Urinary type II collagen C-telopeptide levels are increased in patients with rapidly destructive hip osteoarthritis

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Objective: To compare type II collagen degradation using a new urinary specific marker in patients with rapidly destructive and those with slowly progressive hip OA.

Methods: Twelve patients with rapidly destructive and 28 patients with slowly progressive hip OA were included in a prospective, cross sectional case-control study. Urinary levels of C-terminal crosslinking telopeptide of collagen type II (CTX-II) as a marker of cartilage degradation were measured by an ELISA, and urinary free deoxypyridinoline (free DPD), a marker of bone resorption, was measured by high performance liquid chromatography. One *x* ray evaluation of the hips and urine samples was made in all patients when the diagnosis of OA was established.

Results: Patients with hip OA had higher mean (SD) urinary CTX-II levels than 65 healthy age matched controls (492 (232) *v* 342 (141), p<0.001), but no significant difference was seen for urinary free DPD (p=0.30). Increased urinary CTX-II, but not urinary free DPD, correlated significantly with decreased minimum joint space width assessed by radiograph of the hip. Mean urinary CTX-II levels were significantly higher in patients with rapidly progressive OA than in the slowly progressive group (612 (218) *v* 441 (221), p=0.015), whereas no significant difference of urinary free DPD was seen between the two groups (p=0.55).

Conclusion: Patients with hip OA have increased CTX-II degradation as assessed by a new urinary marker. Increased urinary CTX-II levels are associated with rapidly destructive disease, suggesting that this marker might be useful in identifying patients with hip OA at high risk for rapid progression of joint damage.

The hallmark of osteoarthritis (OA), the most common joint disease, is cartilage loss leading to joint destruction. Hip OA, one of the most common forms of OA, is associated with significant morbidity.[1] To assess the progression of cartilage destruction the most established method is the measurement of joint space width (JSW) by plain radiography. This technique, however, has some limitations. When there is radiological evidence of OA, significant joint damage has often already occurred. Because changes of JSW are relatively small compared with the precision error of *x* ray measurements, at least one year and preferably two years are usually necessary to assess accurately the progression of joint damage or its reduction by treatment.[2] Clearly, for identifying patients at high risk for destructive OA and for monitoring drug efficacy there is a need for non-invasive methods that can be repeated and have better sensitivity than plain radiography.

Molecular markers are molecules, or fragments thereof, of connective tissue matrices which are released into biological fluids during tissue biosynthesis and turnover and which can be measured by immunoassays. Several molecular markers of bone, cartilage, and synovium have been described and their changes have been investigated in patients with OA, mainly in cross sectional studies (reviewed by Garnero *et al*[3]). Because the loss of articular cartilage, the hallmark of OA, is believed to result mainly from increased degradation[4 5] and because type II collagen is the most abundant protein of cartilage matrix, the assessment of type II collagen breakdown is an attractive approach for the investigation of OA.

To assess type II collagen degradation, immunoassays using antibodies recognising either neo-epitopes generated by denaturation of the triple helix domain of type II collagen[6 7] or crosslinked fragments of the telopeptides[8 9] have been recently developed. Using an assay recognising C-terminal crosslinking telopeptide of type II collagen (CTX-II) in urine,[8] we recently found increased levels of urinary CTX-II in patients with knee OA,[10 11] which correlated with the progression of joint damage.[11]

Only a few studies have evaluated the potential value of molecular markers in patients with hip OA and none of them investigated type II collagen degradation. This study aimed at comparing urinary CTX-II levels—a new marker of cartilage degradation—in patients with rapidly destructive and slowly progressive hip OA.

SUBJECTS AND METHODS

Patients with hip osteoarthritis and healthy controls

This prospective cross sectional case-control study was conducted in 40 patients (23 women, 17 men, mean (SD) age 64 (12) years; range 44–95) who met the American College of Rheumatology criteria for primary hip OA.[12] They were recruited from our outpatient clinic and included all subjects who consulted for symptomatic hip OA between 1996 and 1999. Among the 62 patients for whom two hip *x* ray results at a one year interval and urine samples at the second radiograph were available, 12 fulfilled the criteria for rapidly progressive disease and 28 were selected because of a very low joint space narrowing progression. Rapidly progressive hip OA was defined by the following five criteria: (*a*) severe hip pain; (*b*) symptom onset within the past two years; (*c*) annual rate of joint space loss >1 mm; (*d*) erythrocyte sedimentation rate

