SERUM OSTEOCALCIN, BONE ALKALINE PHOSPHATASE AND CATHEPSIN K LEVELS OF PATIENTS WITH POSTMENOPAUSAL RA: CORRELATION WITH DISEASE ACTIVITY AND JOINT DAMAGE

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ABSTRACT

Objective: To investigate serum levels of osteocalcin (OC), bone alkaline phosphatase (BALP) and cathepsin K in patients with postmenopausal rheumatoid arthritis (RA) and to evaluate the relationship between these markers and disease activity and radiological joint damage.

Material and methods: Forty six postmenopausal women diagnosed with RA according to the criteria of American College of Rheumatology and 42 postmenopausal healthy women who have similar age range with the patient group were involved into the study. Disease activity was measured with Disease Activity Score (DAS 28). Furthermore, Larsen score was used to evaluate radiological damage.

Results: OC and BALP levels were not different between postmenopausal patients with RA and control group (p >0.05). However, levels of cathepsin K were elevated in serum of the patients with postmenopausal RA when compared with that of the healthy control group (p<0.05). In addition, the elevated serum levels of cathepsin K were positively correlated with disease activity and joint destruction (p<0.05). No significant relationship was observed between OC and BALP levels and disease activity and joint destruction (p >0.05).

Conclusions: Cathepsin K may be a valuable parameter to assess disease prognosis in postmenopausal RA.

Key words: Bone Alkaline Phosphatase, Cathepsin K, Joint Destruction, Osteocalcin, Rheumatoid Arthritis.

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Introduction

Rheumatoid Arthritis (RA) is a chronic inflammatory disease characterized by inflammation and hyperplasia of the joint lining that results in the destruction of articular cartilage and bone. Focal articular bone erosion is mediated by osteoclasts, progresses rapidly in the absence of treatment and contributes to joint deformity and patient morbidity(1-5).

Osteocalcin (OC) and bone alkaline phosphatase (BALP) are the main markers of bone formation(6). Bone OC is a vitamin K-dependent non-collagenous protein of bone matrix, synthesized by osteoblasts that has been used as indicators of bone turnover(7). BALP activity is the most common marker used for osteoblastic activity. BAP is an enzyme localized on osteoblast membranes and secreted by osteoblasts and participates into the circulation(8). Data on bone formation in RA are contradictory; both high and low levels have been reported(9,10). The predictive value of bone metabolism markers for the development of radiological joint damage is not explored thoroughly yet(11).

Cathepsin K is a cysteine protease that plays an essential role in osteoclast function and in the degradation of protein components of the bone matrix(12). Several lines of evidence suggest that collagenolytic and proteoglycanolytic metalloproteinases and cathepsins are main proteases in the degradation of the principal protein components in cartilage and bone(13). Cathepsin K is characterized by its unique
collagenase activity and predominant expression in osteoclasts and thus thought to be one of the most important proteolytic enzymes in osteoclastic bone and cartilage resorption\(^\text{(14)}\).

The aim of this study was to measure serum levels of OC, BALP and cathepsin K in post-menopausal RA and to investigate the relationship between these markers and disease activity and radiological joint damage.

**Material and methods**

**Patients**

Forty six postmenopausal female patients who have referred to Dicle University Faculty of Medicine, Department of Physical Medicine and Rehabilitation outpatient clinic and have been diagnosed with definite RA according to the criteria of the American College of Rheumatology of 1987 were enrolled in our study\(^\text{(15)}\). Patients were receiving oral prednisolone or disease-modifying anti-rheumatic drugs (DMARDs) (no one was under biologic agents treatment). None of the patients was taking anti-osteoporotic agents treatment. The control group included 42 postmenapausal healthy women who were selected from the same region. The control group consisted of individuals who have referred to molecular haematology laboratory of Dicle University Faculty of Medicine as donors. The individuals of the control group were not taking any drugs known to affect bone metabolism. The present study had a cross-sectional design approved by the local ethics committee. All the recruited subjects were approved with an informed consent form before participating in the study.

Patients with thyroid disease or parathyroid glands disease, other endocrine disorders, serious liver or kidney disease, radiological abnormalities (scoliosis, platyspondyly, and others) were excluded from the study. Patients were also excluded if they had concomitant use of estrogen, androgen, anticonvulsant or anticoagulant drugs. Other exclusion criteria were alcohol users, smokers, and HIV subjects.

**Measurement of disease variables**

Demographic characteristics and disease periods of the patients were recorded. Disease activity was measured by disease activity score 28 [DAS 28, based upon erythrocyte sedimentation rate (ESR) and number of painful and number of swollen joints (both by 28 joint count)] and global pain assessment was measured with visual analog scale (VAS, 0-100 mm)\(^\text{(16)}\). This last index has proved to be a valid index of experimental, clinical and chronic pain\(^\text{(17)}\).

