THE CORRELATION BETWEEN THE VALUES OF URINARY PYRIDINOLINE AND THE RESPONSE TO THERAPY IN OSTEOPOROSIS AS EVALUATED BY DEXA (DUAL ENERGY X-RAY ABSORPTIOMETRY)

Diana Camelia Bonte¹, Marilena Motoc¹, Dana Liana David¹, Rodica Lighezan², Andrei Anghel¹, Ovidiu Horia Bonte³, Ioana Zosin⁴

REZUMAT

Introducere: Pentru ca fibrele de colagen sa se stabilizeze in structura lor trimerica, lanturile polipeptidice trebuie sa se lege intre ele. Doua legaturi majore sunt prezente in matricea ososă si in cartilaje: piridinolinele (PYD) si deoxipiridinolinele (DPD). Ele sunt eliberate in sange in urma degradarii colagenului si excretate apoi prin urina. Obiective: Sa determinam utilitatea masurarii valorilor PYD si DPD in monitorizarea terapiei osteoporotice si sa stabilim posibile corelatii intre valorile acestor markeri si densitatea minerala osoasa (BMD). Material si metode: Lotul de studiu a inclus 87 de paciente (grupul 1) ce sufera de osteoporoza postmenopauzală si primesc terapie antiosteoporotica si un grup de control (grupul 2), alcatuit din 102 femei in postmenopauza, fara osteoporoza. Ele au fost explorate initial, astfel: li s-au determinat densitatea minerala osoasa (BMD)-prin DEXA (absorbtiometrie duala cu raze X), la nivelul coloanei vertebrale lombare L1-L4 si la nivelul soldului si valorile piridinolinelor urinare (tehnica ELISA). Determinarea piridinolinelor s-a repetat la 6 si la 12 luni de la momentul initial, iar DEXA la 12 luni. Rezultate: PYD0/6/12 luni (nmol/mmol creatinina): 52,57 ± 19,92 / 32,61 ± 11,91 / 28,90 ± 12,23 (grupul 1); PYD0/6/12 luni: 29,56 ± 10,15 / 30,03 ± 10,72 / 29,09 ± 10,35 (grupul 2). Concluzii: Rezultatele acestui studiu ne arata ca exista markeri biochimici comuni, care pot fi utilizati ca indicatori ai unui turnover osos crescut, putandu-se astfel interveni terapeutic precoce, prevenind fracturile datorate osteoporozei. Markerii biochimici ai turnoverului osos permit urmarirea in dinamica a remodelarii osoase si ofera astfel informatii despre evolutia masei osoase.

Cuvinte cheie: osteoporoza, piridinoline si deoxipiridinoline, imunodeterminari, postmenopauza

ABSTRACT

Introduction: Pyridinoline (PYD) and deoxypyridinoline (DPD) are the crosslinks of mature type I collagen of bone, being released from the skeleton during collagen degradation, and finally excreted in urine. Objective: To determine the value of urinary PYD and DPD in monitoring osteoporosis and to establish possible correlations between these markers and bone mineral density (BMD). Material and methods: the study group included 87 patients (group 1) suffering of postmenopausal osteoporosis (undergoing treatment) and the control group of 102 postmenopausal women, without osteoporosis (group 2). They underwent a basic examination consisting of: measurement of BMD (dual energy X-ray absorbtiometry - DEXA at L1 – L4 and at the hip) and the evaluation of urinary pyridinoline levels (ELISA). The urinary pyridinoline assessments were repeated at 6 and 12 months after the initial moment, while DEXA was reiterated after 12 months. Results: PYD0/6/12 months (nmol/mmol creatinine): 52.57 ± 19.92 / 32.61 ± 11.91 / 28.90 ± 12.23 (group 1); PYD0/6/12 months: 29.56 ± 10.15 / 30.03 ± 10.72 / 29.09 ± 10.35 (group 2). Conclusions: The results from this study suggests that simple, easy, common biochemical markers could be used as indicators of increased bone turnover, to enable early intervention so as to minimize fracture risk due to osteoporotic changes. The biochemical markers of bone turnover provide dynamic measures of bone remodeling and thus potentially useful in predicting the course of changes in bone mass.

