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Correlation Between Serum Cartilage Oligomeric Matrix Protein (COMP), 25-Hydroxy Vitamin D, and Disease Activity in Patients with Knee Osteoarthritis

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Abstract

Background: The role of vitamin D and cartilage oligomeric matrix protein (COMP) in knee osteoarthritis (OA) still remains controversial.
Objectives: This study aimed to evaluate serum COMP and 25-hydroxy vitamin D concentrations in patients with knee OA in comparison to healthy individuals and to find whether there is a relationship between serum COMP, 25-hydroxy vitamin D, and disease activity in knee OA.
Methods: In a case-control study, 60 patients with knee OA were selected based on the criteria of the American College of Rheumatology referred to the Rheumatology Department of Tabriz University of Medical Sciences, Tabriz, Iran, from October 2017 to February 2018. Also, 28 healthy subjects matched regarding age and sex were selected. The patients were examined, and Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC Index) was assessed. Serum levels of COMP and 25-hydroxy vitamin D were assessed by ELISA method.
Results: The mean ± SD age of the patients and controls was 57.60 ± 10.63 and 56.46 ± 5.58 years, respectively. Serum COMP was significantly higher in OA patients (40.82 ± 10.04 pg/mL) than in healthy controls (27.01 ± 9.64 pg/mL) ($P < 0.001$). There were no statistically significant differences in serum 25-hydroxy vitamin D between the patients and control subjects ($P = 0.361$). Significant correlations were found between serum COMP concentration with WOMAC score ($r = -0.290$, $P = 0.025$) as well as the grade of OA ($r = 0.362$, $P = 0.004$). No statistically significant correlations were found between serum 25-hydroxy vitamin D level with WOMAC score ($r = 0.102$, $P = 0.438$) and the grade of OA ($r = -0.063$, $P = 0.630$) as well as between serum COMP and 25-hydroxy vitamin D levels ($P > 0.05$).
Conclusions: Based on the results of our study, Serum COMP levels correlated with disease characteristics in patients with osteoarthritis. Although further studies are needed to confirm our results, COMP may be used as a possible novel marker to measure osteoarthritis development and progression.

Keywords: Biomarkers, Cartilage Oligomeric Matrix Protein, Humans, 25-hydroxyvitamin D, Joint, Knee, Osteoarthritis, Rheumatology, WOMAC Index

1. Background

Osteoarthritis (OA) is a developing joint disease identified with the deterioration of articular cartilage, unusual sub-chondral bone formation, and inflamed synovium in early phases (1). It causes pain, stiffness, and disability (2). The OA is the most prevalent joint disease in adulthood, influencing almost 12% - 15% of this population (3). Aging leads to a significant increase in OA disease prevalence (3), and it is more prevalent in women than men (4). The knees are the main weight-bearing joints, and they are more often influenced by OA (4). The etiology of OA is ascribed to numerous factors, including genetic, obesity, trauma, and other metabolic reasons (5).

The first changes in OA occur peri-articularly; thus they are not observed well by radiographs. Therefore, there is a need to develop markers e.g., serologic markers to sensitively predict the early diagnosis and initiation as well as the progression of OA. Serum biomarkers are used to

assess joint renewal and disease progression. This would significantly increase the chance of diagnosing the disease easier with more cost-effective tools. Such a biomarker frequently studied to predict OA progression is cartilage oligomeric matrix protein (COMP). The COMP is a glycoprotein synthesized by chondrocytes, which is found in a large amount in the hyaline cartilage but can also be detected in serum and synovial fluid (6, 7). The COMP can activate the complement system, interact with other proteins to maintain cartilage integrity and regulate chondrocyte function and apoptosis (8). The possibility of serum COMP as a marker of ongoing joint destruction, as well as a prognostic indicator of future joint impairment in OA and rheumatoid arthritis, was indicated in previous studies (9, 10). However, the results are controversial.

