

Differences of Urinary Cartilage Type II Collagen C-Telopeptide (CTX-II) and Calcium Levels in Osteoarthritis Patients Based on Severity

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Abstract: *Background: Osteoarthritis (OA) is a slow chronic disease characterized by the degradation of articular cartilage, causing joint space narrowing, loss of joint mobility and pain. Several studies have shown that urinary cartilage type II collagen C-Telopeptide (uCTX-II) is a promising biochemical marker in OA. OA is generally managed with palliative measures and symptomatic treatment, including calcium supplements. Calcium is the most abundant and second most abundant micronutrient that regulates neuromuscular function and skeletal mineralization of the body Objective: To determine the difference between Urinary Cartilage Type II Collagen CTelopeptide (uCTX-II) and Calcium levels in OA patients based on Severity. Methods: This research is an observational study with cross sectional data collection method. This study took blood samples from OA patients who were treated at the Polyclinic of the Department of Orthopedic Surgery and Traumatology Adam Malik General Hospital Medan. As many as 38 patients. Samples were checked for urine CTX II and Serum Calcium. The research was conducted after obtaining ethical approval and informed consent. Results and Discussion: This study was followed by 36 patients with OA. There were 30 female subjects (83.3%). The mean age of the subjects was 64.03 years with the youngest age being 42 years and age. Using the Anova test, it was found that there were significant differences in uCTX-II levels based on the severity of OA ($p=0.043$). Using the Kruskal Wallis test showed that there was no significant difference in calcium levels based on the severity of OA ($p=0.944$). Conclusions and suggestions: There was a significant difference in uCTX-II levels based on the severity of OA ($p=0.043$). On the other hand, Calcium was no significant differences based on the severity of OA ($p>0.005$). Further research needs to be done with a larger sample size and associated using other markers such as Hyaluronic Acid.*

Keywords: Osteoarthritis, u-CTXII, Calcium

1. Introduction

Osteoarthritis is by far the most common joint disease worldwide. This is very much related to age, in the elderly. Osteoarthritis (OA) is a progressive disease with a long period of time showing signs of cartilage degradation, mild to moderate synovial inflammation, and changes in bone structure, resulting in severe damage and impaired function of the affected joint. Osteoarthritis can generally be established based on clinical symptoms and radiographic criteria (Goldring et al, 2010). To detect Osteoarthritis during its early stages, accurate and reliable biomarkers are needed. Several biochemical markers used to detect inflammatory Osteoarthritis such as Urinary Cartilage Type II Collagen C-Telopeptide (uCTX-II), Osteocalcin have been proposed as viable biomarkers for monitoring Osteoarthritis; The incidence of disease or joint complaints in terms of nutrition is associated with calcium consumption habits associated with a person's diet. Osteoarthritis is generally managed with palliative measures and symptomatic treatment to reduce clinical symptoms including lifestyle changes, weight control, exercise, and administration of anti-inflammatory and analgesics along with calcium and vitamin D supplements to control vitamin and mineral deficiency during osteoarthritis (Mouritzen U, et al, 2003). Calcium and phosphorus are the most abundant and second most abundant micronutrients that regulate neuromuscular function and skeletal mineralization of the body. Our bones contain almost 99% calcium, which regulates vascular contraction, vasodilation, glandular secretion, muscle contraction, glycogen metabolism, nerve transmission, and maintenance of bone health and mineralization (Rousseau et al, 2012).

2. Purpose

Knowing the difference between Urinary Cartilage Type II Collagen C-Telopeptide (uCTX-II) and Calcium levels in Osteoarthritis (OA) patients based on Severity.

3. Research Methods

This research was conducted in a cross-sectional way. Sampling was done by consecutive sampling, where the number of samples was limited to a minimum according to the estimated number of samples or until the specified sample collection time limit. Variable measurements were carried out only once. This research was conducted at the Department of Clinical Pathology, FK USU, H. Adam Malik Hospital, Medan in collaboration with the Department of Orthopedic Surgery and Traumatology, Faculty of Medicine, University of North Sumatra / H. Adam Malik Hospital, Medan and Setia Budi Hospital, Medan in the selection of osteoarthritis patients starting from August 2021 to February 2022. The research sample is Osteoarthritis patients who have been diagnosed by an Orthopedic Surgeon and Traumatology Specialist at RSUP H. Adam Malik Medan and Setia Budi Hospital Medan and according to the inclusion and exclusion criteria U-CTX II examination of urine and serum calcium was carried out at the Department of Clinical Pathology USU / H. Adam Malik Hospital Medan using the *Chem-well Analyzer* with the *Sandwich ELISA* method and ARCHITECH ci 4100 tool using the *Arsenazo III* method.

