Correlation between Severity of Knee Osteoarthritis and Serum Levels of *Cartilage Oligomeric Matrix Protein*

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ABSTRACT

Background: The sensitivity of radiographic examination in the diagnosis and severity assessment of knee osteoarthritis (OA) is still low. Various attempts have been made to find more reliable indicators of cartilage damage. One potential marker is cartilage oligomeric matrix protein (COMP), a substance that in previous animal studies had been shown to be released in proportion to the extent of joint cartilage damage.

Objective: To evaluate the correlation between the severity of knee OA and serum level of COMP in human with normal renal function.

Methods: This was a cross-sectional study performed at the outpatient clinic in Department of Internal Medicine, Sanglah Hospital, Denpasar. The diagnosis of knee OA was based on the American College of Rheumatology (ACR) criteria. The degree of knee OA severity was determined by using the Kellgren-Lawrence criteria, while COMP values were checked by enzyme-linked immunosorbent assay (ELISA) method.

Results: Forty five patients who were recruited were examined: 19 (42.2%) were female and 26 (57.8%) were male. The mean age of patients was 64.1 ± 7.1 years. There were 4.4%, 26.7%, 46.7%, and 22.2% patients who had grade 1st, 2nd, 3rd, and 4th degree joint damage based on the Kellgren-Lawrence score, respectively. Mean serum level of COMP was 1081.4 ng/mL. We found a significant correlation of the severity of knee OA with serum level of COMP ($r = 0.41$, $p = 0.005$).

Conclusion: Among the patients in this study, there was a significant correlation between the severity of joint damage in knee OA and serum level of COMP.

Osteoarthritis (OA) is the most common articular disease in humans.[1] World Health Organization (WHO) estimated 25% of people aged 65 years old around the world suffer from this disease.[2] Radiographic studies conducted in America and Europe reported the prevalence of knee OA in people aged 45 years or more is 14% in men and 22.8% in women.[3]

In Indonesia the prevalence of knee OA through radiologic findings reaches 15.5% in men and 12.7% in women.[4] Several studies conducted in Indonesia even shows a higher result compared to America. A study done at the Internal Medicine outpatient clinic in Manado General Hospital (March 1994-November 1995) showed a prevalence of 36.81% and 37.55% in Surabaya.[5] Surveys conducted at the Rhemathology outpatient clinic of Sanglah Denpasar Hospital (2001 – 2002) demonstrated OA as the most frequent rheumatic cases (37%) where the majority of them were knee OA (97%).[6]

Osteoarthritis can affect many joints, more often the weight bearing joints such as hips and knees.[3] The prevalence of OA is expected to increase along with the increase of obesity as its major risk factor and a higher life expectancy. Pain, inflammation, and joint stiffness due to osteoarthritis may cause physical disability. In the United states, OA not only became the major cause of physical disability but also the expense incurred for this disease in 2001 reached 81 million dollars and an indirect cost of approximately 47 million dollars.[7] In Indonesia it is expected that 1-2 million elderly suffer handicap due to OA.[4]

To prevent the increasing number of handicap, an early diagnosis and accurate assessment of OA severity is needed. The current assessment is neither uniform nor objective due to its high dependency to the skill and experience of radiologists. By the time of diagnosis, OA has usually in an advance stage because conventional radiographs have limited capacity in detecting early stages of OA. This condition implies a higher failure in preventing physical disability. Therefore a marker that can detect cartilage damage as an objective assessment of OA is necessary, especially if it can predict early stages of this disease.

During the cartilage matrix turn over, both in normal and pathologic states, metabolites and fragments of the cartilage matrix will be released to blood and synovial fluid. One of these macromolecules is *cartilage oligomeric matrix protein* (COMP). The structure of this cartilage-specific protein is similar to trombospondin. COMP is known to stabilize articular cartilage matrix by binding to collagen and other extracellular components.[7,8,9] According to a study by Geng et al. (2008), Wistar rats deprived of COMP undergoes immediate arthritis reaction and later developing into severe chronic arthritis. Another study conducted in experimental animals that were made

OA, COMP was found to be expressed at the earliest phase of cartilage damage[10] and its level increases along with the grading of OA. [11]

The aim of this study is to evaluate the correlation between knee OA severity with serum levels of COMP in humans.

