Bone Mineral Density, Bone Turnover Markers and Cytokines in Alcohol-Induced Cirrhosis

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INTRODUCTION

Osteoarticular alterations as extrahepatic manifestations of chronic liver disease (CLD) are well known (Diamond et al., 1990; Gallego-Rojo et al., 1998; Rouillard and Lane, 2001; Pares and Guanabens, 2008). Cirrhosis is considered to be a risk factor for osteoporosis. Nevertheless, the pathogenesis of bone mass loss in AC is still unknown (Giouleme et al., 2006). The prevalence of osteoporosis in CLD can reach up to 50% (Bonkovsky et al., 1990; Gallego-Rojo et al., 1998). Several factors are related to the pathogenesis of osteoporosis in cirrhotic patients, i.e. physical inactivity, smoking, malnutrition, vitamin D deficiency or the use of corticoids could affect mineral metabolism in CLD patients (Schiefke et al., 2005; Collier, 2007).

The pathogenesis of bone mass loss in chronic alcoholic subjects without liver disease seems to be related, at least in part, to the reduced osteoblastic activity associated with chronic alcoholism (Garcia-Sanchez et al., 1995; Gonzalez-Calvin et al., 1999; Maran et al., 2001).

In AC patients, other factors related to liver disease could participate apart from the toxic effect of alcohol (Kimble 1997; Santolario et al., 2000). Several evidences show that immune alterations and an increase in production of cytokines are related to the severity of CLD (Neuman, 2003; Garcia-Ruiz et al., 2004; Feng, 2005; Leevy and Elbeshbeshy, 2005; Garcia-Valdecasas et al., 2006). These changes could be involved in other systemic manifestations of liver cirrhosis such as bone mass loss of these patients.

Therefore, we determined serum concentrations of soluble tumour necrosis factor receptor (sTNF-R55), soluble interleukin 2 receptor (sIL-2R) and macrophage product neopterin to study the possible role of immune changes on the pathogenesis of osteoporosis and bone turnover markers in patients with AC.

METHODS

Thirty-three consecutive males outpatients were studied, with a mean age of 53.1 years (SD: 9.59 years); 26 of them were diagnosed by liver biopsy. Patients were not active drinkers for the last 6 months at the moment of the study. Previous alcohol consumption was in a range from 60 to 200 g/day for at least 10 years. The diagnosis of liver cirrhosis was based on liver biopsy in 28 patients; in the remaining four patients, diagnosis was based on the association of clinical, analytical and image data. Patients with malignancy, endocrine, cardiac, immune system or bone disease were excluded. Also, those patients under treatment with corticosteroids, and other medications affecting the immune system or mineral metabolism, were excluded.

Subjects

Thirty-three consecutive males outpatients were studied, with a mean age of 53.1 years (SD: 9.59 years); 26 of them were diagnosed by liver biopsy. Patients were not active drinkers for the last 6 months at the moment of the study. Previous alcohol consumption was in a range from 60 to 200 g/day for at least 10 years. The diagnosis of liver cirrhosis was based on liver biopsy in 28 patients; in the remaining four patients, diagnosis was based on the association of clinical, analytical and image data. Patients with malignancy, endocrine, cardiac, immune system or bone disease were excluded. Also, those patients under treatment with corticosteroids, and other medications affecting the immune system or mineral metabolism, were excluded.

Bone mineral density measurements

Bone mineral density (BMD) was measured in all patients in lumbar spine (LS) and femoral neck (FN) by X-ray absorptio-
metry (Hologic QDR-1500, S/N 1535; Hologic Corp., Waltham, MA, USA). Osteopaenia and osteoporosis have been defined according to the World Health Organization criteria depending on the BMD values (Kanis, 2000).

**Laboratory analyses**

Serum concentrations of sTNF-R55 and sIL-2R were measured by enzyme immune analysis (T Cell Sciences, Cambridge, MA, USA). Neopterin concentrations were measured by the neopterin RIAAcid kit (BRAHMS, Hennigsdorf, Germany). In addition, serum concentrations of insulin-like growth factor I (IGF-I; Nichols Institute Diagnostics, San Juan Capistrano, CA, USA), BGP-osteocalcin and intact PTH (Incstar Corporation, Cat 22280, Stillwater, MN, USA), sexual steroids binding globulin (SHBG; Farmos SF 90460, Oulunsalo, Finland) and 25-hydroxyvitamin D (25OHD; RIA, Incstar) were measured by radioimmunoassay.