Vitamin D plays a leading role in bone metabolism and may have an indirect effect on the development of OA through its influence on the bone (11). In vitro studies demonstrated vitamin D metabolism in articular cartilage, which could show a direct influence of vitamin D on OA vulnerability (12). Furthermore, low vitamin D level is related to the muscle weakness (13), which is related to the OA development (14, 15). Numerous studies examined the relationship between serum 25-hydroxy vitamin D level and OA, which showed conflicting results. Some studies reported significantly lower levels of serum 25-hydroxy vitamin D in patients with OA (16-18); while other studies found no association (19, 20). Therefore, there is a controversy regarding the association between vitamin D concentration and knee OA.

2. Objectives

Considering inconsistency between various studies regarding the role of serum COMP and vitamin D in OA and due to differences between the populations studied (interventional studies vs. population-based cohorts), and differences in sample recruitment, the present study aimed to evaluate serum COMP and 25-hydroxy vitamin D levels in knee OA patients in comparison to healthy subjects and to find whether there is a correlation between serum levels of COMP, 25-hydroxy vitamin D, and disease characteristics in knee OA.

3. Methods

3.1. Subjects

In this case-control study, 60 patients with bilateral primary knee OA (based on the criteria of the American College of Rheumatology) (21) with age of 40 to 70 years that referred to the Rheumatology Department of Tabriz University of Medical Sciences from October 2017 to February 2018, were included. Also, 28 healthy individuals matched regarding age and sex with no history of inflammatory rheumatic diseases were selected. The exclusion criteria were secondary OA (due to a known disorder), inflammatory joint disorders, previous knee trauma or injury, arthroscopy, surgery, or a knee joint injection, cardiovascular disease, diabetes mellitus, liver, renal, thyroid and/or parathyroid disorders, and any other chronic inflammatory disease, and taking glucosamine sulfate and/or chondroitin sulfate supplements. None of the participants were received vitamin D supplement. This research was approved by the Ethics Committee of Tabriz University of Medical Sciences (Iran) with an approval No. IR.TBZMED.REC.1396.278 and all subjects signed written informed consent before the study.

3.2. Sampling

The sampling was done based on a consecutive method. The sample size was calculated using the Power and Sample Size Calculation (PS) software, version 3.1.2. We designed a study of a continuous response varying from independent case and control individuals with 0.5 control(s) per case subject. In a previous study, the response within each subject group was normally distributed with a standard deviation equal to 3.6 (22). If the true difference in the case and control means is 2.5, we will need to include 50 cases and 25 controls to be able to reject the null hypothesis that the population means of the case and control groups are equal with probability (power) of 0.8. The Type I error probability associated with the test of this null hypothesis is 0.05. The dropout rate was considered about 15%, and therefore, the sample size was increased to 60 in the patient and 28 in the control groups.

3.3. Clinical Assessment

At baseline, a complete medical history was taken, and all the participants were examined by a rheumatologist, and the grading of knee severity was performed using knee X-ray based on the Kellgren/Lawrence scale (23). The Persian version of Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC Index) (i.e., Likert version) was completed through face-to-face interviewing (24). This questionnaire has been translated into various languages, and its validity and reliability have been examined, and found to have good validity and reliability (Cronbach's alpha above 80%). Also, the validity and reliability of the Persian translation of this questionnaire have been assessed and it has been determined that it is a valid and reliable instrument (Cronbach's alpha = 0.81 - 0.95) for knee OA evaluation in Iranian population (25). The WOMAC index exam-

ines three dimensions: pain (5 questions), stiffness (2 questions), and physical functions (17 questions).

3.4. Biochemical Measurements

Five mL of venous blood samples were obtained after overnight fasting. The serum samples were separated and kept at -70°C until biochemical measurements. Serum COMP (MyBiosource, San Diego, California, USA), 25-hydroxy vitamin D (Immunodiagnostic Systems, Bioactiva diagnostic, Germany) and C-reactive protein (CRP) (Monobined Inc., Lake Forest, CA) levels were evaluated with ELISA kit according to the manufacturer's recommendations, using a calibrated ELISA plate reader (Model STAT FAX 2100, Multi-detection Multi Plate Reader, USA). Serum calcium and phosphorus concentrations were assessed using the standard enzymatic colorimetric method (Pars Azmoon Co, Tehran, Iran) by an automated chemical analyzer (Abbott analyzer, Abbott Laboratories, Abbott Park, North Chicago, IL).

