High frequency of iron bone deposits in a Mexican population with renal osteodystrophy

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Abstract Renal osteodystrophy (ROD) is a multifactorial disease. Aluminium deposits have been implicated in its physiopathology but iron deposits have seldom been described. The purpose of this study was to investigate the presence of iron on the mineralization front, in 70 patients with ROD. Their mean age was 48 ± 16 years, 36 were female, 34 male, 55 were admitted on peritoneal dialysis (78.5%) and 15 to haemodialysis (21.5%), for a period of 28 ± 22 months. A bone biopsy was obtained from each patient after double tetracycline labelling. Blood samples were also obtained at the time of bone biopsy. The histomorphometric analysis was performed following the criteria of Sherrard et al., with slight modifications; beside the usual stains, aluminium, iron and amyloid stainings were done on all bone specimens. Biochemical findings were: Ca 8.8 ± 0.9 mg/dl, P 6.1 ± 1.5 mg/dl; total alkaline phosphatase 197 ± 258; PTHm 4.9 ± 0.45 ng/ml (normal 0.4–0.7 ng/ml), calcitonin 11 ± 6 pg/ml (normal 1–26 pg/ml). Osteitis fibrosa was found in 31 patients (44.28%), mixed bone disease in two patients (2.28%); mild bone disease in 20 subjects (28.57%), adynamic bone lesion in 15 cases (21.42%) and osteomalacia in two patients (2.28%). Iron deposits were found on the mineralization front in 43 patients (61.4%); in 17, the percentage was < 25 and, in 26, > 25%. The iron deposits in the osteitis fibrosa group were highly significant (25/31). The aluminium deposit at the mineralization front was observed in eight patients (11.4%); in all but one, the percentage of this metal was < 10%. Amyloid deposits were negative in all cases. The results show: (i) a Mexican population with ROD, present a highly significant incidence of siderosis on the bone mineralization front; (ii) in contrast, the aluminium deposits in this group of patients is lower than that reported in other series, and (iii) the spectrum of RO in this Mexican population is similar to that reported in other studies.

Key words: aluminium bone deposits; bone iron overload; iron bone deposits; renal osteodystrophy

Introduction

In the last two decades, aluminium bone deposits have been widely studied in renal osteodystrophy, particularly in patients undergoing haemodialysis [1–3]. Aluminium has been implicated in the genesis of encephalopathy, low bone turnover, hypoparathyroidism, anaemia, osteomalacia and adynamic bone disease in dialysis subjects [4–11]. Although the role of aluminium is beyond question, iron has been less studied, but has occasionally been reported to co-exist with aluminium on the bone mineralization front, and some investigators have proposed that it may have similar toxic effects [12,13]. Recently, other trace elements have been investigated, but their role, particularly the issue of whether clinical disturbances result from accumulation (toxicity) or depletion (deficiency) has remained unclear. Histochemically we frequently have seen iron deposits in the mineralization front in our bone biopsy studies [14,15]. The purpose of this study was to investigate the presence of iron in the mineralization front in patients with renal osteodystrophy.

Subjects and methods

A prospective cross-sectional study was performed in 70 patients, 34 males and 36 women with chronic renal failure (CRF) undergoing dialysis. The mean age was 48 ± 16 years. Fifty five patients were on chronic peritoneal dialysis (CAPD) and 15 on haemodialysis (HD) for a mean period of 28 ± 22 months. A bone biopsy was obtained after double tetracycline labelling in all patients. In the same week, blood samples were studied in order to investigate biochemical markers of bone remodelling.
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Laboratory measurements

Serum calcium was determined with atomic absorption (Perkin Elmer); serum creatinine, urea, phosphorus, total alkaline phosphatase and albumin were quantified with a computerized Technicon Autoanalyzer. The serum concentration of calcitonin and parathyroid hormone (PTH) was estimated with a middle molecule radioimmunoassay (INCSTAR kit; normal range 0.32–0.65 ng/ml).

Bone histomorphometry

After double tetracycline labelling with a 10 day interval between doses, full thickness bone biopsy was obtained from the iliac bone. The non-decalcified bone specimen was fixed with ethanol and embedded in methylnmethallylate and sectioned for histomorphological examination. Bone samples were stained with Goldner trirome, aluminium according to a modification of the method of Maloney et al. method [16], iron with Perl’s method [17] and amyloid with Bennhol’s congo red [18]. We stained all bone sections for aluminium and iron using a control obtained from the iliac bone of rats loaded with both metals. The amounts of iron and aluminium were estimated on the bone spicula peripheral surface with the Merz–Schenk reticle in a minimum of 25 fields. The percentage of the metal present on the surface of the spicula was calculated, multiplying by 100 the number of times that the waves of the reticle made contact with the metal, and the value obtained was divided by the total sum of the number of times that the waves of the reticle contact or touch the peripheral surface of the spicula.