4. Statistical Analysis

Data analysis was performed using SPSS (Statistical Package for Social Sciences, Chicago, IL, USA) software for Windows. The description of the characteristics of the research subjects is presented in tabulated form and described. After the normality test with the Independent T test was carried out for the mean levels of Urinary Cartilage type II collagen C-telopeptide (uCTX-II) and serum calcium, the Man Whitney test was used because the data were not normally distributed. All statistical tests with p value <0.05 were considered significant.

5. Results

This study was followed by 36 patients with osteoarthritis who went to the Polyclinic of the Department of Orthopedic Surgery and Traumatology H. Adam Malik Hospital Medan and the Polyclinic of Orthopedic Surgery and Traumatology Setia Budi Hospital Medan. All patients included in this study met the inclusion criteria. There were 30 female subjects (83.3%) and 6 male subjects (16.7%). The mean age of the subjects was 64.03 years with the youngest age being 42 years and the oldest being 80 years. The subjects' mean BMI was 27.5 kg/cm². Based on the categorization of BMI values, there were 26 people (72.2%) including obesity.

A total of 17 subjects (47.2%) were housewives (IRT). All subjects had complaints of knee pain. The most history of disease was hypertension, amounting to 19 people (52.8%). Of the 30 female subjects, almost all of them as many as 29 people (96.7%) had experienced menopause. Based on the assessment of severity according to Kellgren and Lawrence, most of the subjects amounting to 17 people (47.2%) were in severe severity / grade IV.

Table 1: Characteristics of Research Subjects

Subject Characteristics	n = 36
Gender, n (%)	
Man	6 (16.7)
Woman	30 (83.3)
Age, years	
Average (SD)	64.03 (8.49)
Median (Min – Max)	64 (42 – 80)
Weight, kg	
Average (SD)	66.86 (10.84)
Median (Min – Max)	69 (47 – 100)
Height, cm	
Average (SD)	155.56 (5.87)
Median (Min – Max)	155 (142 – 170)

Average (SD) Median (Min – Max)	27.5 (3.26)
Nutritional Status, n (%)	27.96 (21.33 – 34.6)
Normal	3 (8.3)
Obesity	26 (72.2)
Overweight	7 (19.4)
Occupation, n (%)	
Civil servant	6 (16.7)
Self-employed	7 (19.4)
Farmer	6 (16.7)
IRT	17 (47.2)
Symptoms and Sign, n (%)	
Knee pain	36 (100)
Medical History, n (%)	

Diabetes Mellitus	3 (8.3)
Hypertension	19 (52.8)
Hypercholesterolemia	1 (2.8)
No Risk Factor	13 (36.1)
Severity according to Kellgren and Lawrence, n (%)	
Mild	12 (33.3)
Moderate	7 (19.4)
Severe	17 (47.2)

Of the 36 samples, 30 were female (83.3%) and 6 were male (16.7%).

Table 2: Total Sample of Menopausal Women

Woman	n = 30
Menopause, n (%) No	1 (3.3)
Yes	29 (96.7)

Table 3: Calcium and uCTX-II Levels in Osteoarthritis Patients

Variable	n = 36
Calcium Level, mg/dL	
Average (SD)	8.93 (1.49)
Median (Min – Mak)	9 (2 – 10.6)
uCTX-II, ng/mL	
Average (SD)	5.07 (1.74)
Median (Min – Mak)	4.76 (2.05 – 8.85)

Table 4 shows calcium levels in patients with osteoarthritis based on the severity of osteoarthritis. Calcium levels based on the severity of osteoarthritis did not seem much different. In osteoarthritis patients with mild severity showed a mean calcium of 9.38 mg/dL (SD = 1.118 mg/dL), in moderate degrees with a mean of 9.06 mg/dL (SD = 0.44 mg/dL) and weight with the lowest mean of 8.62 mg/dL (SD = 2.35 mg/dL). Using the Kruskal Wallis test showed that there was no significant difference in calcium levels based on the severity of osteoarthritis (p = 0.934).

Table 4: Differences in Calcium Levels by Severity of Osteoarthritis Calcium, mg/dL

Severity (SD)	n	Average	Median (min-Mak)	p*
Mild	12	6.02 (1.42)	6.13 (3.94-8.85)	0.043a
Moderate	7	4.24 (2.36)	3.3 (2.05-7.81)	
Severe	17	4.77 (1.27)	4.6 (3.09-7.37)	

*Kruskal Wallis

Table 5: Differences in uCTX-II Levels by Severity of Osteoarthritis

Severity Degrees (SD)	n	uCTX-II, ng/mL			Posthocb	
		Average	Median (min-Mak)	p	Currently	Heavy
Mild	12	6.02 (1.42)	6.13 (3.94-8.85)	0.043a	0.023	0.044
Moderate	7	4.24 (2.36)	3.3 (2.05-7.81)			0.454
Severe	17	4.77 (1.27)	4.6 (3.09-7.37)			

