

Evaluating the Cartilage Oligomeric Matrix Protein Levels in Sera of Iraqi Patients with Rheumatoid Arthritis

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Abstract

The biomarker significance of chemokine (Cartilage Oligomeric Matrix Protein) was evaluated in the sera of 80 rheumatoid arthritis (RA) patients and 40 control men and women. A test ELISA kit was used in the Human Reader HS apparatus to measure the serum COMP, CRP, and RF. The Nihon Kohden Europes (MEK-6550K) analyzer apparatus was used for the testing of Complete Blood Count and the erythrocyte sedimentation rate (ESR), The Westergren device was used in this evaluation. The results demonstrated that COMP, CRP, RF, and ESR showed a significantly increased median in RA patients compared to control (COMP: 104.79 ± 1.95 vs. 47.79 ± 1.34 ng/ml; CRP: 2442.09 ± 172.32 vs. 1271.41 ± 35.14 pg/ml; RF: 0.92 ± 0.03 vs. 0.34 ± 0.024 ng/ml; ESR: 33.81 ± 2.52 vs. 15.53 ± 1.13 MM/H). Binary logistic regression analysis revealed that COMP was a significant predictor of RA. The ROC curve analysis also revealed that COMP recorded an area under the curve of 1.00 in RA patients. In conclusion, serum COMP is a reliable diagnostic parameter that may accurately distinguish between those with active RA and control individuals.

Keywords: Complete Blood Count, Cartilage Oligomeric Matrix Protein, C-reactive protein, Rheumatoid factor, Rheumatoid arthritis.

Introduction

Rheumatoid arthritis (RA) is a long-lasting autoimmune inflammatory condition influenced by genetic and environmental elements. The Global Health Organization estimates that RA affects 0.45 to 1% of the population and causes functional disability^{1,2}. About 70–80% of females are affected³. Peripheral-symmetric polyarthritis is its defining feature. As the disease progresses, there might be systemic signs like peripheral neuropathy, vasculitis, blood index abnormalities, and skin, ocular, lung, cardiovascular, and blood involvement⁴. The disease manifests as numerous, symmetrical inflammations and initially affects the minor joints before spreading to the major joints. Disorders outside of the joints might also be a symptom^{5,6}.

The American College of Rheumatology (ACR)⁶ defined RA's clinical and laboratory criteria. The Disease Activity Score in 28 Joints (DAS28) and the presence of rheumatoid factor (RF) are commonly employed measures to evaluate the therapeutic response of patients with rheumatoid arthritis.⁷

The emergence of biologic medicines has significantly enhanced the exploration of prognostic indicators serum cartilage oligomeric matrix protein (COMP) is a promising candidate as a predictive biomarker⁸. A sizable pentameric glycoprotein called the cartilage oligomeric matrix protein (COMP) interacts with other extracellular matrix proteins in cartilage and tissues⁹. Thrombospondin family member COMP is a 434.5-kDa

homopentameric noncollagenous protein containing calcium-binding proteins. For the most part, COMP is located in the hyaline ligament to maintain the integrity of the collagen structure¹⁰. Rheumatoid arthritis (RA) is characterized by the occurrence of synovial inflammation, which initiates the degradation of cartilage matrix components through enzymatic proteolysis. This process is facilitated by a cascade of cytokines.¹¹ COMP has received a lot of attention concerning RA, with some evaluations assuming that it may be used as a diagnostic and prognosis indicator, a measure of the severity of the condition, and a measure of how well a medication is working. However, conflicting opinions on the use of COMP as a biological indicator of RA were expressed by several analysts.¹²

Rheumatoid factor (RF) refers to autoantibodies that specifically recognize and bind to the Fc region of immunoglobulin G (IgG). In clinical practice, the measurement of IgM RF is more commonly performed compared to IgA and IgG RF. Approximately 80% of individuals diagnosed with rheumatoid arthritis (RA) exhibit rheumatoid factor (RF)¹³, although the diagnostic accuracy of RF is constrained due to its potential occurrence in several other inflammatory disorders that lead to continuous antigenic stimulation. Additionally, there is a correlation between smoking and a higher prevalence of RF, while it is true that a portion of individuals may develop rheumatoid factor (RF) at a later stage in the progression of the disease, it is worth noting that approximately 30% to 45% of patients diagnosed with early rheumatoid arthritis (RA) do not exhibit the presence of RF¹⁴. Similar to other diagnostic tests, the positive predictive value of rheumatoid factor (RF) is maximized when administered to those who are at a heightened risk of

developing the disease before testing, such as those who exhibit symptoms of inflammatory arthritis.