3.5. Statistical Analysis

The SPSS Statistical Software for Windows, version 18.0 (SPSS Inc., Chicago, Ill, USA) was used for statistical analysis. The normal distribution of the variables was assessed by the Kolmogorov-Smirnov test. Categorical and normally- and not-normally-distributed quantitative data were displayed as numbers (percentages), means ± SD, and median (interquartile range), respectively. Comparisons between groups were assessed by χ^2, independent-sample *t*-test, and Mann Whitney U test. The Pearson correlation test or Spearman rank correlation analysis were used for correlation analysis. A receiver operating characteristic (ROC) curve analysis was carried out to find the optimal cut-off points of serum COMP levels for predicting knee OA. The area under the curve (AUC) value was calculated to determine the accuracy of the test. $P < 0.05$ was considered statistically significant.

4. Results

General, clinical, and biochemical data of the studied subjects are shown in Table 1. The mean ± SD age in OA patients and the controls were 57.60 ± 10.63 and 56.46 ± 5.58 years, respectively. The mean ± SD WOMAC score in the patients was 66.45 ± 19.40 (range: 10 - 95). There were no radiographic findings of OA in 14 (23.3%) patients. The severity of OA based on the Kellgren/Lawrence scale was mild in 18 (30%) patients, moderate in 20 (33.4%) patients, and severe in 8 (13.3%) patients. As presented in Table 1, serum COMP and phosphorous levels were significantly higher in OA patients in comparison to the healthy controls ($P < 0.001$). No statistically significant differences were observed in general characteristics, as well as serum calcium and 25-hydroxy vitamin D, between OA patients and the controls ($P > 0.05$).

According to Table 2, significant correlations were observed between serum COMP level with WOMAC score ($r = -0.290$, $P = 0.025$) and the grade of OA based on the Kellgren/Lawrence scale ($r = 0.362$, $P = 0.004$). However, no significant correlations were indicated between serum 25-hydroxy vitamin D level with WOMAC score ($r = 0.102$, $P = 0.438$) and the grade of OA based on the Kellgren/Lawrence scale ($r = -0.063$, $P = 0.630$). No statistically significant correlations were seen between serum COMP and 25-hydroxy vitamin D levels with general characteristics as well as biochemical parameters such as serum calcium, phosphorous, and ESR ($P > 0.05$). Furthermore, for the first time, no significant correlation was observed between serum COMP and 25-hydroxy vitamin D levels ($r = 0.101$, $P = 0.441$).

The capability of serum COMP levels to discriminate patients with OA from those without OA was evaluated by ROC curve analysis (Figure 1). The ROC curve was well above the diagonal, showing good sensitivity and specificity. The ROC curve for the presence of OA diagnosis had an AUC of 0.836 (95% CI: 0.751 - 0.921, $P < 0.001$), which established a high probability of correctly prediction of OA.

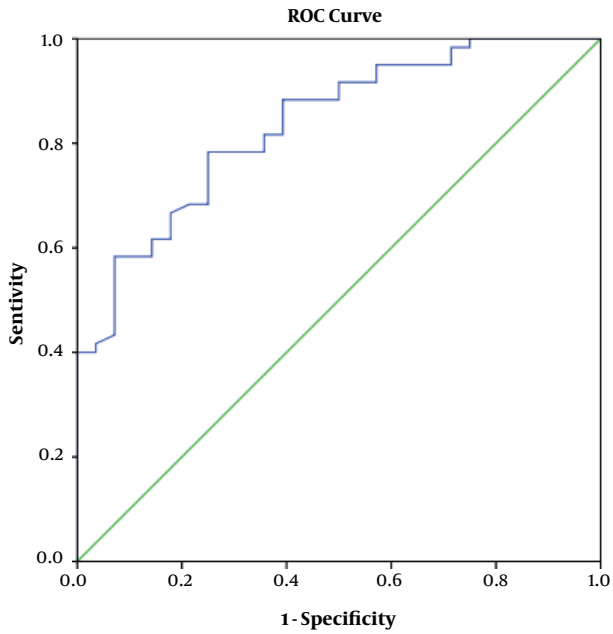

Figure 1. Receiver operating characteristic (ROC) curve analysis for predictive values of serum concentrations of COMP in OA. The area under the ROC curve is 0.836; 95% CI: 0.751 - 0.921, $P < 0.001$.

