Association Between Spine Disc Degeneration and Type II Collagen Degradation in Postmenopausal Women

The OFELY Study

Patrick Garnero,1 Elisabeth Sornay-Rendu,2 Monique Arlot,2 Claus Christiansen,3 and Pierre D. Delmas2

Objective. To investigate whether radiologic spine disc degeneration is associated with increased type II collagen (CII) degradation products in the urine of postmenopausal women, independently of radiologic knee osteoarthritis (OA) and clinical hand OA.

Methods. The study group comprised 324 postmenopausal women ages 58–94 years who had no treatment or disease that could alter bone metabolism. Lateral radiographs of the thoracic and lumbar spine were obtained in each woman and scored for disc space narrowing (DSN) and osteophytes. Fixed-flexion posteroanterior radiographs of the knee were obtained to assess femorotibial knee OA. In all women, hand OA was assessed by clinical examination, and the level of urinary C-terminal crosslinking telopeptide of CII (CTX-II), a biologic marker of CII degradation, was measured.

Results. The prevalences of radiographic lumbar and thoracic spine disc degeneration, knee OA, and clinical hand OA were 84%, 88%, 35%, and 58%, respectively. After adjustment for age and body mass index (BMI), patients with lumbar spine DSN grade >1 had, on average, 34% higher CTX-II levels compared with the other women (P = 0.0005), whereas no association was observed with lumbar spine osteophytes. No significant association between thoracic spine DSN or osteophytes and urinary CTX-II levels was observed. Multivariate analysis of variance showed that, after adjustment for age and BMI, lumbar spine DSN (P = 0.0049), knee OA (P = 0.0055), and clinical hand OA (P = 0.0060) were, independently of each other, associated with CTX-II levels. Thus, patients with lumbar spine DSN, knee OA, and clinical hand OA had CTX-II levels 80% higher (P < 0.0001 after adjustment for age and BMI) than those of patients with no lumbar spine DSN, no radiologic knee OA, and no clinical hand OA.

Conclusion. Postmenopausal women with lumbar spine disc degeneration are characterized by increased CII degradation. The contribution of lumbar spine DSN to CII degradation was similar to, and independent of, the contribution of radiologic knee OA or clinical hand OA. Lumbar spine disc degeneration in elderly patients should be assessed when analyzing levels of CTX-II in studies of knee, hip, and hand OA.

Spine disc degeneration is very prevalent in elderly persons and is associated with significant morbidity (1,2). Spine disc degeneration is characterized by disc space narrowing (DSN) and the presence of vertebral osteophytes. It remains unclear whether this condition constitutes a form of osteoarthritis (OA) or a different disease, although it is often considered as spinal OA. Several risk factors for the development and progression of knee and hip OA have been reported (2), but little is known about the molecular mechanisms leading to intervertebral disc degeneration in humans (3).
The hallmark of OA is the destruction of articular cartilage, which is believed to result mainly from increased degradation of its matrix components, including type II collagen (CII) (4). CII is also present in spine discs and actually constitutes the most abundant protein of the nucleus pulposus. Using human intervertebral discs, Antoniou et al (5) observed increased denaturation of CII with increasing age and disc degeneration. The rate of cartilage turnover can be assessed noninvasively by measuring cartilage matrix protein or fragments released in the circulation and eventually excreted in the urine during the process of cartilage matrix synthesis or degradation (6–9). Because CII is the most abundant protein of intervertebral discs, and because it is highly specific for cartilage tissue, measurement of CII turnover appears to be the most relevant process to investigate in spinal disc degeneration.

To assess CII degradation, immunoassays using antibodies recognizing either neoepitopes generated by denaturation of the triple helix domain of CII (10,11) or crosslinked fragments of the telopeptides (12,13) have been developed recently. Increased levels of CII helix neoepitopes in the urine of patients with OA (14), rheumatoid arthritis (RA) (15), or relapsing polychondritis (16) and in the synovial fluid of patients with inflammatory joint diseases (17) have been reported. In previous studies using assays recognizing the C-terminal crosslinking telopeptide of CII (CTX-II), increased levels of CTX-II in synovial fluid were demonstrated soon after knee injury (12), and increasing levels of CTX-II that were observed in the urine of patients with RA or with knee or hip OA (18–20) correlated with progression of joint damage. However, no study has yet investigated the relationship between intervertebral disc degeneration and CII degradation.

The aim of this study was to investigate the association between radiologic spine disc degeneration and cartilage degradation, as assessed by urinary CTX-II levels, in a large cohort of untreated postmenopausal women participating in the OFELY (Os des Femmes de Lyon) prospective study.