Key Words: osteoporosis, pyridinium crosslinks, immunoassay, bone, postmenopause

INTRODUCTION

Osteoporosis is a systemic disease, characterized by a decrease in the bone mass and alteration of bone microarchitecture, its consequence being the increase of bone fragility and fractures. Due to its significant outcome, osteoporosis became a public health issue of wide interest.¹ ²

The skeleton is a metabolically active tissue and subject to constant changes and adaptative processes.³ From a more physiological point of view,
bone mass may therefore be considered the net result of two opposing metabolic processes, namely bone formation and bone resorption, which in physiological conditions are in equilibrium. There is a wide range of factors that may affect their equilibrium. In the case of an imbalances of this processes, the bone mass and structure change, phenomena identified and quantified by densitometric techniques. Measurement of bone density is important because osteoporosis, the most common metabolic bone disease is defined in individual patients by low bone density and because the risk of fragility fracture is strongly related to bone density.

In contrast to these static methods, the biomarkers of bone metabolism are the ideal tools for the evaluation of bone turnover. They generally show changes in response to antiresorptive treatment within a few months and the percent changes tend to be large. During the past years, the detailed knowledge relating to the structure and dynamics of the skeletal matrix led to the development of different biochemical markers. These markers are measured in blood or urine, and they reflect the activity of bone cells or of the different components of the matrix during bone apposition or resorption. There are two types of markers:

- Markers of bone formation and
- Markers of bone resorption (e.g. pyridinium crosslinks).

The most commonly used, as reflected by references in published literature are shown in Table 1.

Table 1. Bone turnover markers.

<table>
<thead>
<tr>
<th>Formation Markers</th>
<th>Resorption Markers</th>
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<tbody>
<tr>
<td>Serum osteocalcin</td>
<td>Urinary hydroxyproline</td>
</tr>
<tr>
<td>Serum bone specific-alkaline phosphatase</td>
<td>Urinary total pyridinoline</td>
</tr>
<tr>
<td>Serum procollagen type I C-terminal propeptide</td>
<td>Urinary total deoxypyridinoline</td>
</tr>
<tr>
<td>Serum procollagen type I N-terminal propeptide</td>
<td>Urinary free pyridinoline</td>
</tr>
<tr>
<td></td>
<td>Urinary free deoxypyridinoline</td>
</tr>
<tr>
<td></td>
<td>Urinary collagen type I cross-linked N-telopeptide</td>
</tr>
<tr>
<td></td>
<td>Urinary collagen type I cross-linked C-telopeptide</td>
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<tr>
<td></td>
<td>Serum carboxyterminal telopeptide of type I collagen</td>
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</table>

PYD and DPD are the crosslinks of mature type I collagen from bone. As the bone is by far the most important source of collagen, the presence of PYD and DPD in the biological fluids may be considered as having its origins in the bone tissue. Both metabolites of collagen are released from the matrix in circulation, during the process of collagen degradation, and, by excretion, get in urine unchanged, as they are not metabolized in vivo and they are not affected by diet.

Pyridinium cross-links are excreted into urine as free (40%) and peptide-bound (60%) forms (10-13). The objectives of this study were:

- To estimate the value of urinary PYD in monitoring postmenopausal osteoporosis;
- To search a possible correlation between the discussed marker and bone mineral density (BMD) - expressed in g/cm².

**MATERIAL AND METHODS**

The study group included 87 patients suffering from postmenopausal osteoporosis, who were administered bisphophonates (acidum risedronicum - Actonel -5 mg/day + CaD3 for 70 women and acidum alendronicum - Fosamax - 10 mg/day + CaD3, for 17 women), while the control group included 102 postmenopausal women, without osteoporosis. The patients were monitored during March 2007 - November 2008.

All the subjects underwent a basic examination which consisted of measuring the bone mineral density (BMD-assessment I) and urinary pyridinoline levels (PYD 0). BMD was assessed by dual energy X-ray absorbiometry (DEXA), performed at the level of the lumbar spine (L₁ – L₄) and at the femoral neck – at the Timisoara County Hospital no. 1 (Spitalul Județean nr.1). The diagnosis of osteoporosis was expressed according to the WHO criteria:

- T score higher than -1.0 SD = normal;
- T score between -1.0 and - 2.5 SD = osteopenia;
- T score under - 2.5 SD = osteoporosis

The free urinary pyridinoline levels were evaluated by using the METRA PYD ELA kit, based on the competitive enzyme immunoassay principle. The results obtained were corrected for variations in urine concentration by dividing the pyridinium crosslinks value (nmol/l) by creatinine value (mmol/l) of each sample. The evaluations were performed on the first morning void.

**Specimen collection and storage for the measurements of the urinary pyridinoline:** we used preservative free first morning void urine collections. Urine samples were frozen at -70°C until analysis.

