

## RESEARCH ARTICLE

**Association of Cross Linked C-Telopeptide II Collagen and Hyaluronic Acid with Knee Osteoarthritis Severity**John Butar Butar<sup>1,\*</sup>, Zola Wijayanti<sup>1</sup>, Beatrix Tjahyana<sup>1</sup>, Veli Sunggono<sup>2</sup>, Hori Harianto<sup>3</sup><sup>1</sup>Orthopedic Department, Siloam Hospital Lippo Village, Jl. Siloam No.6 Lippo Karawaci 15811, Tangerang, Indonesia<sup>2</sup>Department of Public Health, Faculty of Medicine Universitas Pelita Harapan, Jl. Boulevard Jend. Sudirman Lippo Karawaci, Tangerang, Indonesia<sup>3</sup>Anesthetic and Intensive Care Department, Siloam Hospital Lippo Village, Jl. Siloam No. 6 Lippo Karawaci 15811, Tangerang, Indonesia

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## Abstract

**BACKGROUND:** This study was carried out to investigate the association of Cross Linked C-Telopeptide Type I & II Collagen (CTX-I and II) and hyaluronic acid (HA) with knee osteoarthritis (OA) severity.

**METHODS:** Sixty menopause women with primary knee OA were enrolled in this study during their visits to the Outpatient Department. Patients with knee pain during weight bearing, active or passive range of motion, or tenderness with Kellgren-Lawrence (KL) grade of more than I were included. Patients with injury, inflammatory and metabolic diseases were excluded. Patients were put in a 10-hour fasting prior to withdrawal of morning blood samples for examinations of HA, CTX-I, interleukin 1 beta (IL-1 $\beta$ ), and high sensitivity C reactive protein (hs-CRP) level. Second void morning urine specimens were taken for CTX-II assessment. HA, CTX-I and II levels were measured by enzyme-linked immunosorbent assay.

**RESULTS:** Sixty menopausal female patients were included in this study, 35 with KL grade II, 17 grade III, and 8 grade IV. Means of CTX-II were significantly different between subjects KL grade IV and III ( $p=0.021$ ). Correlation of KL grade was significant with CTX-II ( $p=0.001$ ,  $r=0.412$ ) and HA ( $p=0.0411$ ,  $r=0.269$ ). KL grades were not significantly associated with CTX-I ( $p=0.8364$ ,  $r=-0.0272$ ); IL-1 $\beta$  ( $p=0.5773$ ,  $r=0.0853$ ) and hs-CRP ( $p=0.2625$ ,  $r=0.1470$ ).

**CONCLUSION:** CTX-II and HA were associated with severity of knee OA, suggesting that CTX-II and HA can be used as marker for knee OA severity.

## Abstrak

**LATAR BELAKANG:** Untuk mengetahui hubungan antara *Cross Linked C-Telopeptide Type I & II Collagen* (CTX-I dan II), dan *hyaluronic acid* (HA) dengan keparahan *osteoarthritis* (OA) pada lutut.

**METODE:** Penelitian ini melibatkan 60 wanita menopause dengan OA lutut pada bagian rawat jalan, dengan keluhan nyeri lutut saat mengangkat beban, melakukan gerakan aktif dan pasif, atau tingkat kelembutan yang masuk dalam *Kellgren-Lawrence* (KL) grade lebih dari I. Pasien dengan luka, inflamasi dan penyakit metabolik dieksklusi. Pasien diminta untuk puasa selama 10 jam sebelum pengambilan darah di pagi hari untuk pemeriksaan kadar HA, CTX-I, *interleukin 1 beta* (IL-1 $\beta$ ) dan *high sensitivity C reactive protein* (hs-CRP). Sampel urin kedua di pagi hari diambil untuk pemeriksaan CTX-II. HA, CTX-I dan II diperiksa dengan *enzyme-linked immunosorbent assay*.

**HASIL:** Enampuluh wanita *menopause* mengikuti penelitian ini, 35 dalam kelompok KL tingkat II, 17 tingkat III, dan 8 tingkat IV. Rerata CTX-II berbeda bermakna antara subyek KL tingkat III dan IV ( $p=0,021$ ). Ditemukan korelasi yang bermakna antara tingkatan KL dengan CTX-II ( $p=0,001$ ,  $r=0,412$ ), dan HA ( $p=0,0411$ ,  $r=0,269$ ). Tingkat KL tidak berkorelasi bermakna dengan CTX-I ( $p=0,8364$ ,  $r=-0,0272$ ), IL-1 $\beta$  ( $p=0,5773$ ,  $r=0,0853$ ) dan hs-CRP ( $p=0.2625$ ,  $r=0.1470$ ).