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Abbreviations: BMI, body mass index; CTX-II, C-terminal crosslinking telopeptide of type II collagen; DPD, deoxypyridinoline; JSW, joint space width; OA, osteoarthritis

<20 mm/1st h; and (*e*) absence of detectable inflammatory or crystal induced joint disease, according to the criteria proposed by Lequesne and Ray[13] and used in a previously published study.[14] Examination of the hip arthroplasty specimens confirmed the diagnosis of OA in all 12 cases.

The remaining 28 patients had slowly progressive hip OA defined as ⩽0.20 mm joint space loss during the past year; 0.20 mm being the threshold of detection of relevant change in joint space width (JSW) with the method used in our study.[15] All the patients were prospectively followed up in the department of rheumatology for common hip OA and were monitored for more than one year. They all had at least two pelvic radiograph examinations one year apart, showing a hip joint space narrowing progression of <0.2 mm/year (mean −0.13 mm; from −0.20 to +0.13 mm), allowing them to be considered as slow progressors. Patients with hip OA secondary to alternative arthropathies (that is, infectious or inflammatory arthritis, Paget's disease, aseptic osteonecrosis, and a major congenital abnormality such as congenital dislocation of the hip) were excluded. At entry a full clinical history was taken from all patients and the following information obtained: height, weight, body mass index (BMI), disease duration. Patients in the two groups were following various drug regimens of analgesics and non-steroidal anti-inflammatory drugs, but none of the patients presented clinically detectable disease or were receiving treatment including bisphosphonates[16] that might have interfered with the levels of urinary markers investigated in this study. Furthermore, all women were postmenopausal and none of the patients were receiving treatments that might interfere with bone metabolism, including oestrogen replacement. In each patient with hip OA, all clinical parameters, *x* ray data used in this analysis, and urine samples were collected once on a single study day. In patients with rapidly progressive hip OA, this was the day on which the diagnosis of rapidly progressive disease was established. Patients with a slowly progressive disease were receiving follow up at six month intervals. The study day occurred after at least one year of follow up in order to establish that the annual rate of joint space loss was <0.20 mm, and urine samples were collected at the time of the second hip *x* ray evaluation.

Healthy control subjects comprised 38 postmenopausal women (mean (SD) age 63.2 (8.1) years, range 50–80) and 27 men aged 62 (8.2) years (range 53–79). Menopausal status was defined as the absence of menses for at least 12 months. Healthy women and men were randomly selected from two large population based cohorts taking part in prospective studies on the determinants of bone loss in women (OFELY study) and men (MINOS study).[17] [18] The cohort of the OFELY study comprises 1039 healthy female volunteers, aged 31–89 years, randomly selected from affiliates of the section of a health insurance company (Mutuelle Générale de l'Education Nationale) from the Rhône district in France. The cohort of the MINOS study comprises 842 healthy male volunteers, aged 50–85 years, randomly selected from the affiliated section of a health insurance company (Société de Secours Minière de Bourgogne) in Montceau les Mines, a town located in the same region as that of the healthy women and of the patients with hip OA. All healthy women and men had no evidence of symptomatic OA as assessed by clinical examination of the hands performed by an experienced rheumatologist and by asking the following question: "Has a doctor ever told you that you have osteoarthritis". Furthermore, *x* ray films of the thoracic and lumbar spine were obtained in all subjects. Spine films were graded with a standard atlas to document the severity of disc degeneration and osteophyte formation using the method of Lane *et al*, with a grade of 0 as normal, 1 for mild narrowing and or mild osteophytes, 2 moderate-severe (2–3) narrowing and or moderate-severe (2–3) osteophytes.[19] Subjects with a grade of 2 (moderate-severe) were excluded from the control group. All subjects were healthy without any disease or treatment that might interfere with bone or joint metabolism, including hormone replacement therapy in postmenopausal women.