PATIENTS AND METHODS

Patients. The study group included postmenopausal French women belonging to a population-based cohort. These women were participants in a prospective investigation of the determinants of bone loss, the OFELY study. At baseline, the cohort comprised 1,039 healthy female volunteers, ages 31–89 years, selected from a large health insurance company (Mutuelle Générale de l’Éducation Nationale); this cohort has previously been described in detail (21,22). In the present analysis, we studied the 559 postmenopausal women (menopause was defined as an absence of menses for at least 12 months) after 8 years of followup in the study, when spine disc degeneration, radiographic knee OA, and clinical hand OA were assessed. Of the 559 women, 207 were excluded because of treatment or disease that could influence calcium metabolism, including 156 women who currently used hormone replacement therapy (HRT), which recently has been shown to influence urinary CTX-II levels (23). Among the remaining 352 women, urine specimens were not available for 28. The present study was performed in the 324 untreated postmenopausal women, ages 58–94 years.

Spine and knee radiography. Lateral radiographs of the spine and both knees were obtained in all postmenopausal women. Spine films were graded with a standard atlas to document the severity of disc space narrowing (DSN) and osteophyte formation, using the grading system described by Lane et al (24). According to that system, each level of the spine is assessed for osteophytes (the largest one from both the anterior and posterior areas) and DSN; both osteophytes and DSN are given a score from 0 to 3 (with higher numbers indicating increasing severity). An integrated summary grade of 0–2, based on the presence and severity of osteophytes and DSN, is then assigned to each intervertebral disc level, as follows: grade 0 = normal joint (score of 0 for both osteophytes and DSN); grade 1 = mild DSN (score of 1) and/or mild osteophytes (score of 1); grade 2 = moderate-to-severe DSN (score of 2 or 3) and/or moderate-to-severe osteophytes (score of 2 or 3). For the lumbar spine, intervertebral compartments from the first/second lumbar vertebrae to the fourth/fifth lumbar vertebrae are assessed. For the thoracic spine, because of the number of levels to be evaluated, only the 2 intervertebral levels that are most involved, as subjectively determined by the reader, were scored in detail for DSN and osteophytes, as previously described (24). For both the lumbar and thoracic spine, the final grade corresponds to the grade for the intervertebral disc level with the highest score. All spine radiographs were scored by a single trained rheumatologist (ES-R).

Both intraobserver and interobserver reproducibility of scoring were assessed by the kappa statistic on 32 different radiographs. Kappa values for intraobserver reproducibility for DSN and osteophyte scores, respectively, were 0.80 (95% confidence interval [95% CI] 0.65–0.95) and 0.79 (95% CI 0.64–0.94) for the lumbar spine, and 0.57 (95% CI 0.31–0.83) and 0.75 (95% CI 0.58–0.93) for the thoracic spine. For interobserver reproducibility, the corresponding values were 0.83 (95% CI 0.69–0.97) and 0.61 (95% CI 0.39–0.82) for the lumbar spine and 0.53 (95% CI 0.13–0.93) and 0.94 (95% CI 0.81–1.00) for the thoracic spine.

Radiologic evaluation of the knees consisted of bilateral posteroanterior weight-bearing knee radiographs with fixed flexion using the SynaFlex X-ray Positioning Frame (Synarc, San Francisco, CA), as previously described (25). Radiographs were obtained in a single radiography unit by the same staff of 2 technicians using a standardized technique, as follows: 1) for patient positioning, the great toes of both feet are placed in contact with the anterior wall of the frame, under the film; the index foot is fixed in 10° external rotation against the V-shaped support on the base of the frame. Body weight is distributed equally between the 2 legs, and the patient flexes the knees until the knees touch the anterior wall of frame. The
Table 1. Characteristics of 324 untreated postmenopausal women according to the grade of lumbar and thoracic spine disc degeneration*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Lumbar spine disc degeneration</th>
<th>Thoracic spine disc degeneration</th>
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<tbody>
<tr>
<td></td>
<td>Grade 0</td>
<td>Grade 1</td>
</tr>
<tr>
<td>% of women</td>
<td>15.7</td>
<td>39.6</td>
</tr>
<tr>
<td>Age, years</td>
<td>67 ± 8</td>
<td>68 ± 7</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>62 ± 10</td>
<td>62 ± 11</td>
</tr>
<tr>
<td>Height, cm</td>
<td>159 ± 6</td>
<td>158 ± 6</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>24.4 ± 3.8</td>
<td>25.0 ± 4.1</td>
</tr>
<tr>
<td>Radiologic knee OA, %</td>
<td>25.0</td>
<td>27.0</td>
</tr>
<tr>
<td>Clinical hand OA, %</td>
<td>52.8</td>
<td>52.3</td>
</tr>
</tbody>
</table>

* Except where indicated otherwise, values are the mean ± SD. The grade is the summary radiologic grade, including scores for both disc space narrowing and osteophytes. OA = osteoarthritis.
† P < 0.01 versus grades 0 and 1.
‡ P < 0.05 versus grade 0.
§ P < 0.01 versus grades 0 and 1.

