1. Introduction

Osteoarthritis (OA) is a degenerative joint condition that affects millions of people worldwide.\(^1\) Deterioration of articular cartilage, thinning of subchondral bone, development of osteophytes, and a moderate level of chronic inflammation in the synovium are all hallmarks of OA.\(^1,2\)

Over 528 million people were expected to have osteoarthritis worldwide in 2019, a significant increase of 113% over the numbers observed in 1990. Approximately seven in ten people who have been diagnosed with osteoarthritis are aged 55 and over. In addition, women contribute to about 60% of this population. Approximately 365 million people suffer from conditions affecting the knee joint. The result is widespread effects on the hand and hip joints.\(^3,4\)

Vitamin D is a crucial factor in maintaining optimal bone and cartilage metabolism and is well recognized as a fundamental determinant of skeletal well-being. There is a potential correlation between vitamin D and the development and/or progression of osteoarthritis, which may be mediated through its impact on the periarticular bone.\(^4\)
The primary metabolite that circulates in the body is vitamin D (25(OH)D), but active metabolite that is responsible for the majority of vitamin D’s effect is vitamin D (1,25(OH)2D). They have a strong binding affinity for the vitamin D receptor (VDR), a nuclear receptor that functions as a transcription factor. In 2011, Muraki et al. found that VDR was present in chondrocytes, among other tissues. Metalloproteinase (MMP) and prostaglandin E2 (PGE2) expression in chondrocytes is controlled by VDR 1\textsuperscript{-25(OH)2D3}, as shown by Cao et al. (2012).\textsuperscript{5,6}

Cartilage Oligomeric Matrix Protein (COMP) is part of the thrombospondin family, a group of extracellular calcium-binding proteins originally identified in cartilage. During the process of cartilage matrix turnover, fragments of the cartilage matrix and other products resulting from cartilage metabolism are discharged into both the synovial fluid and the serum. One of the released macromolecules is COMP. Hunter et al. (2014) found that elevated serum concentrations of COMP were associated with worsening cartilage in OA patients.\textsuperscript{7,8}

The aim of this study is to investigate the correlation between vitamin D (25(OH)D) and COMP serum in knee osteoarthritis.

2. Methods

The study was conducted between July and December 2022 in the Rheumatology Department of Dr. Mohammad Hoesin General Hospital in Palembang, using a cross-sectional research methodology. The researchers followed the guidelines established by the Declaration of Helsinki and was approved by Dr. Mohammad Hoesin General Hospital Ethics Committee (No. 112/kepkrsmh/2022).

Study participants

Participants were enrolled in a consecutive method and were screened using the American College of Rheumatology (ACR) 1990 criteria for knee osteoarthritis. After receiving thorough explanations, the participants in this study provided their informed consent to take part in the research. Participants were both male and female and had been diagnosed with OA using the Kellgren and Lawrence (KL) scale, with the guidance of X-rays. Patients who have other autoimmune conditions, elevated C-reactive protein (CRP) or erythrocyte sedimentation rate (ESR) values (more than 40 mm/hr), or positive rheumatoid factor (RF) results are not eligible for this study.

Assessment

Serum vitamin D (25(OH)D) levels were analysed from blood samples collected during a 12-hour overnight fasting period. In the RSMH laboratory, measurements were taken using the chemoluminescent immunoassay methods.

Measurements of COMP levels in the blood are taken at various times during the day, but preferably three hours after waking up and after a rest period of at least 60 minutes. Human Cartilage Oligometric Matrix Protein ELISA Reagent (Biovender, Laboratorni medicina a.s., Czech Republic; RD194080200, Lot E21-093); utilised by measurements in the PRODIA laboratory.

Statistical analysis

The Shapiro-Wilk test was employed to assess the normality of the data. Considering the normal distribution of the data, we used the Spearman and Pearson correlation tests to analyse the correlation between vitamin D (25(OH)D) and COMP serum. A significance level of p<0.05 was employed for determining statistical significance. Multiple linear regression test was conducted to investigate the correlation between the major independent variable (vitamin D (25(OH)D) serum) and the covariate variables (knee OA gradings, physical activity, and age) that affect the dependent variable (COMP serum levels).

3. Results

The study involved a sample of thirty subjects, whose enrollment took place from July to December 2022. The basic characteristics are summarised in Table 1. Insufficient vitamin D levels were found in
33% subjects, while 67% subjects had vitamin D deficiency. Radiographic evaluations of knee OA found that half of the subjects had Grade 2 Kellgren-Lawrence OA (50%), which is statistically similar to the prevalence of Grade 3 disease (50%). The respondents’ average serum COMP concentration was 494.20 ng/ml.