Radiological variables

An anteroposterior radiograph of the pelvis was taken in patients with hip OA on the day of urine collection. A standardised procedure was used: patient in the standing position with the feet turned inward 20°, focal distance 100 cm, and beam aligned on the upper edge of the pubic symphysis. JSW at the narrowest point of the two hips was measured by a digitised image analysis method which has been previously validated.[20] [21] The hip most affected was considered as the target hip. Measurements were made by the same experienced observer. Briefly the radiograph to be analysed was digitised at a resolution of 600×1200 ppi (giving a pixel size of 0.004 mm) and 4096 grey levels. Subtraction and magnification were performed to obtain a very clear outline of the joint space. The joint space contours were delineated with the mouse on the superior convex margin of the femoral head and the inferior margin of the acetabulum within an angle whose summit was the centre of the femoral head (automatically given by the computer from three peripheral points drawn using the mouse) and that included the whole superior joint space from the internal boundary to the external end of the acetabulum. Interbone distance at the narrowest point of the joint space was automatically given by the computer.

Biochemical measurements

Fasting second morning void urine samples were collected in plastic containers. After mixing the whole collection, aliquots of urine were transferred into plastic tubes and frozen at −70°C without acidification. Samples were obtained from controls in the same way. All biological samples were kept frozen at −70°C until assayed.

Molecular marker of cartilage and bone degradation

Type II collagen degradation was assessed by measuring CTX-II using an enzyme linked immunosorbent assay (ELISA) based on a monoclonal antibody raised against a linear six amino acid epitope of the type II collagen C telopeptide.[9] Intra- and interassay coefficients of variation are lower than 8% and 10%, respectively.[9]

Urinary free deoxypyridinoline (free DPD) was measured on non-hydrolysed samples by high performance liquid chromatography as previously described.[22] Intra- and interassay coefficients of variation are lower than 11% and 15%, respectively.

Statistical analyses

All data are expressed as mean (SD) unless otherwise specified. Distribution of urinary CTX-II and free DPD was not normal in both the healthy controls and the patients with hip OA. Consequently, urinary CTX-II and free DPD levels were logarithmically transformed before analyses. Differences in urinary CTX-II and urinary free DPD between controls and between patients with rapidly progressive and slowly progressive hip OA were assessed by unpaired Student's *t* test. Correlations were evaluated by linear regression analyses. Patients with a high rate of cartilage degradation were identified by separating them according to urinary CTX-II levels, using as a cut off point the mean + 1SD of healthy controls, as previously suggested.[11] Before the start of the study no data on urinary CTX-II in patients with OA were available. Retrospectively, with our sample size, we had 90% power to detect an increase of CTX-II between the overall group with hip OA and controls and between the groups with slowly and rapidly destructive hip OA at a significance level of 0.05.

Table 1 Baseline characteristics of patients with hip osteoarthritis and healthy controls. Results are shown as mean (SD)

Parameters	Hip OA (n=40)			Controls (n=65)	Overall hip OA v controls (p value)
	Rapidly destructive (n=12)	Slowly progressive (n=28)	Rapidly v slowly progressive (p value)		
Sex (F/M)	7/5	16/12	0.79	38/27	0.93
Age (years)	70 (8)	63 (9)	0.03	63 (8)	0.81
Weight (kg)	73 (11)	70 (16)	0.85	69 (14)	0.49
Height (cm)	162 (9)	165 (11)	0.49	161 (9.0)	0.14
Body mass index (kg/m^2)	27.5 (3.4)	25.6 (4.2)	0.13	26.2 (3.7)	0.99
Bilateral/unilateral hip OA	6/6	7/21	0.07	–	
Minimal joint space width (x ray, mm)	0.63 (0.67)	1.73 (1.24)	0.008	–	
Urinary CTX-II (ng/mmol Cr)	612 (218)	441 (221)	0.015	342 (141)	<0.001
Urinary free DPD (nmol/mmol Cr)	3.34 (1.02)	3.20 (1.39)	0.55	3.5 (0.9)	0.30
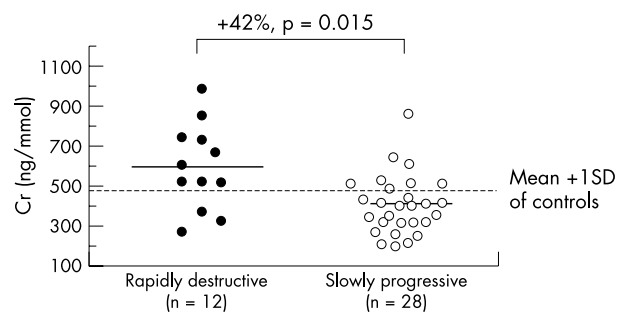
Figure 1 Individual values of urinary CTX-II in patients with hip OA with a rapidly or slowly progressive disease. The dotted line represents the upper limit of the value in the 65 healthy age matched controls defined as the mean value + 1SD. The solid lines represent the mean value in each group.