All histomorphometric analyses were done with the aid of a light microscope with a Merz–Schenk reticle [19] and an eyepiece micrometer. Measurement of cancellous bone was divided into the following normal index values.

Static index

The percentage area of osteoid (OAr: 3.19 ± 0.82%); percentage area of fibrosis (FbAr: 0.32 ± 0.31%); percentage area of mineralized bone (MbAr: 21.03 ± 3.36%); percentage surface of osteoblasts (ObS: 5.40 ± 1.30%); and percentage surface of osteoclasts (OcS: 1.40 ± 0.72%) were measured.

Dynamic index

The mineralized appositioned rate (MAR: 1110 ± 170 μm/day); and bone formation rate (BFR: 1275 ± 168 μm²/mm²/day expresse per unit area of existing trabecular bone) were determined. The normal bone biopsy indexes were obtained in bone specimens taken from 10 normal subjects of the same age and body weight and studied with the same methodology in our laboratory.

Biopsies were classified according to Sherrard et al. [20] and the results are summarized in Table 1.

The five groups were defined quantitatively as: osteitis fibrosa when the percentage of total bone area of osteoid was <12 and when the percentage of total bone area of fibrosis was >0.5; mild when the percentage of total bone area of osteoid was >12 and when the percentage of total bone area of fibrosis was >0.5; mild when the percentage of total bone area of osteoid was >12 and when the percentage of total bone area of fibrosis was <0.5; mixed when the percentage of total bone area of osteoid was <12 with the percentage of total bone area of fibrosis >0.5 and bone formation rate >1100 μm²/mm²/day; adynamic when the percentage of total bone area of osteoid was <12 with the percentage of total bone area of fibrosis <0.5 and bone formation rate <1100 μm²/mm²/day; and osteomalacia when the percentage of total bone area of osteoid was >12 and the percentage of total bone area of fibrosis was <0.5. The 10 normal subjects were submitted to surgery for monostotic benign bone dysplasias. For our histomorphometric data, the nomenclature of the ASBMR histomorphometry committee was followed [21,22]. The study was approved by each Institutional Research Ethic Committee and informed consent was obtained from each patient.

Statistical analysis

All values are expressed as mean ± standard deviation of the mean. The non-paired Student’s t-test was utilized to analyse the differences between the static and dynamic indexes and the normal bone biopsies. The χ² test was used to analyse differences between percentages. P values <0.05 were considered significant.

Results

All patients included in this study were in end-stage chronic renal failure. The causes of the renal insufficiency were: eight, with membranoproliferative glomerulonephritis; two, with membranous glomerulopathy; six, with tubulointerstitial nephritis; seven, with obstructive uropathy; four, with polycystic kidney; five, with diabetes mellitus; two with lupus nephritis; one with polycystosis; and 35 with end-stage renal disease. Table 2 depicts the patient’s demographic data. Of

<table>
<thead>
<tr>
<th>Lesion</th>
<th>Area of osteoid % of total bone</th>
<th>Area of fibrosis % of total bone</th>
<th>Spicula area bone formation rate (μm²/mm²/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osteitis fibrosa</td>
<td>&lt;12</td>
<td>&gt;0.5</td>
<td>X</td>
</tr>
<tr>
<td>Mixed</td>
<td>&gt;12</td>
<td>&gt;0.5</td>
<td>X</td>
</tr>
<tr>
<td>Mild</td>
<td>&lt;12</td>
<td>&lt;0.5</td>
<td>&gt;1100</td>
</tr>
<tr>
<td>Adynamic</td>
<td>&lt;12</td>
<td>&lt;0.5</td>
<td>&lt;1100</td>
</tr>
<tr>
<td>Osteomalacia</td>
<td>&gt;12</td>
<td>&lt;0.5</td>
<td>X</td>
</tr>
<tr>
<td>Normal range</td>
<td>&gt;3 or &lt;5</td>
<td>&gt;0.3</td>
<td>&gt;1100 or &lt;1450</td>
</tr>
</tbody>
</table>

X= is not a diagnostica criteria.