**KESIMPULAN:** CTX-II dan HA berhubungan dengan tingkat keparahan OA lutut, sehingga CTX-II dan HA dapat digunakan sebagai penanda untuk keparahan OA.

**KEYWORDS:** CTX-II, hyaluronic acid, oosteoarthritis, knee

**KATA KUNCI:** CTX – II, asam hyaluronat, osteoarthritis, lutut

*Indones Biomed J. 2013; 5(3): 179-84*

## Introduction

As a chronic progressive disorder, knee osteoarthritis (OA) is marked by damage on cartilage and subchondral bone, and synovial thickening. The damage is a manifestation of imbalance between degradation and repair processes.(1) The extent of OA assessed by radiographic characteristics cannot be used in early diagnosis and prediction of progression due to low sensitivity. Therefore, developing a more sensitive indicator that can be used alongside with radiographic technique is important.(2)

Many efforts have been done to identify specific indicators using imaging and biomarker parameters.(3) Type I collagen, composed of nearly 90% organic matrix of bone, consists of triple helix of the two chains, alpha 1 and alpha 2. Level of urinary cross linked C-telopeptide type I collagen (CTX-I) was found higher in patients with progressive OA compared to non-progressive one and control.(4) C-telopeptide type II collagen (CTX-II) is a constituent of the articular cartilage and hyaline cartilage. Urinary CTX-II correlated with pain, malfunction, joint gap narrowing and subchondral sclerosis.(5) Hyaluronic acid (HA) is a precursor of synovium and cartilage. Increased levels of HA in the blood occurs in osteoarthritis.(6) Several studies have shown that HA and CTX-II related to the severity of OA(7-15), but one study reported no correlation was found(16). Most studies were done on Caucasian subjects.

## Methods

### Subjects

Sixty menopause women with primary knee OA were enrolled to this study. Patients with knee pain during weight bearing, active or passive range of motion, or tenderness with Kellgren-Lawrence (KL) grade of more than grade I were included. Subjects underwent weight-bearing postero-anterior (PA) radiography and weight-bearing lateral radiography. Patients with inflammatory joint disease such as rheumatoid arthritis were excluded.

### Blood Samples Collection and Assessment

Fasting morning blood samples were collected for HA, CTX-I and high sensitivity C-reactive protein (hs-CRP)

assessment. Serum HA was measured with enzyme-linked immunosorbent assay (ELISA) (Cat. No.: 029-001, Corgenix, Broomfield, CO). CTX-I was measured by  $\beta$ -Crosslaps ELISA with sensitivity of 10 pg/ml (Cat. No.: 11972308, Roche, Indianapolis, IN). Meanwhile high sensitivity C reactive protein (hs-CRP) was measured using Cobas Integra, with standard range  $\leq$  10 mg/L.

### Urine Samples Collection and Assessment

Morning second void urine specimens were taken for CTX-II assessment as recommended in a previous study based on diurnal and activity related variation(17,18). Samples were measured using Urine CartiLaps EIA with sensitivity 0.20 mg/ml (Immunodiagnostic Systems Ltd., Boldon, UK).

### Articular fluid

Articular fluid of minimum 1 mL was taken for Interleukin 1 beta (IL-1 $\beta$ ) analysis. Samples were measured using ELISA with sensitivity 1 pg/ml (R&D System, Minneapolis, MN).

### Radiographs

Subjects underwent weight-bearing PA knee radiography in mild flexion.(19) KL grading system was used to assess severity of OA, which included the formation of osteophytes, joint space narrowing, subchondral sclerosis and joint deformity. OA was classified based on KL grading into 5 grades: grade 0 (normal), grade 1 (doubtful OA), grade 2 (minimal OA), grade 3 (moderate OA), and grade 4 (severe OA). X-Ray was read by an orthopedic surgeon and a trained radiologist with inter-rater reliability kappa test 0.797.

### Data Analysis

Data analysis was done by SPSS version 20.0 (IBM Corp., Armonk, NY). Analysis of mean differences of biomarkers among the KL groups was done using Mann-Whitney U Test, and  $p < 0.05$  was defined as significant. The association between biomarkers and KL grades was analyzed using spearman correlation test.

