in six out of nine osteomalacic patients, but in our bone biopsies with osteomalacia we observed no peristoeocytes mottled osteoid and extended intra-trabecular mineralization defects. This is in contrast to the histological features of endemic fluorosis. Thus, the causal role of fluoride overload in mineralization defects in our patients is not fully demonstrated as there were no mineralization defects specific to fluorosis. Another hypothesis is that fluoride might accumulate passively in osteoid seams, since the fluoride contents were higher in both hyperparathyroidism and osteomalacia patients compared with controls. A possible source of fluoride is drinking water. In this series, patients were almost certainly not drinking fluoride-containing water since they were treated by dialysis, although most of them were likely to have been drinking fluoridated water during their pre-dialysis periods.

In conclusion, osteomalacia has become a rare disease in the dialysis population of developed countries, since we found a prevalence of only 3.3%. The patients under study had slightly elevated bone strontium and fluoride contents compared with controls. The increase in bone strontium and fluoride levels was moderate and no relationship between the bone metal contents and bone formation indices was observed. Given the small number of patients studied, these data do not allow a firm causal relationship between metal overload and mineralization defects to be concluded. However, the hypothesis of strontium- or fluoride-induced osteomalacia in renal patients deserves further investigation.

Acknowledgement. The authors are grateful to Dr Pierre Marie for reviewing the manuscript and for critical advice.

References


Received for publication: 26.7.01
Accepted in revised form: 2.11.01