Taken and modified from Sherrard et al. [20].
Table 2. Patients demographic data

<table>
<thead>
<tr>
<th>No.</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>34</td>
<td>36</td>
</tr>
<tr>
<td>Age (years)</td>
<td>48 ± 16</td>
<td>28 ± 22</td>
</tr>
<tr>
<td>Time on dialysis (months)</td>
<td>55 (76.5%)</td>
<td>15 (21.5%)</td>
</tr>
<tr>
<td>Peritoneal dialysis patients</td>
<td>55 (76.5%)</td>
<td>15 (21.5%)</td>
</tr>
<tr>
<td>Hemodialysis patients</td>
<td>55 (76.5%)</td>
<td>15 (21.5%)</td>
</tr>
</tbody>
</table>

The 70 patients, 34 were male, 36 female, and the mean age was 48 ± 16 years. All the patients were in dialysis, 55 on peritoneal dialysis (PD) and 15 on haemodialysis (HD), for a period of 28 ± 22 months before the bone biopsy was performed. Table 3 depicts the biochemical data. The mean serum values were: calcium 8.8 ± 0.9 mg/dl, phosphorus 6.1 ± 1.5 mg/dl, total alkaline phosphatase 197 ± 258 IU (Ref. 50–112), PTHm 4.9 ± 4.05 pg/ml (Ref. 0.4–0.7) and calcitonin 11.6 ± 5.4 pg/ml (Ref. 1–26).

Table 3. Serum biochemical values

| Ca mg/dl | 8.8 ± 0.9 |
| P mg/dl | 6.1 ± 1.5 |
| AlkPh IU | 197 ± 258 (ref. 50–112) |
| PTHm ng/ml | 4.9 ± 4.05 (ref. 0.4–0.7) |
| Calcitonin pg/ml | 11.6 ± 5.4 (ref. 1–26) |

The quantitative histomorphometric results are depicted in Table 4 and the distribution of the lesions can be observed in Figure 1. The most common lesion was osteitis fibrosa in 31 patients (44.28%); mild osteodystrophy was observed in 20 patients (28.57%); adynamic bone lesion in 15 patients (21.42%); and mixed and osteomalacia were observed in two subjects respectively (2.28%).

The quantitative percentages of iron and aluminium distribution on the bone mineralization front were particularly noteworthy. As we can see in Table 5 and Figure 2, 43 patients had iron deposits on the bone mineralization front (61.4%). All patients but one had aluminium deposits on <10% of the bone mineralization front surface, and all of them had also iron deposits (Figure 2). When we compared the presence of iron deposits with the different osteodystrophy lesions, we found a significantly (P<0.05) greater incidence of this metal (25/31) in the osteitis fibrosa group (Table 5). Amyloid deposits were negative in all patients.

Discussion

Our results showed iron deposits on the bone mineralization front in 61.4% of 70 patients with renal osteodystrophy. This number is highly significant when compared with the findings from other series [10,20,23,24]. We reported recently iron deposits at the mineralization front of the bone biopsies from 10/16 patients that have received kidney transplants and had remained with normal renal function after a mean post-graft time of 84 months. Our major question is the source of the iron. The causes of this issue are difficult to explain in our patients. According to the information of the city Health Service Department, the amounts of iron, aluminium and other trace metals in the domestic water supply are within an acceptable range. For many decades, Perl’s stain has been used widely in order to identify iron deposits, and no false-positive results using this histochemical technique have been known. Gokal et al. [25] studied the iron absorption in patients undergoing haemodialysis therapy using 59Fe and a total body counter. They found that iron absorption in fasting maintenance haemodialysis patients was normal, and the mechanisms relating

Table 4. Bone biopsy results

<table>
<thead>
<tr>
<th>Lesion</th>
<th>N</th>
<th>OAr (%)</th>
<th>FbAr (%)</th>
<th>MdAr (%)</th>
<th>ObS (%)</th>
<th>OcS (%)</th>
<th>MAR (µ/µg/day)</th>
<th>BFR (µ²/mm²/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osteitis fibrosa</td>
<td>31</td>
<td>3.61 ± 2.1</td>
<td>1.22 ± 4.1</td>
<td>17.22 ± 7.4</td>
<td>9.94 ± 4.7</td>
<td>4.54 ± 4.6</td>
<td>1.24 ± 0.5</td>
<td>1.389 ± 1.269</td>
</tr>
<tr>
<td>Mixed</td>
<td>2</td>
<td>17.00 ± 6.2</td>
<td>2.67 ± 3.0</td>
<td>7.17 ± 0.5</td>
<td>11.26 ± 4.0</td>
<td>5.84 ± 6.8</td>
<td>1.65 ± 0.3</td>
<td>1.718 ± 1.296</td>
</tr>
<tr>
<td>Mild</td>
<td>20</td>
<td>2.77 ± 2.3</td>
<td>0.11 ± 0.1</td>
<td>13.69 ± 5.1</td>
<td>8.15 ± 4.3</td>
<td>2.15 ± 2.7</td>
<td>1.32 ± 0.8</td>
<td>2.906 ± 2.017</td>
</tr>
<tr>
<td>Adynamic</td>
<td>15</td>
<td>3.50 ± 2.0</td>
<td>0.11 ± 0.1</td>
<td>15.80 ± 6.4</td>
<td>6.66 ± 2.4</td>
<td>1.33 ± 1.6</td>
<td>0.89 ± 0.6</td>
<td>6.82 ± 2.791</td>
</tr>
<tr>
<td>Osteomalacia</td>
<td>2</td>
<td>12.39 ± 0.2</td>
<td>0.22 ± 0.3</td>
<td>15.22 ± 1.5</td>
<td>6.02 ± 0.8</td>
<td>1.80 ± 1.6</td>
<td>1.41 ± 0.7</td>
<td>1.363 ± 8.125</td>
</tr>
<tr>
<td>Normal</td>
<td>10</td>
<td>3.19 ± 0.8</td>
<td>0.32 ± 0.3</td>
<td>21.03 ± 3.3</td>
<td>5.40 ± 1.3</td>
<td>1.40 ± 0.7</td>
<td>1.10 ± 0.170</td>
<td>1.275 ± 1.680</td>
</tr>
</tbody>
</table>

