Clinical Usefulness of Urinary CrossLaps as a Sensitive Marker of Bone Metabolism

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Abstract. CrossLaps peptide [Glu-Lys-Ala-His-Asp-Gly-Gly-Arg], a part of the C-telopeptide of the \( \alpha_1 \) chain of type I collagen of bone, is a recently developed biochemical marker of bone turnover. In this study, the clinical utility of measurement of urinary CrossLaps was investigated in eleven premenopausal women who received a gonadotropin-releasing hormone (GnRH) agonist for 6 months for treatment of adenomyosis (n=1) or leiomyomas (n=10). Along with urinary CrossLaps, the levels of various biochemical markers, and serum estradiol, calcitonin and intact parathyroid hormone (i-PTH) were measured, and lumbar spine bone mineral density (BMD) was also monitored before, during, and at the end of the course of GnRH agonist therapy. Apart from CrossLaps, markers of bone resorption tested were urinary pyridinoline, deoxypyridinoline and hydroxyproline. Markers of bone formation tested were serum osteocalcin and bone-specific alkaline phosphatase (B-ALP). Serum estradiol levels decreased to undetectable levels at 2 months of GnRH agonist therapy. The values for all biochemical markers increased significantly throughout the therapy. The degree of an increase in CrossLaps levels was greater than that in all other markers. Mean lumbar spine (L2-1.4) BMD was decreased by 7.2% at 6 months of treatment. The percent change in BMD at 6 months of treatment correlated inversely with the percent change in CrossLaps levels from the baseline to 1, 2, and 5 months of treatment. These results indicate that measurement of urinary CrossLaps might be a useful tool to predict the risk of bone loss caused by hypoestrogenism including GnRH agonist therapy.

Key words: Bone metabolism, Bone mineral density (BMD), CrossLaps, Gonadotropin-releasing hormone (GnRH) agonist

HYPOESTROGENISM induced by administration of gonadotropin-releasing hormone (GnRH) agonists has been shown to cause some degree of bone loss [1–6]. The mechanism, by which hypoestrogenism brings about bone loss, is an increased bone resorption due to attenuation of estrogen action on bone. At present, GnRH agonists are widely used for the treatment of gynecological disorders, such as endometriosis and fibromyoma [7, 8]. The development of a simple and sensitive means of identifying women at high risk of bone loss by GnRH agonists has therefore been sought.

Thus far, a variety of biochemical markers of bone turnover have been employed to foresee bone loss [9–15]. For instance, serum osteocalcin and bone-specific alkaline phosphatase (B-ALP) have been shown to serve as markers of bone formation. In contrast, urinary markers of bone resorption include pyridinoline, deoxypyridinoline, and hydroxyproline, but these markers are rather lacking in specificity. In addition, they are not sensitive enough to detect early changes in bone metabolism caused by hypoestrogenism.
Urinary CrossLaps is derived specifically from a degradation product of type I collagen of bone and has been shown to be a sensitive and specific marker of bone resorption [9, 17, 18]. A monoclonal antibody-based enzyme-linked immunosorbent assay (ELISA) has been developed to measure urinary excretion of CrossLaps [17, 18]. Early postmenopausal women have been shown to exhibit increased CrossLaps levels [17]. In the present study, we investigated whether urinary CrossLaps may serve as a sensitive marker for predicting the degree of bone loss in women treated with GnRH agonist.

**Materials and Methods**

Subjects consisted of 11 premenopausal women who received a GnRH agonist for 6 months for the treatment of adenomyosis (n=1) or uterine leiomyomas (n=10). The mean age (± SEM) of subjects at the beginning of treatment was 45.1 ± 3.2 years (range 39–49). The body mass index (BMI), calculated as kilograms per square meter, of subjects at the beginning of treatment was 21.7 ± 1.8. None of the subjects enrolled in the study had any diseases or medications known to affect bone metabolism or renal function. Informed consent was obtained from all subjects.

All subjects received a GnRH agonist depot, depot leuproride (Leuprin™, Takeda Chemical Industry, Ltd., Osaka, Japan) at a dose of 3.75 mg/month subcutaneously. The initial dose of GnRH agonist was given on day 2–5 of the menstrual cycle. The mean (± SEM) duration of treatment was 5.8 ± 0.6 months.

Serum and daytime urine specimens were collected on the day of every GnRH agonist administration and 1 month after the last administration. Specimens were stored frozen until assay. Serum levels of osteocalcin, estradiol (E2), calcitonin and intact parathyroid hormone (i-PTH) were determined by radioimmunoassay. Serum B-ALP levels were determined by SDS electrophoretic assessment. Urinary levels of pyridinoline, deoxypyridinoline and hydroxyproline were measured by high pressure liquid chromatography (HPLC). Urinary CrossLaps levels were measured with an ELISA kit (Osteometer, Copenhagen, Denmark) and a monoclonal antibody directed against an immobilized synthetic peptide with an amino acid sequence specific for a part of the C-telopeptide of the α1-chain of type I collagen (Glu-Lys-Ala-His-Asp-Gly-Gly-Arg = CrossLaps peptide) [17, 18]. The intra- and interassay coefficients of variation of the test were 2.3% and 4.6%, respectively. All urinary parameters were corrected by the urinary creatinine (Cr) concentration measured by a standard colorimetric method, and the CrossLaps levels were reported as μg/mmol Cr.