^aAnova, ^bLSD

Table 5 shows the levels of uCTX-II in patients with osteoarthritis based on the severity of osteoarthritis. In osteoarthritis patients with mild severity, the mean uCTX-II was 6.02 ng/mL (SD = 1.42 ng/mL), in moderate grade it was 4.24 ng/mL (SD = 2.36 ng/mL).) and in severity with a mean of 4.77 ng/mL (SD = 1.27 ng/mL). Using the Anova test, it was found that there were significant differences in

uCTX-II levels based on the severity of osteoarthritis ($p = 0.043$). From the follow-up test (Postoc test) using the LSD (Least Square Difference) test, it showed that there were differences in uCTX-II levels in patients with mild and moderate degrees ($p = 0.023$) and between patients with mild and severe degrees ($p = 0.044$). However, there was no difference in uCTX-II levels between moderate and severe patients ($p = 0.454$).

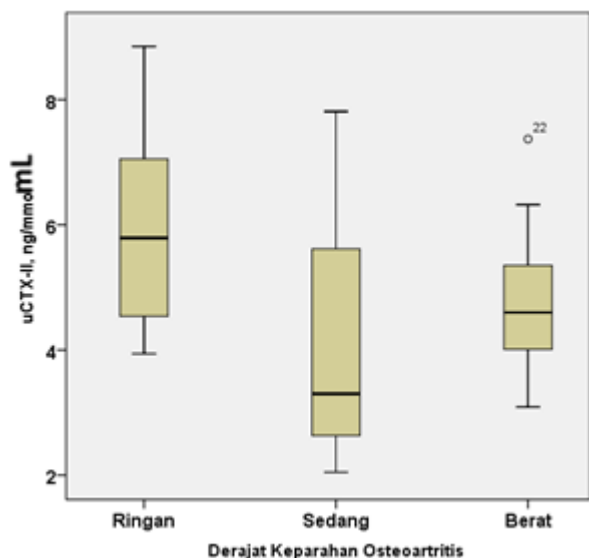


Figure 1: Graph Boxplot Levels for uCTX-II Based on Osteoarthritis Severity

6. Discussion

This study was followed by 36 patients with osteoarthritis. There were 30 female subjects (83.3%). This is in line with the research of Handono et al. From 40 samples, 30 samples were 75% female. Schram et al, stated that the largest distribution of knee OA sufferers is female where women have a greater risk of suffering from knee OA, especially women aged over 50 years. This occurs due to hormones in postmenopausal women, which causes the hormone estrogen to fall, causing a decrease in bone and joint density (Schram B et al, 2022).

The results of this study obtained that the average age of the subjects was 64.03 years with the youngest age being 42 years and the oldest being 80 years. In line with the research of Handono et al, 2015, which obtained an average age of 58.5 years with the youngest age being 42 years and the oldest age being 76 years? Dan Cui et al, 2020, also said that at the age of over 50 years there was a degenerative process and a decrease in functional ability caused by a decrease in protein in the joint cartilage and an excessive workload on the knee joint, so that it would cause the appearance of osteophytes which cause pain in the knee joint. Knee. The aging process has an adverse effect on the ability of joints to protect themselves from exposure to biomechanical stress, due to changes in articular cartilage, such as thinning of non-calcified cartilage.

Based on the characteristics of this study, the average body weight and height of the subjects were 66.86 kg and 155.56 cm, respectively. Subject's mean BMI, based on the categorization of BMI values, there were 26 people (72.2%)

including obesity. In contrast to the study of Handono et al., 2015, the average BMI of subjects based on the categorization of BMI values; there were 9 people (22.5%) including obesity. In addition, in women the fat content in the body is more than 30%, while in men the lower limit is 20-25%. This is because per total body weight in women is more than in men. As you get older, you tend to lose muscle mass and it is easy to accumulate body fat. This is due to a decrease in thyroid hormone which causes fat to be difficult to melt which makes it continue to accumulate in the body and is difficult to burn because the metabolism runs slowly. This results in excessive weight gain. Therefore, more elderly women who experience excess body weight have a greater potential to experience knee OA (Thati S, 2021).

A total of 17 research subjects (47.2%) were housewives (IRT). This is in line with research conducted by Adani et al, 2021. The results of their study showed 61 IRT patients (80%), most of who came to medical rehabilitation at the Islamic Hospital of Jakarta Pondok Kopi with complaints of knee pain with an assessment of severity according to Kellgren and Lawrence. This is because IRT are individuals who often do certain physical activities for a long time while doing their jobs such as kneeling, squatting, climbing stairs, standing for a long time and lifting heavy weights which can increase the risk of OA.