## Results

Sixty menopausal female patients were included in this study. Thirty five subjects (58%) having KL grade II,

**Table 1. Distribution of Demographic, Radiographic and Laboratory Results.**

Variable	Mean ± SD	Minimum	Maximum
Age (year)	58.420 ± 9.803	42	83
BMI (kg/m <sup>2</sup> )	27.000 ± 4.480	20	39.26
Affected Knee			
Left (%)	32.5%		
Right (%)		67.5%	
KL Grade			
Grade II (n%)		35/ 58.3%	
Grade III (n%)	17/28.3%		
Grade IV (n%)		8/13.3%	
CTX -I (ng/mL)	0.480 ± 0.230	0.12	1.18
CTX-II (ng/mmol)	719.630 ± 438.560	107	2213
HA (ng/mL)	213.580 ± 227.940	57	1489
IL-1β (pg/mL)	2.110 ± 2.230	0	11.81
hs-CRP (mg/L)	4.190 ± 4.730	0.20	26.20

**Table 2. Mean Difference Test for Biomarkers in KL Group.**

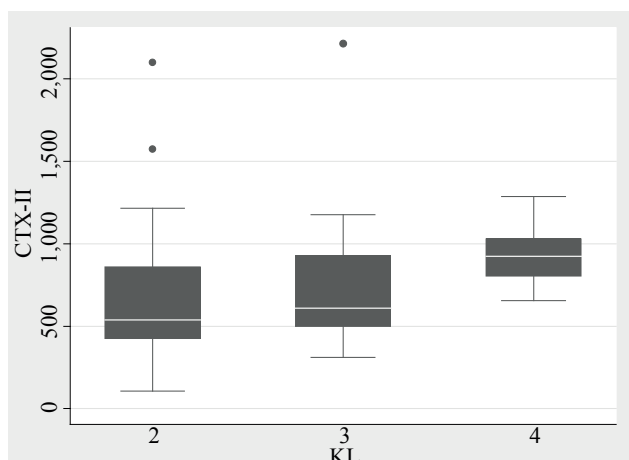
Variable	N	Mean ± SD	<i>p</i>
CTX-I			
KL 2***	35	0.490 ± 0.250	
KL 3	17	0.438 ± 0.210	0.5070*
KL 4	8	0.470 ± 0.180	0.4660**
CTX-II			
KL 2	34	506.940 ± 291.910	
KL 3	16	731.140 ± 428.460	0.0650*
KL 4	8	1004.900 ± 265.060	<b>0.0210**</b>
HA			
KL 2	34	158.800 ± 71.100	
KL 3	16	198.550 ± 103.590	0.2277*
KL 4	8	209.850 ± 93.420	0.1510**
IL-1β			
KL 2	23	2.120 ± 2.170	
KL 3	14	1.350 ± 0.430	0.4701*
KL 4	8	2.180 ± 1.500	0.0983**
hs-CRP			
KL 2	35	1.900 ± 2.520	
KL 3	17	5.420 ± 5.440	0.4293*
KL 4	8	4.270 ± 3.130	0.8714**

\*: Mann-Whitney U Test KL 3 vs. KL 2; \*\*: Mann-Whitney U Test KL 4 vs KL 3

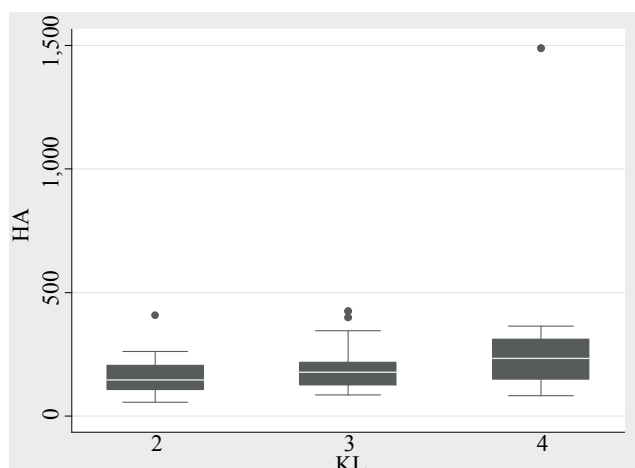
**Table 3. Correlation of Biomarker with Kellgren.**

Variable	KL	
	R	p value
CTX-I	-0.0272	0.8364
CTX-II	0.4120	<b>0.0010</b>
HA	0.2690	<b>0.0411</b>
IL-1 $\beta$	0.0853	0.5773
hs-CRP	0.1470	0.2625

17 subjects (28%) having KL grade III, and 8 subjects (13%) having KL grade IV. Two urine samples for CTX-II examination and two blood samples for HA examination were spoiled hence not eligible for analysis. Fifteen samples of articular fluid for IL-1 $\beta$  examination were not adequately taken, thus not eligible for analysis. The average age of the participants was 58.42 years (42-83 years old) and the average body mass index was 27 $\pm$ 4.48.