Urinary free deoxypyridinoline (D-Pyr) was measured by enzyme immune analysis (Pyrilinks; Metra Biosystems Inc., Santa Clara, CA, USA) according to a previously described method (Delmas et al., 1993). Urinary excretion was expressed as the ratio of D-Pyr to creatinine.

**Statistical analysis**

Statistical analyses were performed using the programme SPSS 15.1 for Windows. All results were expressed as the mean ± SEM. Normal distribution of values in controls and cirrhotic patients were verified with the Kolmogorov–Smirnov test. The mean values in different groups were compared by one-way ANOVA and unpaired two-tailed t-tests or non-parametric Mann–Whitney U-test as appropriate. A one-sample t-test was used to assess the differences between the mean BMD z-score at each site and zero. Correlation studies were performed with Pearson standard linear regression analysis (normal distribution) or the Spearman test (non-normal distribution). Risk factors for the development of osteoporosis were analysed by backward stepwise regression analysis. Variables entered into the model included the patient’s age, Child score, free testosterone index and serum values of 25OHD, IGF-I, PTH, sTNF-R55, sIL-2R and neopterin.

P-values <0.05 were regarded to indicate significant differences between groups or correlations between variables tested.

**RESULTS**

**Table 1** shows biochemical data. Data of the main variables of this study are summarized in **Table 2**.

**Table 1.** Biochemical data in control subjects and alcoholic cirrhotic patients

<table>
<thead>
<tr>
<th>Biochemistry</th>
<th>Controls (n=24)</th>
<th>Viral cirrhosis (n=33)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum albumin (g/dl)</td>
<td>4.50±0.95</td>
<td>3.73±0.12*</td>
</tr>
<tr>
<td>Serum bilirubin (mg/dl)</td>
<td>0.86±0.08</td>
<td>2.14±0.23*</td>
</tr>
<tr>
<td>Prothrombin activity (%)</td>
<td>99.83±0.14</td>
<td>66.97±2.51*</td>
</tr>
<tr>
<td>Serum alkaline phosphatase (U/l)</td>
<td>150.67±5.03</td>
<td>282.91±17.22*</td>
</tr>
<tr>
<td>Serum aspartate transaminase (U/l)</td>
<td>22.96±0.94</td>
<td>56.91±4.97*</td>
</tr>
<tr>
<td>Serum alanine transaminase (U/l)</td>
<td>26.08±2.05</td>
<td>40.00±4.09**</td>
</tr>
<tr>
<td>Serum glutamyltransferase (U/l)</td>
<td>31.83±2.58</td>
<td>130.88±18.05*</td>
</tr>
</tbody>
</table>

Values are the mean±SEM. *P<0.001 vs controls. **P<0.01 vs controls.

**Bone mineral density**

Osteoporosis was found in 39.4% of the patients with AC. When patients were classified according to the severity of liver disease, the percentages of osteoporosis were: Child A 30.0%, Child B 23.1% and Child C 70.0%. BMD in the LS and FN, expressed as the z-score, were significantly lower in patients with liver cirrhosis than in the reference population at both LS (P<0.001) and FN (P<0.05; **Table 2**).

**Serum concentrations of soluble factors and other biochemical markers**

**Table 2** shows that serum values of sTNF-R55 (P<0.001), sIL2-R (P<0.001) and neopterin (P<0.001) were significantly higher in patients than in healthy controls. Child B (P<0.01) and C (P<0.001) patients also had higher serum sTNF-R55 than Child A patients.

Patients with osteoporosis had serum concentrations of sTNF-R55 (8.63±2.42 pg/ml) significantly higher than patients without osteoporosis (5.08±2.33 pg/ml, P<0.001; **Figure 1**). Serum concentrations of sIL2-R were significantly elevated in AC patients with osteoporosis in the LS and FN (1290±474 pg/ml) compared with those without osteoporosis (845±450 pg/ml, P<0.05; **Figure 2**). IGF-I serum concentrations and D-Pyr urinary excretion in AC were significantly higher than in the control group (P<0.001; **Table 2**).

