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Research Article

Efficacy of sCOMP in Diagnosing Knee Osteoarthritis

Sudhir Singh[1]*, Chetan Verma[2] and Lal Shrikant Kaushik[2]

[1]Professor, Department of Orthopaedics, Teerthankar Mahaveer Medical College and Research Centre, Moradabad, Uttar Pradesh, India

[2]Resident, Department of Orthopaedics, Teerthankar Mahaveer Medical College and Research Centre, Moradabad, Uttar Pradesh, India

***Corresponding Author:** Sudhir Singh, Professor, Department of Orthopaedics, Teerthankar Mahaveer Medical College and Research Centre, Moradabad, Uttar Pradesh, India.

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Abstract

Objective: The goal of this study was to establish the utility of serum Cartilage Oligomeric Matrix Protein (sCOMP) as a biomarker for differentiating between disease severity grades of knee osteoarthritis (KOA).

Material and Method: Patients of osteoarthritis of knee were included as Cases (100 subjects) and normal adults were taken as Controls (50 subjects). Clinical, radiological and biochemical assessment was done by WOMAC score, knee radiograph and blood sample for sCOMP respectively.

Results: Results show WOMAC Score was significantly higher in cases (49.97 ± 17.98) than in Controls (11.24 ± 06.07) ($p < 0.001$). Mean sCOMP level was significantly higher in Case group than in Control group (17.38 ± 4.99 U/L vs 1.16 ± 0.39 U/L; $p = 0.001$). sCOMP increases with increasing K-L grades except in grade III. In Case group sCOMP was 14.60 ± 6.47 U/L in K-L grade I; as 17.47 ± 4.99 U/L in K-L grade II; as 17.25 ± 4.63 U/L in K-L grade III and as 19.77 ± 4.65 U/L in K-L grade IV. One-way ANOVA of K-L Grade with sCOMP ($F = 1.55$, $p = 0.02$) and with WOMAC score ($F = 20.18$, $p = 0.001$) show significant association. Pearson Correlation and coefficient (r) value show that Age has moderate positive and significant co-relation with WOMAC Score ($r = 0.43$, $p = 0.001$), with KL grade ($r = 0.40$, $p = 0.001$) and weak positive and significant co-relation with sCOMP level ($r = 0.24$, $p = 0.01$).

Interpretation and Conclusion: The receiver operative curve (ROC) analysis suggested a "Cut-off" value of sCOMP as 9.06 U/L (Sensitivity 99%; Specificity100%; Accuracy100%) between Control group and Case group with excellent discriminatory power ($p = 0.001$) but not for various subgroups of disease severity of KOA.

Keywords: Biomarker; Cartilage Oligomeric Matrix Protein (COMP); Cut-Off Value; Osteoarthritis; Knee

Abbreviations

KOA: Knee Osteoarthritis; sCOMP: Serum Cartilage Oligomeric Matrix Protein; WOMAC: Western Ontario and McMaster Universities Osteoarthritis Index; ROC: Receiver Operative Curve

Introduction

Osteoarthritis is one of the major cause of disability and pain, and with obesity and increasing life expectancy the incidence is increasing [1]. Overall prevalence of knee OA in India has been reported to be 28.7% [2].

Functional disability along with pain and stiffness are the major presenting features of primary knee osteoarthritis (KOA). Currently, recording WOMAC Score, a self-assessment questionnaire score is widely accepted and practiced method to assess the functional disability in KOA [3]. Radiologically the staging is done with the help of Kellgren-Lawrence (K-L) grading system [4].

The Gold standard investigation for diagnosis of KOA is radiographs of the knee joint but radiography has also some limitations like lack of sensitivity [5], degree of flexion at knee, different views of the image, inter and intra observer discrepancies [6], angle of x-ray tube [7] besides radiographs being a historical statement of the damage which has already occurred in the joint. Moreover, most people with arthritis are diagnosed very late in the disease process as the patient reports late to the clinicians when he had already developed clinical symptoms and it is past the stage at which pharmacological or surgical treatments will slow or reverse the progression.