**Radiological examination**

Hands and feet radiographs were evaluated and scored using Larsen method\(^\text{(18)}\). Radiographs of the hands and feet were available for all patients, and were estimated separately by two of the authors (IB, CH) without knowledge of the identity of the patients. A blind consensus result was given when necessary.

**Measurement of serum cathepsin K, BALP and OC**

Blood samples were collected between 9:00 and 12:00 a.m from all RA and control groups. Blood was allowed to clot and then centrifuged. All serum samples were stored at -80°C until the assay.

**Cathepsin K**: Serum cathepsin K levels were measured by using Human Cathepsin Elisa kit (Cosmo Bio, USA) with ELISA method on DSXTM Automated ELISA System (Dynex Technologies Inc. USA) device.

**BALP**: BALP was measured by using Microvue BAP EIA kit (Quidel corporation, San Diego, USA) with ELISA method on DSXTM Automated ELISA System device.

**OC**: OC levels were studied by electrochemiluminescence immunoassay method with Siemens Immulte osteocalcine kit on immulite 2000 (Siemens Healthcare Inc. USA) autoanalyzer.

**Statistical analysis**

Analysis was carried out using the Statistical Package for Social Sciences software version 16.0 for Windows (Chicago, IL, USA). The Kolmogorov-Smirnov test was used to confirm that data were normally distributed. The comparison of data between the two groups was carried out through the Chi-square test and Mann Whitney-U-test. Correlations between the Cathepsin K, AC, BALP and disease related variables were investigated with the help of Spearman’s correlation method. Statistical significance was based on a value of P<0.05 with a 95% confidence interval.

**Results**

Average of age of the RA group (n=46) and the control group (n=42) was found as 56.86 ± 9.8 and 53.64 ± 8.2, respectively. The clinical and demographic characteristics of the patients are listed on Table 1.
Table 1: Demographic, clinical and laboratory parameters of the patients with postmenopausal RA and healthy controls (mean±SD).

<table>
<thead>
<tr>
<th>Disease characteristics</th>
<th>RA patients (n=46)</th>
<th>Controls (n=42)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>56.86 ± 9.8</td>
<td>53.64 ± 8.2</td>
<td>NS</td>
</tr>
<tr>
<td>Menopausal duration (years)</td>
<td>9.83 ± 8.88</td>
<td>8.65 ± 7.63</td>
<td>NS</td>
</tr>
<tr>
<td>Disease duration (years)</td>
<td>4.52 ± 5.48</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pain (VAS, mm)</td>
<td>44.13 ± 26.52</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ESR (mm/h)</td>
<td>31.62 ± 24.95</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRP (mg/dl)</td>
<td>2.45 ± 3.96</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RF (IU/ml)</td>
<td>84.34 ±106.13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-CCP (U/ml)</td>
<td>178.33 ± 277.24</td>
<td></td>
<td></td>
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<tr>
<td>DAS28</td>
<td>3.25 ± 1.38</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Larsen score Hand</td>
<td>28.21 ± 17.68</td>
<td></td>
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<tr>
<td>Foot</td>
<td>8.86 ± 6.95</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>36.71 ± 23.27</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMARD only (%)</td>
<td>58.4%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMARD and Corticosteroid (%)</td>
<td>41.6%</td>
<td></td>
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</tbody>
</table>

Table 2: Mean levels of the cathepsin K, OC and BALP in patients with postmenopausal RA and healthy controls (mean±SD).

<table>
<thead>
<tr>
<th></th>
<th>RA Patients (n=46)</th>
<th>Controls (n=42)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cathepsin K</td>
<td>28.38 ± 9.31</td>
<td>23.95 ± 9.64</td>
<td>0.031</td>
</tr>
<tr>
<td>OC</td>
<td>8.23 ± 4.72</td>
<td>8.70 ± 3.33</td>
<td>NS</td>
</tr>
<tr>
<td>BALP</td>
<td>11.67 ± 8.58</td>
<td>15.45 ± 9.30</td>
<td>NS</td>
</tr>
</tbody>
</table>

Table 3: Correlation between the cathepsin K, OC and BALP levels with disease activity, radiological damage and laboratory parameters in patients with postmenopausal RA.