METHODS

Study Design

This was a cross sectional study. Samples were taken from the Internal Medicine Outpatient Clinic at Sanglah Hospital, Denpasar during 2010-2011.

Samples recruitment

Sample inclusion criteria were patients who fulfilled the American Clinical Rheumatology (ACR) 1996 criteria for knee OA, aged more than 50 years old, and have agreed to participate by signing informed consent. Samples excluded in this study were patients with total articular space damage (no space left) from radiologic examinations, OA in joints other than knee, articular diseases other than OA, patients that were unable to stand without assistance, using or having used steroids for the last 2 weeks, and creatinine clearance below 60 ml/minute.

Assessment

Knee OA severity was assessed according to the Kellgren Lawrence grading scale. Knee OA is classified into 5 grades (figure 1): grade 0 with normal radiologic findings; grade 1 with doubtful narrowing of joint spaces and possible osteophytic lipping; grade 2 with definite osteophytes and definite narrowing of joints space; grade 3 with moderate multiple osteophytes, definite narrowing of joints space, some sclerosis and possible deformity of bone contour; and grade 4 with large osteophytes, marked narrowing of joints space, severe sclerosis and definite deformity of bone contour. Radiographic examination of patients was done in a standing position, where anteroposterior (AP) and lateral projection of both knees were taken. Radiographs were then examined by three qualified radiologists that had been tested with a reability test (kappa test).

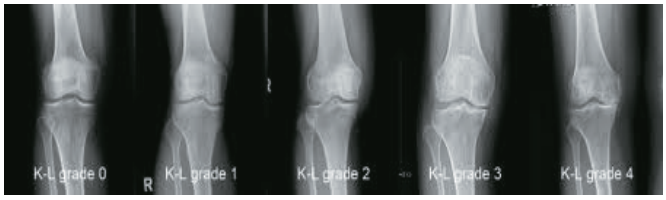

Figure 1. Kellgren Lawrence grading scale of knee OA

Sera for COMP were collected 3 hours after the patient woke up in the morning and rested approximately 30 minutes. To determine serum COMP levels, samples were examined with Human Cartilago Oligomeric Matrix Protein ELISA (Biovendor, Brno, Czech Republic) reagent. The examination using Biovendor reagent had a *limit of detection* (LOD) of 0,4 ng/mL.

Statistical Analysis

Data normality of COMP levels were assessed using the Shapiro-Wilk test. Correlation between serum COMP levels, knee OA gradings, and age were analyzed with the Spearman and Pearson test according to the normality data. Relations of gender and serum COMP levels were assessed by T-test. Statistical significance used was $p < 0,05$. Linear regression multivariate tests were used to estimate serum COMP levels prediction using knee OA severity.

RESULTS

Forty five patients enrolled in this study, of which 19 (42,4%) patients were male and 26 (57,8%) patients were female. Serum levels and knee x-ray were examined. Average age of sample was 63.3±6.8 years with a range between 50 to 85 years old. Most frequent education background was high school (60%) and most frequent occupation was civil servant or retired civil servants.

Most frequent OA grading based on Kellgren-Lawrence grading scale was grade 3. Mean serum COMP level was 1175,4 ng/ml. Mean body mass index (BMI) was 26,2 kg/m^2 also included as grade 1 obesity based on Western Pacific Region of WHO 2000 classification. Sample characteristics are shown in table 1.

Tabel 1 Characteristics of based sample (N = 45)

Characteristics	n (%)*
Gender	
male	19 (42,2)
female	26 (57,8)
Age, years, mean (SD)	63,3 (6,8)
Education backround	
No education	1 (2,2)
elementary	6 (13,3)
junior high school	2 (4,4)
high school	27 (60,0)
University	9 (20,2)
Work	
unemployed	8 (17,8)
labor	0 (0)
farmer	0 (0)
civil servants (PNS)	30 (66,7)
private sector	4 (8,9)
Military-police	3 (6,7)
BMI, kg/m^2, mean (SD)	26,2 (4,2)
COMP level, ng/mL, mean (SD)	1081,4 (3,3)
COMP level, ng/mL, median (range)**	
Grade 0	0 (0)
Grade 1	868,4 (613,5–1123,3)
Grade 2	881,3 (683–1197,5)
Grade 3	11124,3 (673,6–2177,8)
Grade 4	1273,9 (816,9–2278,6)
Degree of Joint damage	
Grade 0	0 (0)
Grade 1	2 (4,4)
Grade 2	12 (26,7)
Grade 3	21 (46,7)
Grade 4	10 22,2)