**Figure 1. Box Plot of CTX-II among the KL Groups.**



**Figure 2. Box Plot of HA among the KL Group.**

The mean differences of CTX-I among KL II, III, and IV were not significant,  $p > 0.05$  (Table 2). The mean of CTX-II in KL IV was 1.004, which was significantly higher (mean=1004.9) than that in KL III (mean=731.14,  $p = 0.021$ ). The result also showed the mean differences of HA levels in KL IV were higher (mean=209.85) than those in KL III (mean=209.85) ( $p = 0.151$ ). No significant mean was found between CTX-I and OA severity. We found no correlation between inflammatory biomarkers (IL-1 $\beta$ , hs-CRP) and OA severity.

The correlation of KL grade in Table 3 are shown significant with CTX-II ( $p = 0.001$ ,  $r = 0.412$ ) and HA ( $p = 0.0411$ ,  $r = 0.269$ ). Table 3 shows that KL grade was not significantly associated with CTX-I ( $p = 0.8364$ ,  $r = -0.0272$ ); IL-1  $\beta$  ( $p = 0.5773$ ,  $r = 0.0853$ ) and hs-CRP ( $p = 0.2625$ ,  $r = 0.1470$ ).

Figure 1 and 2 show the mean difference of CTX-II and HA among the KL grade II, grade III, and grade IV groups. The mean of CTX-II and HA are consistently increasing from KL grade II to grade III group, and from grade III group to grade IV group.

## Discussion

According to OA Biomarkers Network recommendation on osteoarthritis, study on biomarkers should be differentiated on its role in burden of disease, investigation, prognosis, efficacy and, diagnosis of OA.(20,21)

Burden of disease markers assess the severity or extent of disease, typically at a single point of time, among individuals with OA. This can be thought of as severity within articular joint, and/or severity in terms of number of joints involved.(20) Several studies have shown that CTX-II, HA, pyridinoline (PYD), cartilage oligomeric matrix protein (COMP), matrix metalloproteinase (MMP), tissue inhibitor of matrix metalloproteinase (TIMP), and YKL-D can be included in this category.(20,21)

This study showed that CTX-II was significantly correlated with knee OA severity. This result is in accordance to results of most studies (22,23,24,20,4). Conflicting results on CTX II was reported by Kalai who concluded that there was no significant correlation between CTX-II with a degree of joint gap narrowing.(16) Van Spil reported the lack of specificity of CTX-II as a possible cause, finding that changes in CTX-II were not pararel with other cartilage specific biomarkers changes.(25) This could also be due to differences in subgroup populations between studies.

The correlation of KL grade was significant with CTX-II and HA. KL grades were not significantly associated with

## CTX-I, IL-1 $\beta$ and hs-CRP.

In Mann-Whitney U test, no significant mean difference was found statistically between HA concentration and KL grade. These results are different compared to other studies. (7-11,14) We exclude the possibility of HA fluctuation due to inflammatory condition because no significant difference of IL-1 $\beta$  and hs-CRP between groups was found in this study. In our opinion, this conflicting result might be due to small sample size because the trend showed higher HA in KL 3 and KL 4.

The weakness of this study was that biomarkers fluctuation related to joint degradation process could not be adequately assessed by cross sectional method and small sample size might also affect result of the statistical analysis.

Serum levels of CTX-I, CTX-II urine and serum HA might not represent the actual level inside the affected joint, since most of the patients had bilateral knees involvement. To minimize the bias, in this study we excluded subjects with multiple joints involvement and only included those with higher KL score in patients with bilateral knee OA. Thus with those study limitations, results of this study may not represent the general population, in particular due to the small sample size.

## Conclusion

CTX-II and HA were associated with the severity of knee OA, suggesting that CTX-II can be used as a marker for knee OA severity.

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