<table>
<thead>
<tr>
<th></th>
<th>Larsen (hand) r</th>
<th>Larsen (foot) r</th>
<th>Larsen (total) r</th>
<th>DAS28 r</th>
<th>ESR r</th>
<th>CRP r</th>
<th>RF r</th>
<th>Anti-CCP r</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cathepsin K</td>
<td>-0.518**</td>
<td>-0.360*</td>
<td>-0.318*</td>
<td>0.015</td>
<td>0.051</td>
<td>0.108</td>
<td>0.067</td>
<td>-0.176</td>
</tr>
<tr>
<td>OC</td>
<td>-0.014</td>
<td>-0.028</td>
<td>-0.205</td>
<td>0.089</td>
<td>0.161</td>
<td>-0.044</td>
<td>0.120</td>
<td>-0.084</td>
</tr>
<tr>
<td>BALP</td>
<td>-0.084</td>
<td>-0.103</td>
<td>-0.128</td>
<td>0.080</td>
<td>0.124</td>
<td>-0.114</td>
<td>0.176</td>
<td>-0.084</td>
</tr>
</tbody>
</table>

Cathepsin K levels were significantly higher in patients with RA (p<0.05) (Table 2). OC and BALP levels among RA patients and the control group are listed on Table 2.

According to the Spearman correlation analysis, cathepsin K was positively correlated with DAS 28 and Larsen scores (hands, feet and total) (p<0.05). However, no significant correlation was observed between bone formation markers and radiological damage or disease activity (p>0.05) (Table 3).

On RA group, 58.4% of the patients were treated with DMARD and 41.6% patients were treated with DMARD plus corticosteroids. No significant difference has been observed between the treatment modality and bone metabolism markers in patients with RA (p>0.05).

Discussion

The evaluation of levels of bone markers can provide valuable information regarding bone turnover in RA. We initially studied the serum levels of OC, BALP and cathepsin-K in postmenopausal women. There was not any significant difference detected on OC and BALP levels between the patients with RA and the control group. However, OC and BALP levels were not associated with disease activity and joint erosion.

Studies on serum OC and BALP levels in RA patients have shown discrepant findings with reports of increased, reduced, or normal levels of serum OC and BALP compared with healthy controls (19-21). These different results may reflect heterogeneity of bone involvement in RA, or may be due to differences regarding the duration and activity of the disease, the influence of different variables on serum OC and BALP concentrations, or methodological approach in the assays.

Parallel to the previous studies, in our data, there was no significant correlation between serum OC and BALP levels and markers of RA activity such as DAS28, ESH or CRP and joint erosions in postmenopausal women with RA (10,11,20,22,23). In contrast, some studies reported high or low serum bone formation markers values in patients with RA with active arthritis (24-26). Also, according to Garneo et al (27), bone formation was reduced in patients both with and without joint destruction. Therefore, we think that serum markers of osteoblastic bone formation may not indi-
c ate disease activity and joint damage in post-menopausal women with RA.

In RA, synovial fibroblast-like cells, chondrocytes and osteoclasts have been implicated in joint degradation(22-28). These cells destroy cartilage and subchondral bone by secreting proteolytic enzymes(33). Cathepsin K is a proteolytic enzyme produced by osteoclasts and is secreted into the extracellular department. This leads to an impairment of the organic matrix between the osteoclasts and the bone surface(34). In a certain study, cathepsin K has been demonstrated to be increased in the serum of patients with RA. It has been shown in the same study that there was a significant correlation between cathepsin K and Larsen score(35). Similarly, serum cathepsin K levels were significantly higher on patients with RA when compared with the control group. Furthermore, there was significantly positive correlation between scores of Larsen and cathepsin K levels. Moreover, we found positive correlation between disease activity (DAS 28) and cathepsin K levels in post-menopausal women with RA. These results suggest that cathepsin K seems to be a valuable parameter for the assessment of joint destruction and disease activity in postmenopausal women with RA.

Several studies confirm that bone resorption is increased in active RA(23,28,36-38). On the other hand, other studies demonstrated that bone formation is not associated with disease activity as well as with joint destruction(23,29). It appears that bone resorption is positively associated with joint destruction and disease activity, whereas bone formation is not in patients with RA.

The present study has some limitations. It is a cross-sectional design and our study included small sample sizes of patients and controls. Prospective studies including an adequate sample size are needed in order to fully reveal the relationship between the cathepsin K, OC and BALP levels and the radiological damage as well as with disease activity in postmenopausal women with RA.

Conclusion: In this study, the results of OC and BALP, markers of bone formation, are not different between patients with postmenopausal RA and healthy controls. However, our results demonstrate that cathepsin K, marker of bone resorption, is elevated in the serum of patients with postmenopausal RA compared with that of a healthy control group. Furthermore, elevated serum levels of cathepsin K are significantly positively correlated with disease activity and joint destruction. We suggest that cathepsin K may be a valuable parameter for the assessment of prognosis in patients with postmenopausal RA and its measurement may contribute to developing targeted therapies for the prevention of further bone destruction.

References

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