In this study, 19 people had a history of hypertension (52.8%) in line with research conducted by Ishaan Vohra et al in 2015 with the results of the study showing that there was a significant correlation between hypertension and clinical severity of OA with a p value of <0.0001 . This is in accordance with the research of Zhang et al, 2015, many risk factors that cause osteoarthritis, one of which is hypertension due to the effects of vascular damage caused. Where hypertension causes atherosclerosis which can cause arterial occlusion and cause stasis of blood flow in the subchondral vessels, a subchondral ischemia occurs so that the exchange of nutrients and gases is disrupted into the articular cartilage which is a potential initiator of degradative changes in cartilage.

Based on the assessment of severity according to Kellgren and Lawrence, in this study, most of the subjects, amounting to 17 people (47.2%) were in severe severity/grade IV. In contrast to the study conducted by Handono et al, 2012, where they found mild knee OA in 23 patients (57.5%) while moderate and severe degrees were 17 patients (42.5%). The possible cause of the difference is because most people in Medan come to the hospital after complaining of severe pain that has interfered with activities.

By using the Anova test, in this study, a significant difference in uCTX-II levels was obtained based on the severity of osteoarthritis ($p = 0.043$). From a follow-up test (Postoc test) using the LSD (Least Square Difference) test, it was found that there were differences in uCTX-II levels in patients with mild and moderate degrees ($p = 0.023$) and between patients with mild and severe degrees ($p = 0.044$). there was no difference in uCTX-II levels between moderate and severe patients ($p = 0.454$). The results of this study are in line with the study conducted by Handono et al., 2012, where there was a significant difference in urinary CTX-II levels in the

mild OA group (KL grade 2) and the OA group with KL degrees 3 and 4 (240.35 (SB 88).90) ng/mmol and 728.24 (SB 296.15) ng/mmol, $p = 0.001$). All patients with grade 3 and 4 OA were shown to have urinary CTX-II levels > 300 ng/mmol. The results of the Spearman correlation test showed a significant relationship between urine CTX-II levels and the degree of joint damage in osteoarthritis patients with a correlation value (r) of 0.734; $P < 0.0001$. (Wang et al, 2019).

CTX-II is a C-terminal telopeptide cross-linked from type II collagen and contains a dimeric-hexapeptide epitope (EKGPDP) with a pyridine ring as a linker, which is cleaved by matrix metalloproteinases (MMPs). 51 CTX-II diffuses from the joints into the blood and is finally excreted in the urine. Type II collagen is the most abundant matrix protein of articular cartilage and is highly specific for this tissue. Numerous studies have shown that cartilage, especially type II collagen, degradation is an important step in the development of knee OA. (Wang et al, 2019).

In contrast to the study conducted by Xin et al, 2017, the value of uCTX-II concentrations in COA patients was higher than the control group ($p < .001$). Where the concentration of uCTX-II was found from high to low as follows: group IV, group III, group II, and group I ($p < .001$), but when compared with group I and a group of healthy individuals (control group) they did not there was a significant difference ($p > .05$).

Using the Kruskal Wallis test in this study, it was found that there was no significant difference in calcium levels based on the severity of osteoarthritis ($p = 0.944$). This is in line with the study conducted by Yazmalar L, et al, 2013, also did not get a statistically significant difference between calcium levels in COA patients compared to the control group ($p > 0.05$).68 This is different from the study conducted by Nicoleta T, et al. al, 2020, it was found that the total serum calcium level was significantly lower ($p = 0.039^*$) in patients with COA (9.23 ± 0.395) compared to the control group (9.33 ± 0.367). Calcium levels decreased significantly as the degree of COA worsened.

Healthy cartilage matrix mainly contains type II collagen. The type of collagen in cartilage is degraded continuously through matrix metalloproteinases (MMPs), which are synthesized by chondrocytes, osteoclasts and synoviocytes. Osteoarthritis is a disease characterized by continuous joint destruction, including cartilage wear and synovial tissue changes. Studies suggest that increased expression of MMP-1, MMP-2, and MMP-9 proteins may be associated with the pathogenesis of osteoarthritis (OA). MMP is a Zn^{2+} and Ca^{2+} dependent proteinase, and MMP activity is regulated by Ca^{2+} concentration (Xin et al, 2017).

7. Conclusion

A significant difference in uCTX-II levels was found based on the severity of osteoarthritis ($p = 0.043$). From the follow-up test (Postoc test) using the LSD (Least Square Difference) test, it showed that there were differences in uCTX-II levels in patients with mild and moderate degrees ($p = 0.023$) and between patients with mild and severe degrees ($p = 0.044$).

However, there was no difference in uCTX-II levels between moderate and severe patients. This study also showed that there was no significant difference in calcium levels based on the severity of osteoarthritis ($p = 0.944$).

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