The ability to diagnose the disease in initial stages i.e., even before the radiological changes occur can open the door for more successful interventions. Hence, there is an urgent need for reliable and quantitative test which can detect KOA at an early stage. Serological biomarkers have the potential to achieve this objective of detecting early-stage knee OA.

Aims and Objectives

The aim of this study was record and report the serum levels of COMP, a cartilage disruption biomarker in normal adult knee (Control Group) and in knee osteoarthritis (Case Group). The efficacy of this biomarker in predicting the presence or absence of KOA and its ability to predict the severity and progression of disease was also studied.

Material and Method

Cases and controls

The study was designed as a prospective case-control study and included 150 subjects (100 cases and 50 controls). Cases included all those patients who had reported to our Out-patient department with complaints pertaining to primary knee osteoarthritis. Controls were those persons who did not have any complaint pertaining to knee joint. Subjects were excluded if they had a) any other pathology effecting knee joint, b) Secondary osteoarthritis, c) pregnant or lactating females, d) any renal/hepatic disease, rheumatoid arthritis, uncontrolled diabetes, bleeding disorder or malignancy, e) were on treatment of osteoarthritis, f) drug abuse.

All subjects were explained about the purpose and relevance of the study and only those who volunteered were included in the study after signing the consent form. The study proposal was approved by research committee and Institutional ethics committee and was done in accordance with the ethical standards as laid down in the 1975 Declaration of Helsinki and its later amendments (2013) or comparable ethical standards. The study was partially funded by the Institutional research committee. The study was conducted after clearance from Ethical committee from January 2020 till September 2021.

Self-assessment questionnaires and imaging

After taking detailed relevant history of the Cases and Controls, all subjects were asked to fill up WOMAC Score sheet and weight bearing antero-posterior knee radiographs was taken. WOMAC score was calculated and K-L grading of all subject was done and recorded. This scale defines radiographic OA in 5 categories. Radiographs scored as grade 0 (normal) showed no radiographic features suggestive of OA; K-L grade 1 (At risk/questionable) included a minute radiographic osteophyte of doubtful pathologic significance. Radiographs showing an osteophyte but no joint space narrowing were assigned a K-L grade 2 (mild); moderate diminution of joint space was graded K-L grade 3 (moderate); and K-L grade 4 (severe) was defined by severe joint space narrowing with subchondral bone sclerosis [4].

Personal demographic data like: a) Smoking/drinking, b) Quadriceps strength, c) Occupation and daily routine physical activity was not recorded, Only Age, Gender, serum COMP levels, KL grade and WOMAC Score were recorded.

Sample collection and analysis

To estimate Serum COMP level a venous blood sample approximately 5 ml was drawn from antecubital vein of the patient between 12pm-2pm after a rest of 30 minutes and was stored in plain sample vials in freezer at -20° Celsius. Collected blood was incubated undisturbed at room temperature for 20 minutes. Blood sample was centrifuged at 3,000 rpm for 10 minutes at 4°celsius. Immediately the aliquot supernatant (serum) was stored in plain vials at -20^0 Celsius. Stored serum was tested for serum levels of Cartilage Oligomeric Matrix Protein (sCOMP) by enzyme linked immuno-sorbent assay ELISA technique for sCOMP levels. COMP was quantified with a sandwich-ELISA (AnaMar Medical, Lund, Sweden).

The data was analyzed by SPSS (Statistical Package for Social Sciences) Version 25.0 Statistical Analysis Software and ROC Curve analysis.

Data Analysis and Discussion

Several studies conducted in past suggested that COMP is an important degradation product of articular cartilage. It may prove to be a promising diagnostic and prognostic marker in serum for diagnosis of knee OA [8-11]. This inspired us to study association of serum COMP levels in normal knee and in a case of knee OA.