Testing individuals with osteoarthritis, myalgia, or non-specific arthralgia is not advised. There exists a positive correlation between rheumatoid factor (RF) and higher titers, which is associated with an elevated likelihood of developing rheumatoid arthritis (RA), while the administration of effective treatment for rheumatoid arthritis (RA) may lead to a decrease in rheumatoid factor (RF) titers, it is important to note that alterations in RF titers do not necessarily imply a corresponding modification in disease activity¹⁵. It is not recommended to engage in serial RF level monitoring. When making a selection for rheumatoid arthritis (RA) treatment, the presence of rheumatoid factor (RF) positivity may enhance the probability of a favorable response to B-cell-depleting monoclonal antibodies, such as rituximab¹⁶.

C-reactive protein (CRP) is frequently utilized as a biomarker for assessing systemic inflammation in individuals diagnosed with rheumatoid arthritis (RA). However, it also functions as an immunological regulator that plays a substantial role in the inflammatory pathways associated with rheumatoid arthritis (RA) and contributes to the development of atherogenic outcomes¹⁷. Comorbidities related to systemic inflammation are more prevalent in individuals with rheumatoid arthritis (RA). C-reactive protein (CRP) levels have been linked to an increased susceptibility to various health conditions, including cardiovascular disease, diabetes, metabolic syndrome, pulmonary disorders, and depression. The relationship between systemic inflammation, C-reactive protein (CRP), and comorbidities in rheumatoid arthritis (RA) is intricate.¹⁸

Materials and Methods

Eighty RA patients were enrolled in this study (74 females and 6 males). The age of the patients was (48.84±1.39 years). Samples were collected from Baghdad Teaching Hospital from March 1 to June 8, 2023. Forty people ages were (40.10±1.81 years), and gender (F:M 33:7) acted as healthy controls.

Before being a part of the study, The National Center for the Development of Humans and Medical City's Ethics Committee both gave their approval to the study. The following laboratory examinations were done on each patient: serum level of cartilage

oligomeric matrix protein (COMP), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), rheumatoid factor (RF), and complete blood count (CBC) indices (platelets, hemoglobin, hemoglobin concentration, and WBC and RBC). Some records were based on the clinical disease activity index (CDAI).

Rheumatoid arthritis (RA) patients were found to have gout, osteoarthritis (OA), polycystic ovarian syndrome (PCOS), disabilities, and cancer. pregnant

women were excluded, as were patients with another autoimmune disease.

Five milliliters of blood were withdrawn using a disposable syringe, each sample was centrifuged for 5 minutes at 3000 Xg after the blood was then placed into gel tubes and left aside to coagulate for 30 minutes at standard room temperature.

The serum was then divided into Eppendorf tubes and kept at -20°C (for a maximum of three months) for analysis of COMP, CRP, and RF. The quantitative sandwich enzyme immunoassay method employed in this assay (ELISA) was utilized to assess COMP, CRP, and RF levels (normal range of tests, respectively, (≤ 50 ng/ml), (≤ 2000 pg/ml), and (≤ 6.0 ng/ml) in serum using commercially available kits (Pepro Tech, USA) and according to the manufacturer's instructions.

In a nutshell, 12x8-well plate wells were coated with an anti-COMP, CRP, and RF antibody (arresting antibody). The appropriate wells were then loaded with serum 80 μ l a standard. An anti-human chemokine antibody that is bioavailable (detection

antibody) was added. Horseradish peroxidase (HRP), coupled with avidin, is added after the wells have been cleaned. After washing the wells to eliminate the unstructured avidin enzyme reagent, it interacts with the substrate solution, changing color before stopping. indirectly proportional to the quantity of COMP, CRP, and RF bound during the first phase. (The wavelength of the tests, as mentioned in the procedure in their kits, is 450 nm.)

Statistical analysis

Statistical analysis was accomplished with the statistical program for social sciences (SPSS 26). The data were expressed as mean \pm standard error (SE) with 95% confidence intervals. and the person correlation test has been utilized to highlight the distinction between COMP and other parameters within the patients' group. A significance level of 0.05 or lower was deemed as statistically significant. The ROC curve methodology was used to examine the optimal cut-off values for serum COMP, RF, and CRP.