RESULTS

In postmenopausal women, urinary CTX-II levels increased slightly, but significantly, with age (r = 0.18, P = 0.0009). Urinary CTX-II levels were significantly and positively associated with BMI (r = 0.17, P = 0.0014). Women with osteoporosis based on the World Health Organization definition (bone mineral density values below the mean ± 2.5SD for healthy young women) for total hip only (P = 0.75), lumbar spine only (P = 0.92), or either hip or spine (P = 0.41) had CTX-II levels similar to those of women without osteoporosis, after adjustment for age and BMI. Consequently, all subsequent analyses were adjusted for age and BMI.

As shown in Table 1, 84% and 88% of this population of postmenopausal women had radiographic evidence of lumbar and thoracic spine disc degeneration, respectively, when disc degeneration was defined by the summary grade of ≥1. Among women with lumbar spine disc degeneration, those with a grade of 2 were significantly older and had a higher BMI compared with the other women, and a larger proportion of them also had concomitant radiologic knee OA. Among women with thoracic spine disc degeneration, those with a grade of 2 were heavier and had a higher BMI.

As shown in Figure 1, after adjustment for age and BMI, a significant increase in urinary CTX-II levels associated with lumbar spine disc degeneration was observed; however, there was no significant difference between the levels in women with grade 1 and grade 2
degeneration. In contrast, no significant association was observed between the grade of thoracic spine disc degeneration and urinary CTX-II values after adjustment for age and BMI. We then investigated the association between lumbar spine disc degeneration and CTX-II levels, considering DSN and osteophyte scores separately. As shown in Figure 2, after adjustment for age and BMI, there was a significant association between lumbar spine DSN scores and urinary CTX-II levels, with women with a score of 1 or 2 having 34% higher levels than did women with a score of 0 ($P = 0.0005$), although no significant difference was observed between scores of 1 and 2. In contrast, there was no significant relationship between lumbar spine osteophyte scores and CTX-II values. For the thoracic spine, no significant association was observed between urinary CTX-II levels and either DSN or osteophytes, after adjustment for age and BMI (data not shown). Because we found no association between lumbar spine osteophytes, thoracic spine DSN, or osteophytes and CTX-II levels, the following analyses were performed regardless of the presence or absence of alterations at these sites.

We investigated whether the association between lumbar spine DSN and urinary CTX-II levels could be confounded by the concomitant presence of radiologic knee OA or clinical hand OA. Regardless of the presence of lumbar spine disc degeneration, patients with knee OA had increased CTX-II levels compared with the other individuals ($P = 0.02$) after adjustment for age and BMI (Table 2). After adjustment for age and BMI, the 59 patients with unilateral knee OA had increased levels of CTX-II (238 $\pm$ 114 ng/mmoles creatinine) compared with the 212 women with no knee OA (210 $\pm$ 100 ng/mmoles creatinine; $P = 0.047$). The 53 patients with bilateral knee OA had significantly higher urinary CTX-II levels (309 $\pm$ 155 ng/mmoles creatinine) than women with no knee OA ($P < 0.0001$) and patients with unilateral knee OA ($P = 0.0054$) after adjustment for age and BMI. Patients with clinical hand OA had significantly higher CTX-II levels ($P = 0.0002$) than did women with no clinical hand OA, after adjustment for age and BMI (Table 2). As shown in Table 2 and Figure 3, women with lumbar spine DSN (score $\geq$1) only, knee OA only, or hand OA only had higher urinary CTX-II levels than did women with no lumbar spine DSN, no knee OA, and no clinical hand OA, although the difference reached statistical significance only for spine DSN after adjustment for age and BMI. Patients with abnormalities in 2 skeletal locations (spine and knee, spine and hand, or knee and hand) had higher CTX-II levels than did patients with only 1 involved joint.