In many of the previous studies the authors have subdivided the study groups arbitrarily, not as per actual K-L grading system. K-L grade I has not received much significance and has been included either as normal controls or not included at all. Furthermore, "mild cases" at times would include K-L I and II; "moderate case would include K-L grade II and III and "severe case" would include K-L grade III and IV Cases of KOA [12-16]. The significance of this sizeable population belonging to this group was noticed and study of this group as a separate entity has also been suggested by many researchers [2,17-21]. K-L grade I cases have been differently labelled as "Pre-radiological", "Sub-threshold" population [2,22]. We labelled K-L grade I (questionable) as "At Risk" group as these subjects are likely to progress to clinically symptomatic stage. It has been reported that synovial fluid provides a more accurate picture of cartilage damage, we preferred estimation of serum levels of COMP so as to avoid unwanted chance infection in knee joint [23].

Demographic profile

Present study included 150 subjects (100 Cases and 50 Controls). Cases included 36 males and 64 females with a mean age 53.91 ± 10.36 years and Controls had 34 males and 16 females with mean age 33.80 ± 9.48 years. Subjects in Control group were significantly younger than subjects of Case group ($p = 0.001$). An ideal study design should have BMI, age and gender matched group in both case group and control group.

Analysis of results shows that WOMAC Score was significantly higher in cases (49.97 ± 17.98) than in Controls (11.24 ± 06.07) (t = 14.78, p < 0.001). (Table 1). Further, in Case group the mean *s*COMP level (17.38 ± 4.99 U/L) was significantly higher than in Control group (1.16 ± 0.39 U/L) (t = 22.90, p = 0.001) (Table 1).

The sCOMP levels were significantly higher with increasing age group in both Cases (F = 402.70, *p* = 0.001 and Controls (F = 248.72, p = 0.001). Within each subgroup of age category, the level of sCOMP was higher in cases than controls (p = 0.001) (Table 2). There was no case in >70-year category in controls hence could

	Controls (n- = 50)		Cases (100)		Significance
	Min. -Max.	Mean ± SD	Min. -Max.	Mean ± SD	
WOMAC Score (%)	0.0 – 24.0	11.24 ± 6.07	6.25 ± 87.50	49.97 ± 17.98	t = 14.78 *p* = 0.001
sCOMP level (U/L)	0.56 - 2.01	1.16 ± 0.39	7.88 ± 30.48	17.38 ± 04.99	t = 22.90 *p* = 0.001

Table 1: WOMAC score (%) and sCOMP (U/L) between Cases and Controls.

Age in years	Case (n = 100)			Controls (n = 50)			test and *p* value
	n	Mean	SD	n	Mean	SD	
< 50	34	16.35	5.78	45	1.16	0.39	t = 17.59 and *p* = 0.001
50-59	31	16.77	3.34	3	1.07	0.37	t = 07.60 and *p* = 0.001
60-69	27	18.99	4.73	2	1.10	0.51	t = 05.24 and *p* = 0.001
≥70	8	20.19	5.60	0	0	0	t = a and *p* = a
Among Age categories	F = 402.70, *p* = 0.001			F = 248.72, *p* = 0.001			

Table 2: *s*COMP levels (U/L) in different age groups in Cases and Controls.

(*p* < 0.05 Significant level, S: Significant), a- not computed, #-Computed between 3categories).

not compared with case group. Increase in COMP level with age has also been reported earlier [14,24]. Though one author has reported on the contrary [25].

Among 100 cases there were 8 subjects with of KL grade I, 50 cases with KL grade II, 33 cases with KL grade III and 9 cases with KL grade IV KOA. (Table 3).

K-L Grade	*s*COMP Levels (U/L)				WOMAC Score (%)		
	n	Min	Max	Mean ± SD	Min	Max	Mean ± SD
0	50	0.56	2.01	1.16 ± 0.39	0.0	24.0	11.24 ± 6.07
I	8	19.48	27.36	14.60 ± 6.47	6.25	42.70	22.90 ± 11.0
II	50	10.25	28.50	17.47 ± 4.99	15.6	83.30	45.93 ± 13.48
III	33	11.63	30.48	17.25 ± 4.63	42.7	87.50	57.39 ± 16.48
IV	9	12.47	27.15	19.77 ± 4.65	48.9	83.30	69.90 ± 11.78
One-way ANOVA (Case group only)	F = 1.55, *p* = 0.02 Between KL grade I/II/III/IV				F = 20.18, *p* = 0.001 Between KL grade I/II/III/IV		