The bone mineral density (BMD) of the lumbar vertebrae (L2–L4) was measured with a Lunar DPX, a dual-energy x-ray absorptiometer (Lunar Co. Wisconsin, USA). Measurements were made within weeks before the treatment, and at 4 and 6 months of the therapy. The precision of BMD measurements was 1% [16].

The volume of leiomyomas was measured by using transvaginal ultrasound (with a 5 MHz probe). The volume of each nodule was calculated by the formula used for the volume of ellipsoid tumors: \( V = \frac{4}{3} \pi \times R1 \times R2 \times R3 \) where R1, R2 and R3 were the three largest diameters. The value for the maximum nodule in each patient was used for statistical analysis.

All data are expressed as the mean ± SEM. To estimate the longitudinal changes in all variables, the individual baseline values were defined as 100%, and all subsequent values were calculated as a percentage of the baseline value. The Wilcoxon signed-rank test was used to determine the significance of the percent changes in the values of all serum and urinary parameters, BMD, and the volume of leiomyoma. The percent changes in the values for biochemical markers at each time point were compared with each other by the Sheffe’s F test (multiple comparison test). The Pearson’s correlation coefficient was calculated to determine the relationship between BMD and biochemical markers percent change from the baseline. A P value of less than 0.05 was considered statistically significant.

**Results**

As for the baseline levels of markers for bone metabolism, CrossLaps was 140.0 ± 20.2 μg/mmol Cr, pyridinoline 30.2 ± 3.9 μmol/mmol Cr, deoxypyridinoline 4.2 ± 0.6 μmol/mmol Cr, hydroxyproline 17.0 ± 1.8 μmol/mmol Cr,
CrossLaps as a marker of bone metabolism

The levels of all serum and urinary biochemical markers of bone turnover increased significantly after 6 months of GnRH agonist treatment, compared with the baseline levels (Table 1). As shown in Fig. 1, the values for CrossLaps, Osteocalcin and Deoxypyridinoline increased significantly throughout the therapy. The other biochemical markers showed a similar tendency. The degree of increase in CrossLaps levels was greater than that in all other markers. Interestingly, a significant increase in CrossLaps levels was observed after as early as 2 months of therapy.

The baseline calcitonin and i-PTH values were 21.4 ± 1.7 pg/dl and 33.5 ± 5.2 pg/dl, respectively, and neither changed significantly throughout the treatment period. At the end of therapy, their values were 22.8 ± 1.3 pg/dl and 32.5 ± 1.6 pg/dl, respectively.

Serum E2 levels were 44.6 ± 9.7 pg/ml at the entry time point, and 10.6 ± 0.3 pg/ml at 1 month of treatment. Then E2 levels fell below the detection limit (<10 pg/ml) at 2 months of treatment, and remained suppressed for the rest of the treatment. Lumbar spine (L2–L4) BMD was 1.176 ± 0.042 g/cm² at the beginning of treatment. BMD decreased significantly to 94.4 ± 2.5 and 92.8 ± 2.1% (P < 0.05, <0.01) of baseline at 4 and 6 months of treatment, respectively (Fig. 2).

The percent change in BMD at 6 months of treatment was correlated inversely with the percent change in the CrossLaps levels from baseline to 1 month (r = -0.74, P < 0.01), 2 months (r = -0.68, P < 0.05) and 5 months (r = -0.72, P < 0.05) of treatment (Table 2). As for the percent change in osteocalcin levels, a significant negative correlation with the percent change in BMD at 6 months of treatment was also found at 1 month (r = -0.67, P < 0.01), 2 months (r = -0.63, P < 0.05), 5 months (r = -0.89, P < 0.001) and 6 months (r = -0.80, P < 0.01) of treatment. The percent change in hydroxyproline levels only at 5 months (r = -0.65, P < 0.01) of treatment was correlated inversely with the percent change in BMD after 6 months of treatment.

The volume of leiomyomas decreased significantly to 52.0 ± 0.07, 41.1 ± 0.07, 37.4 ± 0.06, 32.7 ± 0.06 and 27.4 ± 0.07% (P < 0.001) of the baseline at 2, 3, 4, 5 and 6 months of the treatment, respectively.