Table 1. Characteristics of the Patients with Knee OA and Controls[a,b]

Characteristics	Patients with Knee OA (N = 60)	Control Group (N = 28)	P Value[c]
Gender			0.084
Male	13 (21.7)	11 (39.3)	
Female	47 (78.3)	17 (60.7)	
Age, y	57.60 ± 10.63	56.46 ± 5.58	0.514
COMP, ng/mL	40.82 ± 10.04	27.01 ± 9.64	< 0.001
25-hydroxy vitamin D, ng/mL	30.58 ± 15.61	33.53 ± 9.79	0.361
Calcium, mg/dL	9.2 (9.00 - 9.50)	9.2 (9.10 - 9.37)	0.871
Phosphorous, mg/dL	3.91 ± 0.27	3.61 ± 0.13	< 0.001
ESR, mm/h	17.93 ± 15.01	-	-
CRP			-
Negative	32 (53.3)	-	
+1	15 (25.0)	-	
+2	6 (10.0)	-	
+3	7 (11.7)	-	
WOMAC score	66.45 ± 19.40	-	-

Abbreviations: CRP, C-reactive protein; COMP, cartilage oligomeric matrix protein; ESR, erythrocyte sedimentation rate; OA, osteoarthritis; WOMAC, Western Ontario and McMaster Universities Osteoarthritis.
[a] Data were presented as mean ± SD and median (IQR) for normally- and non-normally-distributed quantitative measures, or the number (percent) of categorical variables.
[b] P < 0.05 was considered significant.
[c] P values indicate the comparison between groups (χ^2 or independent-sample *t*-test).

Table 2. Correlation Between Serum COMP and 25-Hydroxy Vitamin D with WOMAC Score and the Grade of Osteoarthritis in Patients with Knee OA (N = 60)

	WOMAC Score		Grade of Osteoarthritis	
	r	P Value[a]	r	P Value[b]
COMP, ng/mL	-0.290	0.025	0.362	0.004
25-hydroxy vitamin D, ng/mL	0.102	0.438	- 0.063	0.630

Abbreviations: COMP, cartilage oligomeric matrix protein; OA, osteoarthritis.
[a] Pearson correlation test.
[b] Spearman rank correlation analysis.

5. Discussion

Cartilage destruction is the main characteristic of OA, with increased serum concentrations of cartilage degradation products such as COMP (9). In the current research, we observed that serum COMP levels were significantly elevated in patients with OA in comparison to the controls, which was in line with Darwish et al. (26) study who found higher serum COMP levels in patients with OA. Furthermore, a systematic review by Hoch et al. (27) concluded that serum COMP was increased in patients with radiographic knee OA in comparison to the healthy subjects; additionally, higher serum COMP concentrations correlated with a trend toward greater radiographic OA severity. These findings declared the function of COMP as a joint marker, which is difficult to be increased in healthy participants. Furthermore, it has been suggested that COMP is up-regulated in late phases of OA either to renovate damage or freshen the matrix, whereas high serum COMP levels in the early phases of OA are due to the cartilage degradation (28). In the present study, we reported significant correlations between serum COMP with WOMAC score and OA grade, which was consistent with Vilim et al. (29) study who reported a significant correlation between serum COMP level and a change in joint space width (JSW). Also, Clark et al. (30) found a relationship between serum COMP concentrations and knee OA severity based on Kellgren and Lawrence scale. Similar to our study, Wisowska and Jabonska (22) found a significant correlation between serum COMP level and WOMAC index. Moreover, a recent meta-analysis demonstrated higher serum COMP levels in pa-

tients with incident Knee OA (31). Therefore, serum COMP has the possibility to be used as a prognostic marker of disease development, and it can be stated that COMP level is a reflection on the clinical or functional situation of patients since it may indicate early cartilage destruction, which is not detectable by functional parameters. However, in contrast to the present study, Bruyere et al. (32) could not report any relationship between serum COMP and the WOMAC index. Moreover, Darwish et al. (26) found no correlation between serum COMP level with the WOMAC index.