Table 3: Comparison of *s*COMP level with WOMAC Score and KL Grade.

sCOMP levels

Analysis of data showed sCOMP level as 1.16 ± 0.39 U/L in K-L grade 0 (normal); as 14.60 ± 6.47 U/L in K-L grade I (At Risk); as 17.47 ± 4.99 U/L in K-L grade II (mild); as 17.25 ± 4.63 U/L in K-L grade III (moderate) and as 19.77 ± 4.65 U/L in K-L grade IV (severe). (Table 3). In our study increasing level of the sCOMP is seen with increasing K-L grades except in grade III and grade IV.

The level of sCOMP increases with increasing K-L grade and this difference is statistically significant when compared as group (F = 1.55, p = 0.02). The difference between Controls (K-L grade 0) and Cases (K-L grade I/II/III/IV) is statistically significant. But there is no significant difference in *s*COMP levels when compared individually between each K-L grade I and II, between K-L grade II and grade III and between K-L grade III and grade IV.

We did not find a significant increase within K-L subgroups but other authors have reported significant difference within K-L subgroups [8,12-16,18,25,26]. The reason for this variation might be because in all of these studies there were only three study groups.

Segregation of subjects in Case group did not actually follow K-L grading norms. Controls group at times included subjects with no radiographic evidence but history of pain was present. In one study K-L grade II and III was taken as "moderate OA" and in another study K-L grade III and IV are taken "severe OA case".

Despite all the variations of allocating the study subjects in to a particular disease severity group, they all have reported that sCOMP level is significantly higher in a group with clear signs and symptoms of radiographic KOA (K-L grade II/II/IV) than a group of subjects with knee pain and possible osteophyte lipping or doubtful joint space narrowing (K-L grade I) which in turn is again significantly higher than controls (K-L grade 0). Similar result has been shown in our study. Further, the sCOMP values do not show any significant increase between K-L grade II, II and IV indicates that sCOMP levels are a poor indicator of the disease progression.

Co-relation of biomarker with other parameter

One-way ANOVA was computed to find the association between K-L Grading and *s*COMP levels with WOMAC score. Our results showed that K-L Grade is significantly associated with *s*COMP level ($F = 1.55$, $p = 0.02$) and with WOMAC score ($F = 20.18$, $p = 0.001$). (Table 3).

Pearson Correlation and coefficient (r) value was calculated for Age with WOMAC score and K-L grade. Age showed moderate positive and significant co-relation with WOMAC Score ($r = 0.43$, $p = 0.001$) and with KL grade ($r = 0.40$, $p = 0.001$) but showed weak positive and significant co-relation between Age with sCOMP level ($r = 0.24$, $p = 0.01$). (Table 4) Pearson correlation and coefficient (r) value was calculated for sCOMP with WOMAC score and KL grade. It showed weak positive co-relation of sCOMP with WOMAC Score ($r = 0.1$, $p = 0.14$), and with K-L grade ($r = 0.16$, $p = 0.11$) though not significant. (Table 4)

SN	Variable	Respondents		Pearson Correlation and coefficient (r) value	P value
		Mean	Stan. deviation		
1	Age (years)	53.75	10.60	0.40	0.001
	K-L Grade	2.43	0.76		
2	Age (years)	53.75	10.60	0.43	0.001
	WOMAC Score (%)	49.97	17.98		
3	Age (years)	53.75	10.60	0.24	0.01
	*s*COMP levels(U/L)	17.38	4.99		
4	*s*COMP levels(U/L)	17.38	4.99	0.14	0.14
	WOMAC Score (%)	49.97	17.98		
5	*s*COMP levels(U/L)	17.38	4.99	0.16	0.11
	K-L Grade	2.43	0.76		

Table 4: Co-relation among Age, K-L grade, WOMAC Score and *s*COMP levels.