The measurements of the urinary pyridinoline were repeated 6 and 12 months following the initial moment, DEXA was performed again 1 year after the first measurement – BMD₂ for all the patients.
RESULTS

Tables 2 and 3 show the descriptive statistics for the subjects taking part in the study.

Postmenopausal women with osteoporosis (study group) were 2.0 years older on average (p<0.05 S) compared to the control group and the age of menarche was 1.7 years later (p<0.001 S) compared to the control group.

8 spine BMD increase of 9.1%, and an average hip BMD increase of 4.8% were noticed (after 12 months of antiresorptive therapy) – see TAB. II. During the first year of treatment, none of the patients developed osteoporotic bone fractures.

The initial values of PYD were significantly higher (p<0.05 S) in postmenopausal women suffering from osteoporosis (to be undergoing treatment), indicating a higher bone resorption rate compared to the control group. The average PYD value was also significantly higher (p<0.05 S) due to a major contribution of the initial values to the average. The resorption rate was similar to the control group (p>0.05 NS) after 6 and 12 months of therapy. (Table 3 and Fig. 1)

In the study group, there was a relative decrease of 37.9% (p<0.001 S) in PYD levels after 6 months, and another decrease of 7.05% (p=0.001 S) from 6 months to 12 months, with an overall decrease of 44.9% (p<0.001 S) during the first year of treatment. (Figures 2, 3)

The association between PYD levels and age, BMI and BMD was estimated using linear regression models.

Table 2. Characteristics of subjects from the two groups.

<table>
<thead>
<tr>
<th>Group of women</th>
<th>Statistical parameter</th>
<th>Age (years)</th>
<th>Age of first period (years)</th>
<th>Body mass index -BMI (kg/m2)</th>
<th>BMD*I L.s. (g/cm2) / T score</th>
<th>BMD*I II L.s. (g/cm2) / T score</th>
<th>BMD** I hip (g/cm2) / T score</th>
<th>BMD** II hip (g/cm2) / T score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Postmenopausal control group</td>
<td>Average</td>
<td>59.21</td>
<td>12.06</td>
<td>24.74</td>
<td>1.176 / 0.02</td>
<td>1.152 / 0.14</td>
<td>1.084 / 0.24</td>
<td>1.071 / 0.23</td>
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<tr>
<td>(n=102)</td>
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<tr>
<td>Postmenopausal study group</td>
<td>Average</td>
<td>61.17</td>
<td>13.8</td>
<td>26.34</td>
<td>0.687 / -2.78</td>
<td>0.749 / -2.36</td>
<td>0.650 / -1.93</td>
<td>0.681 / -1.59</td>
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<td>(n=87)</td>
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* Bone mineral density - lumbar spine, expressed in grams of mineral per unit area scanned (g/cm²) and T scores;
** Bone mineral density - hip, expressed in grams of mineral per unit area scanned (g/cm²) and T scores.

Table 3. PYD values in the control group vs study group.

<table>
<thead>
<tr>
<th>Group of women</th>
<th>Statistical parameter</th>
<th>PYD initial (nmol/mmol creat.)</th>
<th>PYD 6 months (nmol/mmol creat.)</th>
<th>PYD 12 months (nmol/mmol creat.)</th>
<th>PYD Avg (nmol/mmol creat.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Postmenopausal control group</td>
<td>Average</td>
<td>29.56</td>
<td>30.03</td>
<td>29.09</td>
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<td>(n=102)</td>
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<td>Postmenopausal study group</td>
<td>Average</td>
<td>52.57</td>
<td>32.61</td>
<td>28.90</td>
<td>38.03</td>
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<td>(n=87)</td>
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</table>

Figure 1. PYD levels (average) in postmenopausal women suffering from osteoporosis vs postmenopausal women of the control group, initially and after 6 / 12 months of antiresorptive therapy

The BMD values in the control group were normal. The corresponding values in women with osteoporosis were much smaller. In the study group an average lumbar spine BMD increase of 9.1%, and an average hip BMD increase of 4.8% were noticed (after 12 months of antiresorptive therapy) – see Table 2. During the first year of treatment, none of the patients developed osteoporotic bone fractures.

The initial values of PYD were significantly higher (p<0.05 S) in postmenopausal women suffering from osteoporosis (to be undergoing treatment), indicating a higher bone resorption rate compared to the control group. The average PYD value was also significantly higher (p<0.05 S) due to a major contribution of the initial values to the average. The resorption rate was similar to the control group (p>0.05 NS) after 6 and 12 months of therapy. (Table 3 and Fig. 1)
We obtained a weak positive association between PYD and age in both groups. (Figure 4)

Our results showed a negative correlation between PYD and BMI (Figure 5).