Table 1. Baseline characteristic

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total n:30 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender</strong></td>
<td></td>
</tr>
<tr>
<td>Woman</td>
<td>28 (93)</td>
</tr>
<tr>
<td>Man</td>
<td>2 (7)</td>
</tr>
<tr>
<td><strong>BMI</strong></td>
<td></td>
</tr>
<tr>
<td>Severe Underweight (&lt; 17)</td>
<td>-</td>
</tr>
<tr>
<td>Underweight (17-18.4)</td>
<td>-</td>
</tr>
<tr>
<td>Normal (18.5-25)</td>
<td>6 (20)</td>
</tr>
<tr>
<td>Overweight (25.1-27)</td>
<td>12 (30)</td>
</tr>
<tr>
<td>Obese (&gt;27)</td>
<td>12 (30)</td>
</tr>
<tr>
<td><strong>Physical Activity</strong></td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>9 (30)</td>
</tr>
<tr>
<td>Moderate</td>
<td>21 (70)</td>
</tr>
<tr>
<td>Severe</td>
<td>-</td>
</tr>
<tr>
<td><strong>OA gradings</strong></td>
<td></td>
</tr>
<tr>
<td>Grade 2</td>
<td>15 (50)</td>
</tr>
<tr>
<td>Grade 3</td>
<td>15 (50)</td>
</tr>
<tr>
<td><strong>Vitamin D (25(OH)D) Serum</strong></td>
<td></td>
</tr>
<tr>
<td>Sufficient (&gt;= 30ng/mL)</td>
<td>-</td>
</tr>
<tr>
<td>Insufficiency (20.1-29.9 ng/mL)</td>
<td>10 (33)</td>
</tr>
<tr>
<td>Deficiency (&lt;20 ng/mL)</td>
<td>20 (67)</td>
</tr>
<tr>
<td><strong>COMP Serum Level, ng/mL, mean (SD)</strong></td>
<td>494.20 (203.03)</td>
</tr>
</tbody>
</table>

The present study investigates the potential correlation between serum of vitamin D (25(OH)D) and COMP.

Table 2. Correlation between serum levels of vitamin D (25(OH)D) and COMP

<table>
<thead>
<tr>
<th>Variable</th>
<th>COMP Serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>vitamin D</td>
<td>-0.862*</td>
</tr>
<tr>
<td>(25(OH)D) Serum Level</td>
<td>0.000**</td>
</tr>
</tbody>
</table>

*Spearman’s Rho test; **P< 0.05. If the value of r= 0.0 to <0.2; very weak, r= 0.2 to <0.4; weak, r= 0.4 to <0.6; moderate, r= 0.6 to <0.8; strong, r=0.8 to 1=very strong.

The correlation between vitamin D (25(OH)D) and COMP serum was analysed using Spearman’s rho. There was a strong, statistically significant negative correlation between these two variables (r = -0.862; p = 0.000), as shown by Table 2. Serum COMP increase in knee osteoarthritis, and that has been correlated with lower vitamin D (25(OH)D) serum (Figure 1).

Multiple linear regression test was used to analyse the correlation between the independent variable (vitamin D (25(OH)D) serum) and confounding factors
(grades of knee osteoarthritis by KL, BMI, levels of physical activity, and age). The serum concentrations of COMP were the dependent variable, and these variables were found to be potential predictors.

We were able to include serum vitamin D and knee OA grades by KL (p <0.25) in the multivariate analysis because the study's criteria were met. Additional variables (BMI, physical activity, and age) were not incorporated into the analysis due to p-values exceeding the threshold of 0.25.

Vitamin D (25(OH)D) serum and grades of knee osteoarthritis by KL were found to be significant predictor variables of serum COMP in the final linear regression analysis. Vitamin D (25(OH)D) serum interacted with grades of knee osteoarthritis by KL (p<0.05). This finding indicates that there is a correlation between vitamin D (25(OH)D) serum, grades of knee osteoarthritis by KL, and COMP levels.

![Figure 1. Spearman's rho correlation test between Vitamin D (25(OH)D) and COMP Serum](image)

4. Discussion

Osteoarthritis is the most commonly observed form of arthritis, characterized by the degradation of cartilage and impairment of joint functionality. Vitamin D affects not just bone metabolism but also non-osseous tissues. Despite the established association between vitamin D and OA cartilage regeneration, the underlying mechanism is still unknown. Vitamin D deficiency has been associated in multiple studies with a higher risk of developing OA.8,9

Our results showed a strong, statistically significant negative correlation between vitamin D (25(OH)D) and COMP serum (r = -0.862; p = 0.000) when analysed with Spearman's Rho. This indicates a correlation between low levels of vitamin D (25(OH)D) and increased COMP serum in knee osteoarthritis.