Results and Discussion

The result indicates a statistically significant rise in age, whereas no statistically significant differences were observed in BMI between patients with rheumatoid arthritis (RA) (29.71 \pm 0.67) and control participants (27.73 \pm 0.81). Table 1.

Table 1. The Statistical distributions between patients and controls in AGE; BMI

Parameter	Mean \pm SE		p-value
	RA patients (N = 80)	Control (N = 40)	
Age(years)	48.84 \pm 1.39	40.10 \pm 1.81	<
BMI (kg/m ²)	29.71 \pm 0.67	27.73 \pm 0.81	0.0001

SE: Standard error, N: Number, p-value; +ve: Positive; -ve: Negative

The four examined parameters revealed a significantly increased serum level mean in RA patients compared to the control group with a p-value of less than 0.0001 (COMP: 104.79 \pm 1.96 vs. 47.79 \pm 1.34 (ng/ml); ESR: 33.81 \pm 2.53 vs. 15.53 \pm 1.14 (MM/H); CRP: 2442.09 \pm 172.32 vs. 1271.41 \pm 35.14 (pg/ml); RF: 0.93 \pm 0.03 vs. 0.35 \pm 0.02 ng/ml, respectively) Table 2.

Table 2. Serum level of COMP, ESR, CRR, and RF Rheumatoid arthritis patients and control.

Parameter	Mean \pm SE		p-value
	RA patients (N = 80)	Control (N = 40)	
COMP (ng/ml)	104.79 \pm 1.96	47.79 \pm 1.34	< 0.0001
ESR(MM/H)	33.81 \pm 2.53	15.53 \pm 1.14	< 0.0001
CRP (pg/ml)	2442.09 \pm 172.32	1271.41 \pm 35.14	< 0.0001
RF (ng/ml)	0.93 \pm 0.03	0.35 \pm 0.02	< 0.0001

SE: Standard error, N: Number, p-value; +ve: Positive; -ve: Negative

The findings indicate that there was no statistically significant difference observed in the platelet count (PLT) between rheumatoid arthritis (RA) patients (mean \pm SE: 274.23 \pm 8.77) and the control group (mean \pm SE: 274.8 \pm 10.33). Similarly, there was a non-statistically significant difference observed in the hematocrit (HCT) between RA patients (mean \pm SE: 37.3 \pm 0.46) and the control group (mean \pm SE: 38 \pm 0.57). Furthermore, no statistically significant differences were found in the white blood cell count (WBC), red blood cell count (RBC), and hemoglobin (HGB) levels between RA patients (mean \pm SE: 7.97 \pm 0.25, 4.50 \pm 0.07, 12.07 \pm 0.17, respectively) and control subjects (mean \pm SE:

7.60±0.29, 4.37±0.1, 12.59±0.25, respectively).
 Table 3.

Table 3. The Statistical distributions between patients and controls in CBC (complete blood count)

Groups	Mean ± SE		
	RA patients (N = 80)	Control (N = 40)	p-value
WBC(10 ³ /μL)	7.97±0.25	7.6±0.29	0.377
RBC(10 ⁶ /μL)	4.5±0.07	4.37±0.1	0.271
HGB(g/dL)	12.07±0.17	12.59±0.25	0.087
HCT(%)	37.3±0.46	38±0.57	0.361
PLT(10 ³ /μL)	274.23±8.77	274.8±10.33	0.969

Demonstrates the Personal correlation between RA patients and control groups. In the RA patient group, COMP levels do not show a significant correlation with any of the listed parameters. During the observation of the control group, it was shown that there exists a significant positive association between COMP levels and CRP (correlation coefficient of 0.440, p-value of 0.005) as well as PLT (correlation coefficient of 0.363, p-value of 0.021) Table 4, Fig. 1,2

Table 4. Correlation between COMP and other parameters in control and RA patients.

Parameters	RA patients (N = 80)		Control (N = 40)	
	R	P	R	P
Age	0.101	0.373	0.028	0.864
BMI	0.099	0.382	0.112	0.491
ESR	-0.069	0.543	-0.067	0.681
RF	0.217	0.053	0.188	0.245
CRP	0.003	0.980	0.440*	0.005
WBC	-0.095	0.400	0.162	0.317
RBC	-0.002	0.985	-0.036	0.825
HGB	0.004	0.971	-0.310	0.052
HCT	-0.045	0.690	-0.114	0.483
PLT	-0.169	0.135	0.363*	0.021

The strength COMP of correlations between variables is estimated by linear regression analysis.