Association of radiological grading (K-L grading) with WOMAC [15,27,29], with Age [15,28, 29] and with COMP [12,15,26, 29]; of COMP with age [8,15,24,26], with K/L grading[24], and with WOMAC [25] has been reported earlier as well by different authors in last decade. Another author reported no association of COMP level with age of the patient as well as with staging of the disease [25]. No significant association of COMP level with radiological grading has also been reported by some authors [8,26].

Diagnostic potential of biomarker

The receiver operative curve (ROC) analysis was done. A "Cut-off" value of sCOMP as 9.06 U/L (Sensitivity 99%; Specificity100%; Accuracy100%) between control group and Case group is suggested. Similarly, the "Cut-off" point as 12.79 U/L (Sensitivity 82%; Specificity 62.5%; Accuracy 69.6%) between K-L grade I and K-L grade II; "Cut-off" point as 17.13 U/L (Sensitivity 45.5%; Specificity 64%; Accuracy 49.2%) between K-L grade II and K-L grade III and "Cut-off" point of 18.52 U/L (Sensitivity 66.7%; Specificity 66.3%; Accuracy 66.3%) between K-L grade III and K-L grade IV KOA is suggested. But the discriminating power of sCOMP level does not have enough sensitivity, specificity and accuracy to discriminate individually between various K-L grades of knee O.A as shown by ROC curve analysis ($p > 0.05$). (Table 5).

Previous studies done by other researchers have reported that sCOMP level can discriminate between the normal knee joint and an KOA/osteoarthritic knee joint irrespective of disease severity as is also shown in our study [8,12,14,15,26,29,30]. Though the sCOMP levels are higher in higher disease severity yet the ROC curve analysis did not show sufficient discriminating power (sensitivity, specificity and accuracy) of sCOMP level to differentiate between K-l grade II, III and IV in our present study. Small sample size might be cause of this**.** Detailed search of Literature show only

Area Under Curve Test Result Variable(s): sCOMP levels(U/L)										
	Area	Std. Error[a]	Asymptotic Sig. b (p-value)	Asymptotic 95% Confidence Interval		Cut-off value	Sensitivity	Specificity	Accuracy	*P* - value
				Lower Bound	Upper Bound					
Case vs. Control	1.00	0.001	0.001	1.000	1.00	9.06	99%	100%	100%	P = 0.001
KL I vs. KL II	0.696	0.124	0.07	0.454	0.939	12.79	82%	62.5%	69.6%	P > 0.05
KL II vs. KL III	0.492	0.064	0.90	0.366	0.619	17.13	45.5%	64%	49.2%	P > 0.05
KL III vs. KL IV	0.663	0.102	0.137	0.462	0.864	18.52	66.7%	66.3%	66.3%	P > 0.05
A. Under the nonparametric assumption. b. Null hypothesis: true area = 0.5										

Table 5: Cut-off values of sCOMP levels in various K-L grades.

two authors who have reported "Cut off points" of sCOMP in knee OA patients, but in one study, subjects with K-L grade II, III and IV were taken as one group (knee pain with radiographic evidence) and in another study subjects with K-L grade II and III were taken as "moderate cases" [12,15]. The "Cut-off"/threshold value levels of sCOMP as 9.06 U/L (Sensitivity 99%; Specificity 100% and Accuracy of 100%) is suggested to differentiate knee osteoarthritis patients from healthy Controls.

The present study has some limitations, one of them is that study groups were not age, gender and BMI matched. Secondly, sample size is relatively small specially in K-L grade I group when each subgroup is studied individually. The study was time bound as per the condition of the funding agency and severe decrease in inflow of patients due to occurrence of pandemic forced both of these limitations.

Conclusion

Our findings revealed that the *s*COMP is a highly effective laboratory measure for distinguishing between healthy knee joints and those affected by KOA. However, its value in discriminating between various subgrades of K-L grading system fails, making it a less reliable biomarker for predicting the severity of disease in KOA.

The present study suggests a cut-off value of *s*COMP value as 9.06 U/L to differentiate between healthy controls and knee OA patients. Our findings must be validated by a study done in multiple centers with wide global separation careful selection of cases as per K-L grading system with gender, age and BMI.

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Conflict of Interest

None.

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