RESULTS

The patients with rapidly destructive and slowly progressive hip OA, considered as a single group, did not differ from controls in age, sex distribution, and BMI (table 1). There was no significant association between urinary CTX-II or free DPD and age in either the patients with hip OA or controls (data not shown). In patients and controls urinary CTX-II correlated moderately with urinary free DPD ($r=0.47$, $p=0.002$ and $r=0.45$, $p=0.04$ in hip OA and controls, respectively). Compared with healthy controls, the 40 patients with hip OA had increased urinary CTX-II (492 (232) v 342 (141) ng/mmol Cr, $p<0.001$) whereas urinary free DPD excretion did not differ significantly between the two groups ($p=0.30$) (table 1). When patients with rapidly and slowly progressive hip OA

were considered separately, both groups had significantly higher urinary CTX-II levels than healthy controls ($p<0.001$ and $p=0.015$ for rapidly and slowly progressive hip OA, respectively) (table 1). Patients with rapidly progressive hip OA were older than the total population of healthy controls (table 1). When urinary CTX-II levels of patients with rapidly progressive hip OA were compared with those of a subgroup of controls matched for age (n=41, mean age 70 (5) years), the patients with hip OA still had significantly increased values (612 (218) v 370 (67) ng/mmol Cr, $p<0.001$).R

As shown in table 1, patients with rapidly destructive hip OA were older and had a more severe joint space narrowing than those with slowly progressive OA but did not differ in the ratio of men to women and BMI. The proportion of patients with a bilateral disease was higher (50% v 25%) in those with a rapidly destructive disease, although the difference did not reach significance ($p=0.07$).

Patients with rapidly progressive hip OA had 40% higher urinary CTX-II levels ($p=0.015$) than those with a slowly progressive disease (table 1, fig 1), whereas no significant difference was observed for free DPD levels (table 1). In the whole group of patients with hip OA, increased urinary CTX-II ($r=-0.48$, $p=0.003$), but not free DPD ($r=-0.17$, $p=0.32$), was associated with decreased minimal JSW; JSW was assessed by x ray examination of the most affected hip on the day diagnosis was established and the day when urine samples were collected (fig 2). Patients with hip OA were then separated into those with low and high urinary CTX-II, using as a cut off point the mean + 1SD of the healthy controls. When that cut off point was used, 17/40 patients with hip OA were identified as having high urinary CTX-II levels. Among patients with a rapidly destructive disease, 9/12 (75%) had increased urinary
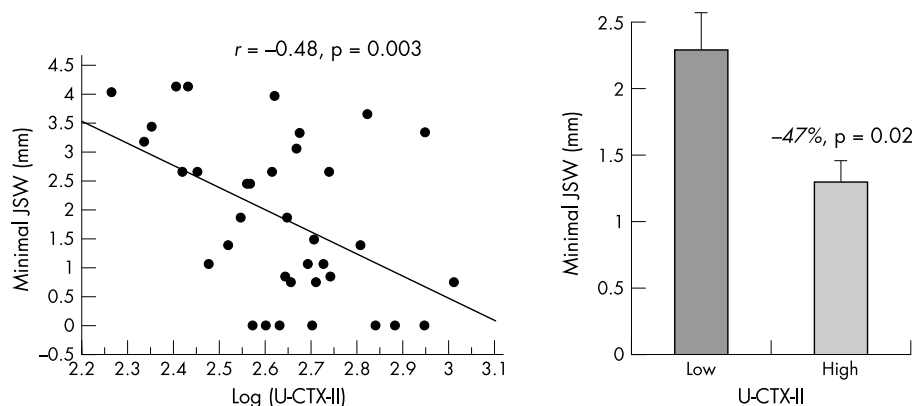
Figure 2 Relationship between urinary CTX-II levels and minimal joint space width (JSW) in patients with hip osteoarthritis (OA). The left panel shows the correlation between logarithmic transformed urinary CTX-II levels (x axis) and minimum joint space width of the hip (y axis). In the right panel, patients with hip OA were categorised into those with low and high urinary CTX-II levels using as a cut off point the mean + 1SD of healthy controls. The bars represent the mean value (SEM) of the minimal JSW in patients with low or high urinary CTX-II levels.