* unless otherwise noted

** data distribution is not normal therefore described in median and range

Reability of knee radiographs in determining severity of knee OA

Three radiologists were tested with a kappa test (k) before assessing knee radiographs to determine OA grade based on Kellgren-Lawrence criteria. Assays were based on the readings of 20 samples of knee radiographs by two radiologists. The kappa value attained was 0,595. This was included an adequate criteria. [12]

Correlation between severity of knee OA and serum COMP levels

Positive correlations were found statistically significant, using the Spearman correlation test, with r = 0,41 and p = 0,005. Therefore there was a tendency of increasing grade of knee OA along with increase COMP serum levels (figure 2).

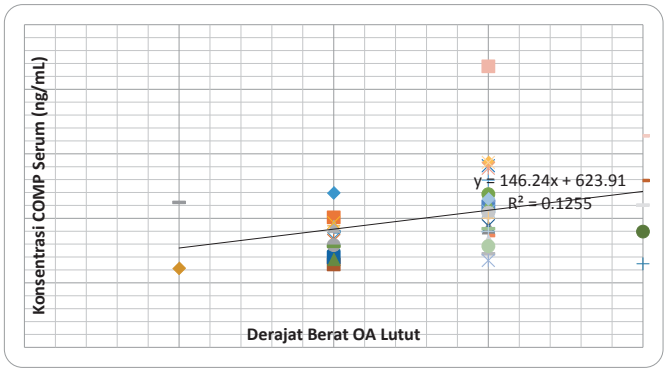

Figure 2 Relationship between knee OA severity grade (K/L) with COMP serum

Correlation of Age and COMP serum level

Two methods were used to test the relationship between age and COMP serum levels. The first test was done by correlation test. The second test was done by test comparing COMP serum levels after they were categorized into 2 age groups of < 65 years and ≥ 65 years old. In the correlation test a tendency of increase COMP serum levels with age was found, but with no significant correlation (r = 0,103; p = 0,5) (Figure 4).

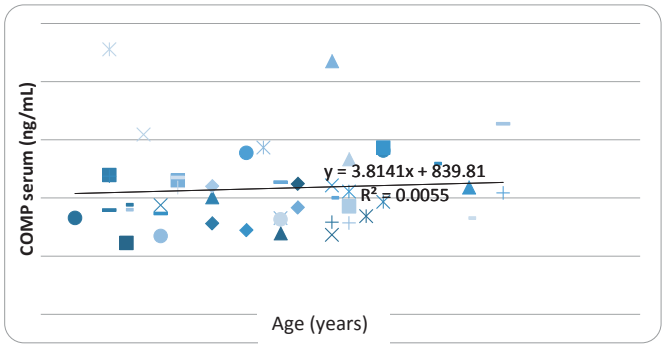

Figure 4 Relationship between age and COMP serum

The compare test of COMP serum in the age groups < 65 years old and ≥ 65 years displayed no significant difference (t = -8,53; p = 0,398).

Correlation of gender and COMP serum level

In this study, no significant difference was found of log10-COMP serum between male and female (t = 0,802, p = 0,427). However, the results show a higher COMP serum levels in male than female (Table 2)

Table 2 COMP serum levels based on gender

Sex	Mean COMP serum (ng/mL)	p
Male	1.135,6 (391,4)	0.427
Female	1.041,7 (318,8)	

Multivariate Analysis

Linear regression was used as the multivariate analysis to look at factors influencing COMP serum. From the analysis results, only knee OA severity grade (p <0,25) were eligible to be included in the multivariate analysis. Other variables such as gender and age could not be included because their p values were > 0,25. Based on the linear regression analysis, equation of COMP was obtained, COMP = 573 + 177,3 (knee OA severity grade). The above equation is feasible because ANOVA test display p = 0,005. However, knee OA severity grading can only explain COMP serum concentration of 15,1% based on *adjusted R square* linear regression test dan *Anova* value.

