

MARKERS OF TYPE II COLLAGEN SYNTHESIS AND DEGRADATION AS A PREDICTOR OF RADIOGRAPHIC PROGRESSION IN KNEE OSTEOARTHRITIS

**Yasser A. Abd El-Hamid MD, Khaled M. Belal MD*,
Azza Abo Senna MD* and Tohamy, H. El-Kholy MD****

*Rheumatology & Rehabilitation, *Clinical Pathology and **Radiodiagnosis Departments; Faculty of Medicine; Benha University, Egypt*

Abstract

The aim of this study was to evaluate serum PIIANP and urinary CTX-II as a parameters of type II collagen synthesis and degradation, respectively, in patients with OA knees and to investigate whether the use of these two molecular markers could predict the progression of joint damage evaluated by radiography during a period of 3 years.

Sixty patients had symptomatic primary knee OA of Kellgren-Lawrence (K-L) grade I-III and met ACR criteria. These patients were evaluated prospectively for 3 years. Serum PIIANP and urinary CTX-II levels were measured by ELISA at baseline and at study end and their levels compared according to the changes in joint space width (JSW), K-L grade and WOMAC index, over 3 years. Also, we assessed the diagnostic value of those molecular markers and their performance for prediction of radiological progression. Serum and urinary levels also compared with 40 matched healthy subjects as a control group.

There were significant decrease in the baseline serum PIIANP ($P < 0.001$) and increase in the baseline urinary excretion of CTX-II ($P < 0.001$) in knee OA patients in comparison with the control, in bilateral than unilateral cases ($P < 0.05$), ($P < 0.05$) and also with increasing the K-L radiological severity of the disease ($P < 0.05$), ($P < 0.001$), respectively. There were significant decrease in the mean baseline serum PIIANP and highly significant increase in the mean baseline urinary excretion of CTX-II in progressors (JSW narrowing ≥ 0.5 mm) and in patients showed increase in K-L grading either of the signal or both knees ($P < 0.05$), ($P < 0.001$), respectively. There were significant decrease in the mean

study end serum PIIANP and highly significant increase in the mean study end urinary excretion of CTX-II in progressors (JSW narrowing ≥ 0.5 mm) and in patients showed increase in K-L grading either of signal or both knees ($P < 0.05$), ($P < 0.001$), respectively. There were insignificant correlation between serum PIIANP and urinary CTX-II either at the baseline or study end and also insignificant correlation between those molecular markers with disease duration, BMI and WOMAC index ($P > 0.05$). Urinary CTX-II showed a higher diagnostic sensitivity and specificity (75% - 92%) than serum PIIANP (60% - 90%), respectively. The diagnostic specificity was greatest when both tests were found in combination (96%). Also, combination of tests showed higher diagnostic sensitivity (92.3%) and specificity (55.3%) for predicting the radiological progression over 3 years than either one alone.

In conclusion: using specific molecular markers serum PIIANP and urinary CTX-II, we found that patients with knee OA are characterized by depressed type II collagen synthesis and increased type II collagen degradation. Combining these two molecular markers allows the identification of patients with a high risk of subsequent progression of joint damage.

Introduction

والمعنى ←
The hallmark of osteoarthritis (OA), the most common joint disease, is cartilage loss leading to joint destruction. Knee OA, one of the most common forms of OA, is associated with significant morbidity (Garnero and Delmas., 2003). To assess the progression of cartilage destruction, the most established method is the measurement of joint space width (JSW) using plain radiographs. However, when there is radiographic evidence of OA, there is

often already significant joint damage (Ravaud et al., 1998). Clearly, for identifying patients at high risk for destructive OA and for monitoring drug efficacy, there is a need for noninvasive method that can be repeated and that have improved sensitivity compared with plain radiographs (Garnero et al., 2002).

Molecular markers are molecules or fragments of connective tissue matrices which are released into biologic fluids during the pro-

Type II A procollagen amino terminal propeptide

cess of tissue biosynthesis and turnover and which can be measured by immunoassays (Punzi et al., 2005). 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 (Garnero et al., 2000); (Solignac, 2004); (Garnero et al., 2005); (Jordan et al., 2006). Because the loss of cartilage is believed to result from the combination of a decreased reparative process and an increased degradative phenomenon (Poole, 1997), thereby limiting the capacity for cartilage repair (Kim et al., 1991) and because type II collagen is the most abundant protein of cartilage matrix, the assessment of type II collagen synthesis and degradation is an attractive approach for the investigation of OA. Actually, in vitro studies performed on cartilage tissue from patients with OA and controls have shown both altered synthesis (Nelson et al., 1998) and increased degradation (Hollander et al., 1994); (Hollander et al., 1995) of type II collagen in OA.