CTX-II levels, whereas only 8/28 (29%) with a slowly progressive hip OA had high CTX-II levels (χ^2=5.6, p=0.015, fig 1).

DISCUSSION

In this study, using a newly developed urinary molecular marker, we found that patients with hip OA had increased type II collagen degradation compared with healthy age matched controls. More importantly, among patients with hip OA, those with rapidly progressive hip OA had 40% higher levels than patients with a slowly progressive disease.

As far as we know this is the first study reporting increased type II collagen degradation in patients with hip OA using a new highly specific urinary marker. These data are in agreement with recent findings showing increased levels of urinary CTX-II in patients with knee OA.[10 11] In contrast, no significant difference of urinary free DPD was found between patients with hip OA and healthy controls. Conflicting data have previously been reported for bone resorption markers in patients with OA, some studies finding increased levels,[23–26] other showing no difference[27] or even depressed levels,[10] although most studies investigated patients with knee and not hip OA.

One of the main potential uses of biochemical markers is to identify patients at high risk for rapid progression of joint destruction. Indeed, clinical indices such as pain and physical function score are poorly related to the destruction of joint structure. When patients with hip OA were considered as a single group, we found that increased levels of urinary CTX-II were associated with decreased minimal JSW, suggesting that a sustained increased rate of type II collagen destruction, as assessed by this specific urinary marker, would lead to more rapid destruction of cartilage. Such findings are corroborated by the 40% higher levels of urinary CTX-II in patients with a rapidly destructive disease than in those with a slowly progressive disease. Recent studies have shown that among different molecular markers of bone, cartilage, and synovium turnover, urinary CTX-II was the most predictive of the progression of joint damage in patients with knee OA.[10 11] We found no significant association between the urinary excretion of free DPD, a specific marker of bone resorption, and joint damage in agreement with the recent findings we obtained in patients with knee OA.[10] Levels of bone resorption markers mainly reflect the overall skeletal change of bone resorption, which can be altered by various factors besides abnormalities of the subchondral bone turnover.[3]

Very few studies have looked at the prognostic values of molecular markers in patients with hip OA. Serum C reactive protein, measured by a highly sensitive immunoassay, has been reported to be increased in patients with a rapidly destructive disease.[14] Increased serum cartilage oligomeric matrix protein[28] and decreased levels of the tissue inhibitor of matrix metalloproteinases-1[29] were reported to be associated with more rapid progression of hip OA in one year studies, whereas no significant association was found with serum bone sialoprotein[14] or hyaluronic acid.[29] However, in these previous studies, the markers investigated were not specific for cartilage,[3] and none of them evaluated the degradation of type II collagen molecules, which form the framework of articular cartilage.

Our study has some limitations. The most important limitation is its cross sectional design and thus we could not investigate whether baseline urinary CTX-II might predict progression of joint damage. The number of patients was limited and clearly these data need to be confirmed in larger longitudinal studies. We did not perform x ray evaluation of the knees, spine, and hands in patients with hip OA, although none of them had symptomatic OA of these joints. Thus we could not investigate the potential contribution of these other joints to the urinary levels of the markers. Similarly, we did not perform x ray evaluation of the hips, knees, and hands

of the healthy controls and did not exclude subjects with grade 1 spine OA because of its high prevalence in a population of that age. Consequently, we cannot exclude the possibility that some of the controls had OA. However, this would have resulted in an underestimation of the difference in urinary CTX-II levels between patients with hip OA and controls.

In conclusion, using a newly developed molecular marker, we found that patients with hip OA are characterised by increased type II collagen degradation. Increased urinary CTX-II levels were found in patients with a rapidly destructive disease, suggesting that this molecular marker might be useful for identifying patients with hip OA at high risk of rapid joint destruction.

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