**Table 3** shows significant differences between parameters in the Child groups.

**Relationship between variables**

Serum concentrations of sTNF-R55 were inversely correlated with the BMD in LS (r=0.364; P<0.05) and positively with the urinary concentrations of D-Pyr/creatinine (r=0.354; P=0.01).

Serum concentrations of sIL-2R were also inversely related with the BMD in LS (r=0.383; P<0.05) and in FN (r=−0.309; P<0.05).

We found a negative correlation between neopterin and BGP (r=−0.33; P<0.05). No correlation was found between neopterin concentrations and BMD. We also found a highly significant positive correlation between IGF-I and serum parathyormone concentrations (r=0.500; P<0.01).

**Regression analysis**

The results of the backward stepwise regression analysis determining the main predictor of LS and FN osteoporosis are shown in **Table 4**. Concentrations of sTNFR-55 and sIL-2R and Child score were the most important independent variables of both LS and FN (P<0.05).

**DISCUSSION**

Osteoporosis is a complication of advanced liver disease. We found decreased BMD in patients with AC compared with the controls, and this difference is even higher in advanced liver disease. Bone mass loss in viral cirrhosis has been related to an increased bone resorption (Corazza et al., 2000; Crawford et al., 2003; Gonzalez-Calvin et al., 2004; Schiefeke et al., 2005). However, the pathogenesis of bone mass loss in alco-
holic liver diseases is not fully understood, and it has been published that a decreased osteoblastic activity could be involved (Turner, 2000).

IGF-I is a polypeptide that enhances bone growth, and it is essential for normal development of different tissues, including bone, and serum IGF-I levels is known to be a major determinant of BMD in healthy men (Johannsson et al., 1994). The decrease in serum concentrations of IGF-I found in our patients could be involved in bone mass loss. We found increased D-Pyr urinary excretion in patients comparing to control, which probably reflects an increased bone resorption in our patients as D-Pyr is a selective marker of bone resorption that is only found in bone and dentine (Tsuneoka et al., 1996; Gonzalez-Calvin et al., 2004). This result suggests that patients with alcoholic cirrhosis have high bone turnover osteoporosis, as it has also been described in patients with viral cirrhosis (Gallego-Rojo et al., 1998; González-Calvín et al., 1999).

Table 2. Serum concentrations of soluble factors and biochemical parameters of bone mineral metabolism in control subjects, patients with cirrhosis and patients classified according to Child–Pugh score

<table>
<thead>
<tr>
<th>Variable</th>
<th>Controls (n=24)</th>
<th>Cirrhotic patients (n=33)</th>
<th>Child A (n=10)</th>
<th>Child B (n=13)</th>
<th>Child C (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>52.08±2.38</td>
<td>53.09±1.67</td>
<td>57.00±2.44</td>
<td>52.85±2.75</td>
<td>49.50±3.23</td>
</tr>
<tr>
<td>sTNF-R55 (pg/ml)</td>
<td>1.28±0.12</td>
<td>5.94±0.48*</td>
<td>3.50±0.65 *****</td>
<td>6.23±0.60******</td>
<td>8.06±0.75***</td>
</tr>
<tr>
<td>sIL-2R (pg/ml)</td>
<td>545.33±37.30</td>
<td>953.06±85.03*</td>
<td>706.70±127.24</td>
<td>998.08±134.89*</td>
<td>1140.90±168.68****</td>
</tr>
<tr>
<td>Neopterin (nmol/l)</td>
<td>4.91±0.30</td>
<td>7.69±0.74*</td>
<td>9.67±1.59</td>
<td>6.85±0.97***</td>
<td>6.81±1.30***</td>
</tr>
<tr>
<td>z-score LS</td>
<td>0.05±0.09</td>
<td>−1.33±0.19*</td>
<td>−0.74±0.30****</td>
<td>−1.42±0.28*****</td>
<td>−1.80±0.39******</td>
</tr>
<tr>
<td>z-score FN</td>
<td>0.02±0.12</td>
<td>−0.58±0.17***</td>
<td>−0.57±0.30***</td>
<td>−0.27±0.30</td>
<td>−1.01±0.26****</td>
</tr>
<tr>
<td>25OHD (pg/ml)</td>
<td>45.51±5.32</td>
<td>26.42±4.68***</td>
<td>914.2±2.46</td>
<td>78.79±3.29</td>
<td>356.8±2.42</td>
</tr>
<tr>
<td>IGF-I (ng/ml)</td>
<td>161.0±69.3</td>
<td>82.6±48.2*</td>
<td>110.56±18.87*****</td>
<td>74.11±1.43*</td>
<td>63.68±10.21****</td>
</tr>
<tr>
<td>BGP (ng/l)</td>
<td>2.70±0.48</td>
<td>3.36±1.91</td>
<td>2.68±1.31</td>
<td>4.21±2.22</td>
<td>2.95±1.74</td>
</tr>
<tr>
<td>SHBG (nmol/l)</td>
<td>31.32±1.83</td>
<td>70.92±7.44*</td>
<td>56.48±12.12***</td>
<td>74.11±1.43*</td>
<td>63.68±10.21****</td>
</tr>
<tr>
<td>PTH (pg/ml)</td>
<td>9.64±0.72</td>
<td>7.71±1.23</td>
<td>9.14±2.46</td>
<td>8.79±3.29</td>
<td>7.56±2.42</td>
</tr>
<tr>
<td>Urinary D-Pyr (nmol/mmol)</td>
<td>4.22±0.49</td>
<td>7.47±0.97*****</td>
<td>5.13±0.92</td>
<td>7.66±1.08****</td>
<td>9.57±2.67*****</td>
</tr>
<tr>
<td>Urinary calcium/creatinine (mg/mg)</td>
<td>0.08±0.01</td>
<td>0.14±0.28</td>
<td>0.08±0.15</td>
<td>0.19±0.05</td>
<td>0.14±0.05</td>
</tr>
</tbody>
</table>