DISCUSSION

In a study done by Clark et al[11] COMP serum obtained from the control group was 1.061,83±370.83 ng/mL , whereas OA patient group was 1.208,57±487,47 ng/mL. Another study was done by Jordan et al[13] examining COMP serum levels corresponding the knee OA severity grade (K/L). After transforming the data using natural logarithm, the mean COMP serum levels in knee OA (±SD) is as follow; grade 0 of 6.68±0.40, grade 2 of 6.79±0.40, grade 3 of 6.87±0.34, grade 4 of 7.00±0.48. In this study, the distribution value of COMP levels was not normal with a mean COMP level in knee OA patients of 1.081,4 ng/mL ranging between 613,5 to 2.278,6 ng/mL. This data was lower when compared with the previous two studies.

Previous studies investigating the relationship between arthritis and serum COMP levels have been conducted in both experimental animals and humans. Experimental animals that were made to have arthritis demonstrated an increased level of COMP in late phase after further cartilage damage, but not at the beginning when inflammation peaked.[14,15] This study showed that increased COMP in blood was due to articular cartilage damage. In another study, elevated levels of COMP were found higher in experimental animals that had severe arthritis damage compared with mild damage. [14,15] This study implied severe degree of arthritis meant more COMP.

Research studying the relationship between OA and COMP serum levels in humans by Petersson et al[16] exhibited samples with no OA in previous radiologic examinations then later on had OA. They showed a higher concentration of COMP serum compared with those without OA after being follow up for three years.

A tendency of increasing knee OA severity grade along with COMP serum levels was found in this study and showed a correlation that was statistically significant. In Indonesia, research on the correlation between knee OA severity grade with COMP serum has never been conducted. The research done by Conrozier et al[17] was on the correlation between hip OA severity grade with COMP serum. In the study a significant correlation was found between COMP and joint space width (JSW) (r = 0,40; p = 0,001). COMP value was also connected to yearly mean narrowing (YMN) of the hips (r = 0,38; p = 0,002).

Researches related to knee OA had been done before, comparing the average levels of COMP serum in patients with different OA grading. One the researches was conducted by Clarck et al (1990) who compared COMP serum levels in OA patients and control group. As a result, patients with grade 3 and 4 knee OA had an average COMP serum of 21,3% higher (p = 0,013) compared with the control group and patients with grade 2 knee OA had an average COMP serum 11,5% higher than the control group (p = 0,045). Although average COMP serum levels tended to be higher in grade 3 - 4 knee OA than to grade 2 but this did not differ significantly. [11]

In this research, the average COMP serum levels were compared between groups of severe knee OA group (grade 3 and 4) with mild knee OA (grade 1 and 2) and obtained a significant difference (t = -3,04; p = 0,004). This data supported previous theory stating that higher grade OA had higher COMP serum level.

However, the use of serum COMP as a tool to assess the severity of knee OA, based on these results, can not be fully utilized because the levels of serum COMP vary in each grade of OA (a sample of grade 4 knee OA was found having a COMP level below the average COMP level of grade 2) (Figure 3).

Multivariate analysis (linear regression) showed that, although the equation on the influence of knee OA severity grading with COMP serum could be used, it could only explain as much as 15,1% COMP serum levels. Possibly, there are other factors influencing COMP levels that has not been investigated. Another cause may be the reability (or kappa) of determining knee OA from radiographs is not included as good criteria. In this study, the kappa value of radiographic readings in determining knee OA grade based on Kellgren Lawrence criteria was moderate. For further research, kappa values should be increased by training. Another alternative is by using better modalities such as MRI in assessing the severity of OA.

Besides the grading of knee OA severity, several other factors may affect serum COMP levels, such as age, sex, and impaired excretion due to kidney disease. Effect of impaired renal function can be ignored because patients with these conditions are excluded first.

There were a few of limitations in this study. One of the criteria used for inclusion OA samples was clinical in which the diagnosis of OA can only be made if there was a complaint. OA patients without clinical complaints are unlikely to be included as a study sample therefore affecting the COMP serum levels obtained. Moreover, assessment of OA grade with knee radiographs using Kellgren-Lawrence criteria only achieve a reliability (kappa value= 0.6) of moderate.

CONCLUSION

This study concluded that there was a significant correlation between knee OA severity grades with COMP serum levels. Strength criteria included moderate correlation (r= 0.41). However, using serum COMP level to assess the severity of knee OA is not applicable because of varying level of serum COMP in each grade of OA. Further efforts to a better and objective assessment of OA severity can be made by increasing the value of kappa or using better modalities should another research is planned to cover the limitations in this research.

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