as a procollagen molecule including the N- and C-propeptides (PIINP and PIICP, respectively) at each end. Type II procollagen is produced in two forms as the result of alternative RNA splicing (Ryan and Sandell, 1990). One form (IIA) includes and the other form (IIB) excludes a 69-amino-acid, cysteine-rich globular domain encoded by exon 2 in the PIINP. On the one hand, Type IIB procollagen is expressed at high levels in well-differentiated chondrocytes, forming the framework of normal adult cartilage. On the other hand, type IIA procollagen is temporally expressed in early cartilage (Oganesian et al., 1997) and can be re-expressed later in development at the onset of cartilage hypertrophy (Nah et al., 2001). In addition, it has been shown that type IIA procollagen is re-expressed by adult articular chondrocytes of affected human OA cartilage (Aigner et al., 1999). During secretion and before incorporation of type II collagen molecules into cartilage matrix, the N- and C-propeptides are removed by specific enzymes and released in part into the synovial fluid and cleared into the blood. The serum levels of

Type II collagen is synthesized

*splicing: removal of RNA sequences (introns) that don't code for protein from primary Transcript
Exons: code for protein*

Telopeptide: portions of a.c.a sequence of a protein that are removed in maturation of protein
ex: N₂ & C-terminal telopeptide of procollagen

Yasser A. Abd El-Hamid, et al....

these propeptides are thus believed to represent an adequate index of the rate of type II collagen synthesis (Garnero and Delmas, 2003). To assess type II collagen degradation, immunoassay have been developed that use antibodies recognizing crosslinked fragments of the C-terminal telopeptide in urine (Christgau et al., 2001).

crosslinked C-terminal telopeptide

The aim of this study was to evaluate serum PIIANP and urinary CTX-II as a parameters of type II collagen synthesis and degradation, respectively, in patients with OA knees and to investigate whether the use of these two molecular markers could predict the progression of joint damage evaluated by radiography during a period of 3 years.

Patients and Methods

Sixty patients had symptomatic primary knee OA were recruited to this 3 years prospective study. All fulfilled the criteria of the American College of Rheumatology (ACR) (Altman et al., 1986). They were selected from the attendant of the outpatient clinic of the Rheumatology and Rehabilitation

Department of Benha University Hospitals, they were followed up between May 2004 and April 2007. They were 45 females (75%) and 15 males (25%). Their ages ranged between 40 and 50 years, with a mean of 41.3 ± 3.2 years. Their disease duration ranged between 1 and 5 years, with a mean of 3.6 ± 1.5 years.

Each patient had a weight bearing post. ant. plain X-ray for both knees flexed at 30° (Schuss view) (Piperno et al., 1998) and scoring was done using Kellgren-Lawrence (K-L) scale (Kellgren and Lawrence, 1957). In case of bilateral knees OA, the patient was classified according to the grade of the worst knee. According to this grading all the patients at the entry were divided into 3 groups (grade I-III).

Joint space width (JSW) measurements and K-L grading were performed on both the baseline and end radiographs after 3 years by one radiologist (blinded to the patients date and chronology of the radiographs). Radiographic progression of joint destruction was defined as an

المرحلة الأولى من المرض

increase of ≥ 0.5 mm in joint space narrowing between baseline and study end.

The knee radiographs were digitized with an image analyzer (Mediscan, Hologic, Massachusetts, USA). The digitized images of the radiographs were prepared with a specially programmed computer system (VAIO, Sony, Tokyo, Japan). The digitized images were modified by the computer software using edge detection and magnification methods to obtain a very clear outline of the tibiofemoral joint space. The joint space contours were delineated using the computer mouse on the margin of the femur condyle and the margin of the tibial plateau according to the methods proposed by Buckland-Wright, (1999).

All the patients were interviewed to obtain the Western Ontario MacMaster (WOMAC) index, which is a multidimensional measure of disease severity. The WOMAC index assesses pain (five items), stiffness (two items) and physical functional activities (17 items) related to OA of the knees (Bellamy et al., 1988). In this

study, the WOMAC was used in its visual analogue scale (VAS) format. The range of WOMAC scores was: Function (0 - 170), pain (0 - 50) and stiffness (0 - 20).

The signal knee was defined without knowledge of the radiographic data as the worst of the two knees on the basis of subjective complaints and objective clinical findings. At entry, patients were also examined by a single rheumatologist, who clinically assessed three signs of knee joint inflammation (tenderness - non-bony swelling - warmth), on which basis a determination of synovitis was made.

Body mass index (BMI) was determined for all patients according to Lukaski, (1987).

As regard the medical treatment, all the patients were received short courses of non steroidal anti-inflammatory drugs (NSAIDs).

Forty apparently healthy subjects were selected as a control group, matched for age and sex with the patients. They were 29