Finally, patients presenting with lumbar spine DSN, knee OA, and hand OA had the highest CTX-II levels (80% higher than those of women with no spine DSN, no knee OA, and no clinical hand OA; $P < 0.0001$).
after adjustment for age and BMI) (Table 2 and Figure 3). There was no significant interaction between lumbar spine DSN, knee OA, and/or clinical hand OA on CTX-II levels (Table 2), indicating that joint abnormalities in these 3 sites contributed independently of each other to increased CTX-II levels.

**DISCUSSION**

This study is the first to analyze the relationship between CII degradation and degenerative joint diseases concomitantly in several skeletal locations, including the spine, knees, and hands, in postmenopausal women. We demonstrated that postmenopausal women with lumbar spine DSN are characterized by an increase in CII degradation products, with levels similar to those observed in patients with knee OA or clinical hand OA, the other most common forms of OA in elderly persons.

In our population of postmenopausal women with a mean age of 70 years, radiologic evidence of lumbar or thoracic spine disc degeneration was very common, and the prevalence was similar to that reported in other populations of postmenopausal women of similar age (28–31); however, comparisons between studies are difficult due to differences in the grading systems used. A recent study in postmenopausal women, in which the same grading system used in this study was applied, showed the prevalence of lumbar spine DSN and osteophytes to be 68% and 91%, respectively (32); these values are also similar to our findings.

**Figure 3.** Association of urinary levels of C-terminal crosslinking telopeptide of type II collagen (CTX-II) with radiologic lumbar spine disc degeneration, radiologic knee osteoarthritis (OA), and clinical hand OA in untreated postmenopausal women. Women were categorized according to the presence or absence of lumbar spine disc space narrowing (DSN) and/or radiologic knee OA and/or clinical hand OA. Bars show the mean and SD level of urinary CTX-II in each group.

**Table 2.** Urinary CTX-II level according to location of degenerative joint disease

<table>
<thead>
<tr>
<th>Group</th>
<th>CTX-II, ng/mmoles creatinine</th>
<th>( P ), univariate analysis†</th>
<th>( P ), multivariate analysis‡</th>
<th>( P ), interaction§</th>
</tr>
</thead>
<tbody>
<tr>
<td>No lumbar spine DSN, no knee OA, no clinical hand OA (n = 44)</td>
<td>166 ± 68</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Lumbar spine DSN, no knee OA (n = 223)</td>
<td>254 ± 129</td>
<td>&lt;0.0001</td>
<td>0.0049</td>
<td>–</td>
</tr>
<tr>
<td>Knee OA, no clinical hand OA (n = 112)</td>
<td>272 ± 139</td>
<td>0.002</td>
<td>0.0055</td>
<td>–</td>
</tr>
<tr>
<td>Clinical hand OA, no lumbar spine DSN, no knee OA (n = 189)</td>
<td>256 ± 126</td>
<td>0.0002</td>
<td>0.0060</td>
<td>–</td>
</tr>
<tr>
<td>Lumbar spine DSN only (n = 49)</td>
<td>205 ± 89</td>
<td>0.049</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Knee OA only (n = 12)</td>
<td>192 ± 62</td>
<td>0.18</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Clinical hand OA only (n = 39)</td>
<td>195 ± 94</td>
<td>0.20</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Lumbar spine DSN + knee OA (n = 33)</td>
<td>256 ± 152</td>
<td>0.021</td>
<td>–</td>
<td>0.92</td>
</tr>
<tr>
<td>Lumbar spine DSN + hand OA (n = 71)</td>
<td>247 ± 117</td>
<td>0.0009</td>
<td>–</td>
<td>0.74</td>
</tr>
<tr>
<td>Knee OA + hand OA (n = 7)</td>
<td>237 ± 84</td>
<td>0.026</td>
<td>–</td>
<td>0.59</td>
</tr>
<tr>
<td>Lumbar spine DSN + knee OA + hand OA (n = 56)</td>
<td>299 ± 146</td>
<td>&lt;0.0001</td>
<td>–</td>
<td>0.92</td>
</tr>
</tbody>
</table>

* Values are the mean ± SD. All \( P \) values were adjusted for age and body mass index. CTX-II = C-terminal crosslinking telopeptide of type II collagen; DSN = disc space narrowing (score ≥1); OA = osteoarthritis.

† Women with degenerative disease in ≥1 location versus women with no lumbar spine DSN; women with no knee OA; women with no clinical hand OA; and women with no lumbar spine DSN, no knee OA, and no clinical hand OA.

‡ Associations between urinary CTX-II and lumbar spine DSN, knee OA, and clinical hand OA in a model including all 3 skeletal sites together.