Table 1. Values for biochemical markers of bone turnover before and at the end of therapy and value for each marker at the end of therapy (% of baseline levels)

<table>
<thead>
<tr>
<th>Marker</th>
<th>before</th>
<th>at the end of therapy</th>
<th>(% of baseline levels)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CrossLaps (µg/mmol Cr)</td>
<td>140.0 ± 20.2</td>
<td>551.7 ± 39.4*</td>
<td>(502.1 ± 87.3%)</td>
</tr>
<tr>
<td>Pyridinoline (µmol/mmol Cr)</td>
<td>30.2 ± 3.9</td>
<td>42.5 ± 4.0*</td>
<td>(152.9 ± 22.8%)</td>
</tr>
<tr>
<td>Deoxypyridinoline (µmol/mmol Cr)</td>
<td>4.2 ± 0.6</td>
<td>8.2 ± 0.8*</td>
<td>(221.60 ± 10.4%)</td>
</tr>
<tr>
<td>Hydroxyproline (µmol/mmol Cr)</td>
<td>17.0 ± 1.8</td>
<td>25.7 ± 1.5*</td>
<td>(172.1 ± 19.5%)</td>
</tr>
<tr>
<td>Osteocalcin (ng/ml)</td>
<td>3.7 ± 0.3</td>
<td>7.6 ± 2.1*</td>
<td>(203.0 ± 9.5%)</td>
</tr>
<tr>
<td>B-ALP (IU/l)</td>
<td>50.0 ± 3.5</td>
<td>93.3 ± 7.3*</td>
<td>(191.4 ± 13.2%)</td>
</tr>
</tbody>
</table>

Data are expressed as the mean ± SEM. ∗: P < 0.05.

![Fig. 1. Values for CrossLaps, Deoxypyridinoline and Osteocalcin (% of baseline levels) during GnRH agonist treatment. Vertical lines indicate the SEM.](image)
The administration of GnRH agonist provides a model for studying the effects of a steep but reversible decrease in ovarian estrogen secretion on bone remodeling. There is an increase in both biochemical markers of bone resorption and formation after menopause or GnRH agonist therapy [4, 5], as shown in this study. Among all biochemical markers tested, CrossLaps was shown to be the most responsive and sensitive to an increase in bone turnover caused by a rapid drop in ovarian estrogen production.

A significant inverse correlation was observed between an increase in urinary CrossLaps levels or serum osteocalcin levels at 1 month of GnRH agonist therapy and a decrease in lumbar spine BMD after 6 months of therapy. Such a lag time makes sense because bone markers reflect currently occurring events in the bone tissue, whereas BMD manifests the cumulative consequence of bone turnover. It may therefore be that the measurement of the levels of biochemical markers is a more sensitive assessment of ongoing bone turnover than BMD. The good correlations between the early changes in biochemical markers and the later changes in BMD suggest that the biochemical markers could be recommended to monitor the status of bone turnover during GnRH agonist therapy. Particularly a specific marker of bone resorption such as urinary CrossLaps may have clinical utility in predicting the future skeletal status of women receiving GnRH agonists or any medication which affects bone metabolism. Further studies are necessary to support a closer association between the change in CrossLaps levels and the change in BMD in other clinical settings where bone resorption is enhanced. Current data also support the notion that the combination of CrossLaps, a marker of bone resorption, and osteocalcin, a marker of bone formation, might offer a more accurate prediction of BMD than any of the currently used markers alone.

At present, the duration of GnRH agonist therapy has been restricted to up to 6 months because of concern about long-standing bone loss due to hypoestrogenism. Recently, in order to minimize the adverse effects, adding back low doses of estrogen and/or progestogen has been proposed to allow long-term GnRH agonist therapy [4, 7, 19-22]. Standard postmenopausal estrogen replacement therapy with 0.625 mg of conjugated estrogen...
estrogens may not consistently prevent spinal bone loss in women treated with GnRH agonist [19], but the use of higher doses of estrogen may counteract the therapeutic effect of GnRH agonists toward endometriosis and leiomyoma, etc. Biochemical markers of bone resorption such as CrossLaps could be used to determine the lowest dose of estrogen individually that will minimize or protect bone resorption.

It has been reported that urinary excretions of markers of bone resorption such as total pyridinolines were relatively greater in the early morning sample, presumably reflecting increased rates of bone resorption at night, but that daytime urinary values were quite constant [12, 14]. It is likely that the levels of bone markers in the morning void urine vary depending on the time of waking. In addition, from the practical point of view, fresh daytime urine is preferable as a sample for measuring bone markers. For these reasons, in this study, we used the daytime urine as specimens.

The present study demonstrates that determination of urinary CrossLaps levels is useful for the prediction of ensuing bone loss caused by GnRH agonist therapy. Unlike the cumbersome measurement of pyridinoline, deoxypyridinoline and hydroxyproline by HPLC [18], CrossLaps ELISA is an assay for routine and simple clinical use. CrossLaps can therefore be recommended as a sensitive and convenient biochemical marker of bone resorption.

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References