After reviewing the current literature, we determined that no studies have been conducted to establish a direct correlation between the concentration of vitamin D and any subsequent changes in the serum COMP concentration in knee osteoarthritis. The study conducted by Zaki et al. (2022) provided a description of vitamin D supplementation (alpha-calcidol) and alterations in joint cartilage, reflected by changes in serum COMP. Consistent with the present study, previous research has shown that patients achieving sufficient serum 25(OH)D levels have better joint cartilage healing than those with insufficient or deficient levels. As a result, there is a reduction in COMP concentrations.10

Moreover, this study demonstrates that the concentrations of COMP are additionally influenced by the Kellgren-Lawrence grades of knee osteoarthritis. This is related to a Brazilian study by Fernandes et al. (2007) that examined the association between COMP serum levels, knee OA pain, and radiographic abnormalities. These results were compared to those of a healthy control group of the same age and sex. Knee OA symptoms had substantially higher COMP
serum concentrations compared to controls.

Vilim et al. (2002) conducted a three-year prospective research. The study involved a sample of forty-eight patients diagnosed with primary knee osteoarthritis, grades I to III OA (classified according to the radiological Kellgren-Lawrence classification). The main objective of the study was to assess the accuracy of COMP serum in predicting the onset of OA. Zaki et al. (2021) observed a positive correlation between a greater pre-intervention (baseline) concentration of COMP serum and a higher radiological grade of knee osteoarthritis according to the Kellgren-Lawrence classification.\textsuperscript{11,12,13}

Vitamin D has an important function in maintaining optimal bone and cartilage metabolism and is widely recognized as a significant determinant of skeletal well-being. There is a theory suggesting that the activation of the Nrf2-KEAP1 antioxidant pathway may confer resistance to oxidative stress. As a result of this phenomenon, it is possible that the antioxidant properties of vitamin D may be expressed through the enhancement of specific antioxidant enzymes. The regulation of specific matrix metalloproteinases (MMPs) can also be influenced by it. Knee osteoarthritis sufferers with low serum vitamin D may experience accelerated cartilage loss, structural abnormalities in their cartilage, weakening quadriceps muscles, and increased knee discomfort\textsuperscript{9,14,18}

A recent study suggests that vitamin D may slow the progression of OA. In OA, catabolic mechanisms predominate over anabolic ones, leading to the degeneration of articular cartilage. Hypertrophy and subsequent degradation of articular cartilage are the results of this interaction. Vitamin D receptors have been shown in numerous studies to have higher expression levels in damaged cartilage (Figure 2). Through interactions with the vitamin D receptor and the retinoic acid receptor (RXR), vitamin D is able to exert its genomic effects in chondrocyte, indicating that chondrocyte proliferation was triggered. On the other hand, vitamin D can lessen the inflammatory response by inhibiting the production of proinflammatory cytokines and matrix metalloproteinases\textsuperscript{14,15}

In a prospective cohort experiment conducted by Bassiouni et al., a total of fifty people were included in the study. Among these participants, fifty percent were diagnosed with knee OA, while the remaining fifty percent did not exhibit any signs of knee OA. The study followed these individuals for a duration of one year. They showed that a higher level of VDR expression was seen in erosion-prone areas of OA.

Figure 2. The Effect of the Vitamin D in the Pathogenesis of Osteoarthritis. COMP: Cartilage Oligometric Matrix Protein, IL: interleukin, NF-κB: Nuclear factor kappa B, MMP: Matrix Metalloproteinase, VDR: Vitamin D receptor. (The figure was created and generated on the online platform BioRender.com).
patients. Tetlow et al. conducted an in vivo study where they observed an overexpression of MMPs 13, 3, and 9 in chondrocytes. This mechanism accelerates the degradation of cartilage. Studies employing a variety of imaging modalities have associated vitamin D deficiency with thinner cartilage. Ultrasonographic research by Malas et al. found that women aged 20–45 with vitamin D deficiency had significantly thinner femoral cartilage. Vitamin D deficiency also had a twofold greater risk of radiographic hip OA compared to normal levels of vitamin D in separate longitudinal cohort research conducted in the United States.16-18

These results provide further evidence associating low vitamin D (25(OH)D) levels with cartilage breakdown, as measured by increased COMP levels. The disease-modifying effects of vitamin D supplementation in OA have been demonstrated, with ability to reduce pain, inflammation, and cartilage deterioration. Vitamin D may have a protective effect on OA, but further research is needed to determine the precise mechanisms of action.

The limitations of this study involve a small sample size and the absence of a longitudinal analysis. Furthermore, the assessment of MMPs' activity was not conducted in the present study.

5. Conclusion

In summary, the findings of our study indicate a strong negative correlation between vitamin D (25(OH)D) and COMP serum in knee osteoarthritis. Nonetheless, more research is required to establish a more complete understanding of the connection between the two variables.

6. References