Data are the mean±SEM. FTI, free testosterone index.

Table 3. Backward stepwise regression analysis defining the main predictor of BMD in alcohol cirrhotic patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>β coefficient</th>
<th>SE</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>sTNF-R55</td>
<td>−0.37</td>
<td>0.01</td>
<td>0.037</td>
</tr>
<tr>
<td>sIL-2R</td>
<td>−0.38</td>
<td>0.04</td>
<td>0.028</td>
</tr>
<tr>
<td>Child score</td>
<td>−0.35</td>
<td>0.10</td>
<td>0.043</td>
</tr>
</tbody>
</table>

Only variables with P<0.05 are listed.
significantly increased in patients with osteoporosis, comparing to patients without osteoporosis, and serum levels of both cytokines correlated inversely with BMD and inversely with D-Pyr urinary excretion. TNF is an important bone resorbing cytokine, and its high serum values and its inverse correlation with BMD suggest that continuous stimulation of the immune system can play an important role in the evolution of cirrhosis and other associated processes like osteoporosis.

During the inflammatory response associated with the activation of T lymphocytes, IL-2 receptors are expressed in cellular membranes of mononuclear cells, increasing the shedding of the soluble receptor (sIL-2R). Increased concentrations of sIL-2R have been reported in many liver diseases (Kitaoka et al., 2003). In our patients, increased concentrations of sIL-2R, in addition to sTNF-R55, their relationship with the severity of liver disease and their association with bone mass loss suggest a role of altered immune response in the demineralization process of these patients. Nevertheless, it could be also a parallel process without causative relation.

Neopterin is considered to be a marker of cellular immune activation as well as a marker of the activity of macrophages in different processes. It reflects one part of cellular immune activation, proliferation and differentiation (Murr et al., 2002). Increased neopterin concentrations have been reported in cirrhotic patients of different aetiology including alcoholic cirrhosis (Díez-Ruiz et al., 1995; Wilmer et al., 1995). Our findings suggest that the activated macrophages can play a role in the pathogenesis of liver cirrhosis, but from our data we could not confirm previous reports about the value of neopterin as a prognostic marker of cirrhosis (Gonzalez-Reimers et al., 1993) nor as a marker of the alterations in BMD associated with cirrhosis.

Some studies have described an association between immune system alterations and osteoporosis (Oelzner et al., 1999; Sugai et al., 2002). Our findings suggest that the decrease in bone mass in AC patients could be related to a persisting activation of the cellular immunity found in these patients. Bone mass loss in AC in our study may be due not only to a decreased osteoblastic activity but also to an increased osteoclastic activity, as shown by the high bone turnover found in our patients.

Conflict of interest statement. None declared.

REFERENCES