At the level of lumbar spine, in patients where the BMD registered an increase (due to the treatment), the urinary pyridinoline showed a significant decrease 12 months after the beginning of treatment. The two aspects did not show a strong correlation (r = -0.183).

DISCUSSIONS

PYD and DPD are pyridinium crosslinks that are formed between neighboring mature collagen molecules during the extracellular maturation of collagen fibrils. PYD is present in many skeletal tissues, including cartilage, bone, ligaments and skeletal muscle, whereas DPD occurs only in bone and dentin.

DPD is a more specific marker of bone resorption, correlating with the rate of bone resorption estimated by bone biopsy and calcium kinetics.11,16

The significance of bone markers is controversial. The American Association of Clinical Endocrinologists stipulates that:

- The precise role of markers in osteoporosis is not yet established but they may be useful for assessing fracture risk in the elderly and therapeutic response to antiresorptive therapy;
- They may be useful in evaluating patients with osteoporosis suspected of having a secondary cause.17

National Osteoporosis Foundation considers that bone markers:

- May assess fracture risk, predict bone loss, predict reduction in fracture risk following 3 to 6 months of antiresorptive therapy and predict BMD response to
antiresorptive and anabolic therapies.\textsuperscript{18,19}

In a recent study, Caulfield et al. have shown that an early response to therapy by means of bone markers predicts a positive increase of bone mineral density, an increase in bone strength, and a decreased risk of osteoporosis related fractures. A significant negative correlation between the levels of certain markers and the bone mineral density was reported.\textsuperscript{20}

The group of C. de la Piedra showed that the determination of urinary pyridinoline is, with carboxyterminal telopeptide of collagen I (CTX) and aminoterminal crosslinked telopeptides of collagen I (NTX), the biochemical marker of bone resorption which presents the best diagnostic accuracy in the study of postmenopausal osteoporosis.\textsuperscript{21}

The EPIDOS study found that NTX, CTX and urinary pyridinoline were significantly higher in control postmenopausal women than in control premenopausal women, but only CTX and urinary pyridinoline were higher in patients with hip fracture than in age-matched controls. The authors concluded that values of CTX and urinary pyridinoline higher than the upper limit (mean + 2.5 SD) of the premenopausal women are associated with an increased risk of hip fracture in the elderly.\textsuperscript{22}

Our study has proved a weak correlation between PYD levels and BMD. This finding is in agreement with other studies. Several studies have examined the relationship between marker changes and BMD changes in women with osteoporosis undergoing drug treatment. For example, Marcus R and al. have shown that correlation coefficients of NTX, CTX or PYD vs BMD in postmenopausal women vary from $r = -0.3$ to -0.5. There are large errors in predicting the bone density for an individual person.\textsuperscript{10}

In another study, Chesnut et al. found that women with the greatest changes in percentage from baseline to 6 months in urinary PYD had the greatest changes in spinal BMD in response to therapy at 1 year.\textsuperscript{23} Hesley et al. have also reported a significant relationship between changes in urinary PYD after 6 months and changes in lumbar spine BMD after 12 or 18 months in response to therapy.\textsuperscript{19}

Not all trials have found a relationship between changes of markers and changes in BMD. For example, in a smaller study comprising 36 women treated with hormone replacement therapy for 1 year, urinary PYD were not correlated with change in BMD.

If BMD represents the quantitative component of the skeleton, bone markers offer data about the bone turnover (qualitative component of skeleton).

Bone markers show precociously changes of bone turnover after treatment, thus proving useful tools for clinicians.

The BMD increase occurs later after antiresorptive therapy and it is not unconditionally linked with an improvement of bone quality.

**CONCLUSIONS**

- The evaluation of urinary pyridinoline levels is useful, as it may help clinicians in assessing the efficacy of the treatment in osteoporosis patients, much before assessing BMD.

- BMD follow-up as a measure of treatment effects is the standard approach to monitor treatment of osteoporosis patients. Changes in mineral density occur slowly and a therapeutic effect is not detectable before several years of treatment. Within a year of treatment, we observed small changes in bone density.

- Biochemical markers of bone metabolism are useful tools to evaluate therapeutic effects after a relatively short period of time.

- Serial measurements of bone markers may help to decide whether or not a patient responds to a specific treatment (responders or non-responders).

In case of non-responsiveness or non-compliance, bone marker measurements have the potential to save medication-related costs.

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