§ Interaction between degenerative diseases in the different skeletal locations on urinary CTX-II levels in a multivariate model.
including osteoporosis and other bone diseases; thus, their interpretation in spine disc degeneration is unclear. In our study, we chose to investigate specifically CII degradation, because CII is highly specific for cartilage, including the annulus fibrosus and nucleus pulposus of the intervertebral disc (5,35). We found that urinary CTX-II levels were strongly associated with spine DSN but not osteophytes. A recent prospective study in postmenopausal women identified clinical risk factors for lumbar spine DSN progression that were different from those of osteophyte progression, suggesting that different pathophysiologic processes may be involved in the individual features of spine disc degeneration (32).

The increase in urinary CTX-II levels was already apparent in women with a DSN score of 1, which corresponds to mild involvement. This indicates that increased CII degradation is probably an early process in lumbar spine disc degeneration, which may be detected by measuring urinary CTX-II levels. We could not find a significant association between thoracic spine DSN and CTX-II levels. Although the reasons for this are unclear, the lack of sensitivity in the radiologic assessment of thoracic compared with lumbar spine DSN could be involved. Indeed, using the grading system described by Lane et al (24), 4 intervertebral discs of the lumbar spine are evaluated, whereas only the 2 most involved levels are considered for the thoracic spine. The intraobserver and interobserver reproducibility of lumbar spine DSN scoring is higher than that of thoracic spine DSN scoring. However, this lack of association could also be related to a lower total cartilage volume in the thoracic compared with the lumbar spine and/or differences in the rate of CII degradation.

We investigated whether the increased CTX-II levels in women with lumbar spine disc degeneration could be confounded by the concomitant presence of knee and/or hand OA. Indeed, among women with a score for lumbar spine DSN of ≥1, 43% also had knee OA, and 61% had clinical hand OA. In multivariate analysis, there was no interaction between lumbar spine disc degeneration, knee OA, and hand OA on the CTX-II levels, indicating that these 3 sites contribute independently of each other to CTX-II levels in postmenopausal women. These results indicate, as expected, that urinary levels of CTX-II reflect whole-body cartilage degradation.

Our findings may be of relevance for the clinical interpretation of urinary CTX-II levels in relation to aging and OA. It has previously been shown that urinary CTX-II levels were 96% higher in early postmenopausal women (mean age 51 years) than in age-matched premenopausal women, although OA was not assessed in that report (23). In our study, postmenopausal women with no spine disc degeneration, no radiologic OA, and no clinical hand OA also had, on average, 33% higher CTX-II levels than did the 147 healthy premenopausal women in the same OFELY cohort (data not shown). Thus, menopause appears to induce an increase in CII turnover independently of degenerative joint diseases, probably resulting from estrogen deprivation (23). Although the measurement of urinary CTX-II was able to discriminate groups of women with degenerative joint disease at different locations, clearly the overlap of the distribution is too large to accurately identify individual women with spine DSN, knee OA, or clinical hand OA based on this biochemical marker. In this report, as in previous studies, radiologic OA was frequently combined with prevalence and progression of spine disc degeneration, and there is a possible genetic relationship between generalized OA and spine disc degeneration (32,36). Thus, the association between increased urinary CTX-II levels and a higher rate of progression that we previously reported in patients with knee (18) or hip (19) OA may, in part, be confounded by the higher prevalence of concomitant spine disc degeneration in those patients with the highest CTX-II levels. It should, however, be noted that in patients with knee OA and in those with hip OA, a direct relationship between CTX-II levels and joint space narrowing of the signal joint was demonstrated.

Our study has strengths and some limitations. We investigated a large, well-characterized sample of postmenopausal women representative of the general Caucasian population. We assessed degenerative disease at the major skeletal sites, i.e., spine, knees, and hands. We analyzed one of the most specific and sensitive biochemical markers of cartilage degradation. Limitations of our study included its cross-sectional design and the fact that we did not obtain radiographs of the hands and hips, and thus could not adequately assessed OA at these 2 locations. For hands, we performed a careful clinical examination, which, in addition to Heberden’s nodes, included Bouchard’s nodes and swelling of the first carpometacarpal joint. Although this clinical assessment has been shown to correlate with radiologic hand OA (27), agreement between the presence of Heberden’s nodes and radiographic OA is poor (37,38). Consequently, we may have underestimated or overestimated the true association of hand OA with knee OA and spine disc degeneration. Finally, this study was performed in a subset of the original OFELY cohort, mainly because we analyzed only postmenopausal women and excluded...
ACKNOWLEDGMENT

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REFERENCES


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