Vitamin D is essential for regular bone and cartilage metabolism. Vitamin D insufficiency affects calcium metabolism, osteoblastic activity, bone density, and articular cartilage turnover unfavorably (33). Vitamin D can decrease bone turnover and cartilage destruction; therefore, likely may prevent the development of knee OA (34). Multiple investigations investigated the association between serum 25-hydroxy vitamin D level and OA, which reported paradoxical results. Based on our study, no significant difference was observed in serum 25-hydroxy vitamin D levels between OA patients and the controls.

Furthermore, there were no significant correlations between serum 25-hydroxy vitamin D level with clinical parameters, including WOMAC score and the grade of OA, which was similar with Cakar et al. (35) study which indicated no correlation between knee pain and vitamin D levels in patients with knee OA. Moreover, Konstari et al. (36) found no significant relationship between vitamin D and the risk of knee or hip OA. Felson et al. (37) also showed that vitamin D concentrations were not associated with the radiographic exacerbation and cartilage deterioration or radiologic symptom of the OA. In contrast to the present study, Bergink et al. (11) demonstrated that low serum 25-hydroxy vitamin D levels were associated with intense knee pain, higher prevalence of radiographic knee OA, and a higher risk of progression. Our results were also different from Heidari et al. (18) who reported a high prevalence of vitamin D deficiency and a significant positive relationship between this deficiency and knee OA in patients less than 60 years of age. In another study, Ding et al. (38) indicated that vitamin D levels were related to knee pain and reduced knee cartilage loss, which was different from our study. Furthermore, Muraki et al. (39) reported that lower vitamin D concentrations might be associated with elevated knee pain rather than radiographic changes. This controversy between our results with previous reports might be due to differences in study subjects, disease length, baseline disease severity, and COMP and 25-hydroxy vitamin D status as well as the type, dose, and length of therapies.

The novelty of the current study compared with previous reports was that no significant differences were observed in serum calcium and 25-hydroxy vitamin D between OA patients and control subjects. In addition, for the first time correlations between serum 25-hydroxy vitamin D level with clinical parameters, including WOMAC score and the grade of OA, as well as serum COMP, were assessed and no significant correlations were observed.

5.1. Strengths and Limitations

The limitations of this study were the relatively small sample size and that the majority of our patients were female. Also, because of the cross-sectional nature of the research, we did not monitor the patients' knee OA progression and vitamin D levels. The strength of the current study was that we included a control group and compared mean serum COMP and 25-hydroxy vitamin D levels between OA patients and normal individuals.

5.2. Conclusion

In conclusion, our research demonstrated increased serum COMP levels in OA patients in comparison to the healthy subjects, which indicated a correlation with disease severity, whereas it lacks a significant correlation with serum 25-hydroxy vitamin D levels. Although further studies are needed to confirm our results, COMP may be used as a possible novel marker to measure OA development and progression.

Footnotes

Authors' Contribution: Mohammad Reza Jafari Nakhjavani, Amir Ghorbanihaghjo, and Ozra Dabagh Asadollahipour designed the study and participated in data collection, data analysis, and the measurement of biochemical values. Sima Abedi Azar, Tala Pourlak, and Aida Malek Mahdavi provided assistance in the design of the study. Aida Malek Mahdavi prepared the first draft of the manuscript and edited the manuscript. All authors participated in data interpretation and read and approved the content of the manuscript.

Conflict of Interests: The authors declare that they have no conflict of interest.

Ethical Considerations: This research was approved by the Ethics Committee of Tabriz University of Medical Sciences (Iran) with an approval No. IR.TBZMED.REC.1396.278 and all subjects signed written informed consent before study.

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Patient Consent: All subjects signed written informed consent before study.

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