The nomenclature is from [21,22] N, number of patients; % area, the percentage of tissue area that is occupied by osteoid (OAr), fibrosis (FbAr) and mineralized bone (MdAr); % surface, the percentage of the peripheral spicule surface occupied by osteoblasts (ObS) and osteoclasts (OcS); MAR, mineral apposition rate, is the average width of mineral deposited in bone-forming sites per day; BFR, bone formation rate, is the amount of mineral deposited in µg per mm² of tissue area per day; normal, 10 normal subjects are used as reference from our laboratory.
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Fig. 1. Spectrum of bone lesions in a Mexican population with renal osteodystrophy.

Table 5. Percentage of iron deposits at the bone mineralization front

<table>
<thead>
<tr>
<th>%</th>
<th>OF</th>
<th>Mixed</th>
<th>Mild</th>
<th>Ady</th>
<th>OM</th>
<th>Total %</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;25</td>
<td>6/19</td>
<td>0/1</td>
<td>6/2</td>
<td>4/3</td>
<td>1/2</td>
<td>17/25/60/6411/7</td>
</tr>
<tr>
<td>&gt;25</td>
<td>19/25</td>
<td>1/1</td>
<td>2/2</td>
<td>3/3</td>
<td>1/1</td>
<td>17/26/70/70/70/70</td>
</tr>
</tbody>
</table>

%, percentage of iron deposits; OF, osteitis fibrosa; mixed, mixed osteodystrophy; mild, mild osteodystrophy; Ady, adynamic osteodystrophy; OM, osteomalacia.

*P < 0.05 vs all the other lesions.

The presence of aluminium deposits on the mineralization front of our patients was significantly low (P < 0.05) compared with those reported in other series [10, 20, 23, 24].

Cannata et al. [27, 28] have suggested that intestinal aluminium absorption occurs via iron-specific, transferrin-dependent pathways and that iron deficiency enhances the fractional absorption of aluminium. This could partially explain the significantly low frequency of aluminium deposits in our patients on the bone mineralization front. However, recently, Ittel et al. [29] raised doubts about this point of view. They mentioned that the absorption of neither iron nor aluminium is mediated via transferrin receptors, but seems to be due to increased paracellular permeability of the intestine in uraemic rats. Nevertheless, the results obtained in this study showed high iron deposits and low aluminium deposits on the mineralization front.

The bone iron deposits were observed in the majority of the different osteodystrophy diseases. Furthermore, it was observed that the osteitis fibrosa group contains a high incidence of iron deposits when compared with the other renal osteodystrophies. The high incidence of iron on the bone mineralization front of our patients with renal osteodystrophy encouraged us to investigate the causes of this issue.

The histomorphometric spectrum of the different renal osteodystrophies was similar to the majority of the previous recent series (Figure 1). Furthermore, when we compared our data with those reported a

Fig. 2. Percentage of metal deposit on the bone mineralization front in a Mexican population with renal osteodystrophy.
decade ago, we found a decrease in the incidence of osteomalacia (2.28%) and a significant increase in the incidence of adynamic bone lesion (21.42%) [10,30–32]. The difference between our classification and that of Scherrard et al. [20] is due to the use of a minor limit of the percentage of osteoid area of the total bone in our studies, as we believe that four times from our normal reference, is enough for this appreciation. On the other hand, our data on bone formation rate are augmented because they are expressing per unit area of existing trabecular bone and not per unit area of tissue.

In conclusion, the results of this study show: (i) a Mexican population with renal osteodystrophy presents a highly significant incidence of siderosis on the bone mineralization front; (ii) in contrast, the aluminum deposits in this group of patients is lower than that reported in other studies, and (iii) the spectrum of renal osteodystrophy in the Mexican population is similar to that reported in other studies.

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References