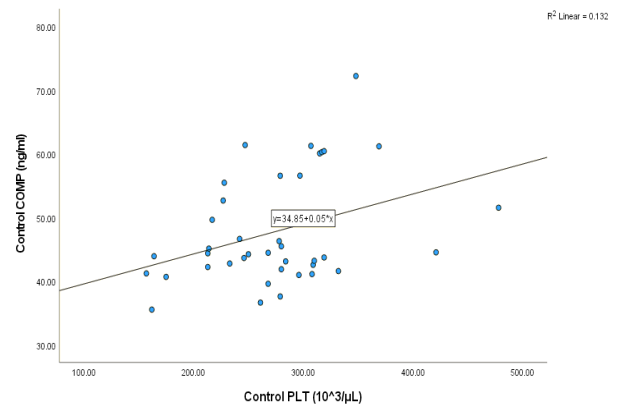


Figure 1. Linear regression between Log COMP and PLT in the control group.

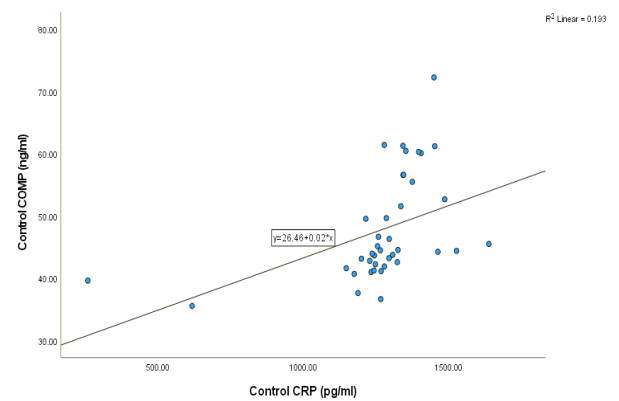


Figure 2. Linear regression between Log COMP and CRP in the control group.

ROC analysis revealed that the level of COMP in serum could discriminate between RA with a sensitivity of 100 and a specificity of 1. (Area under curve= 1) Fig. 3, Table 5.

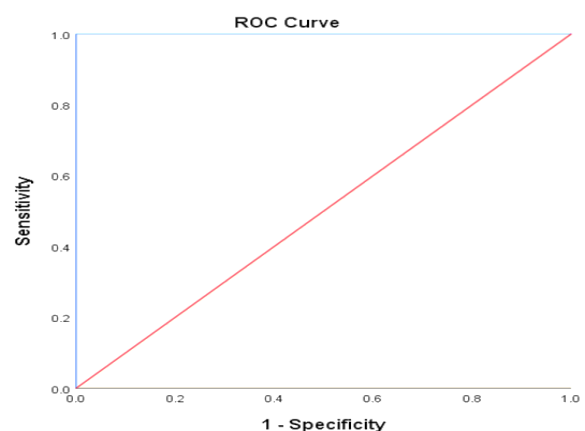


Figure 3. The ROC curve is used to diagnose serum COMP rheumatoid arthritis (RA) in patients.

Table 5. Area under the curve of ROC curve for using of serum COMP (RA) in patients.

Test Result Variable(s): COMP	Area Under the Curve				
	Area	Std. Error ^a	Asymptotic Sig. ^b	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
	1.000	.000	.000	1.000	1.000

a. Under the nonparametric assumption
 b. Null hypothesis: true area = 0.5

ROC analysis revealed that serum ESR, CRP, and RF levels could discriminate between RA (area under curve= 0.777,0.993,0.980 respectively) Fig. 4, Table 6.

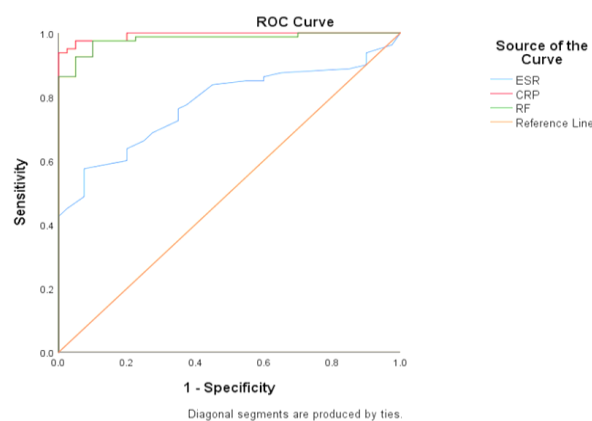


Figure 4. The serum ESR, CRP, and RF ROC curves are used to diagnose rheumatoid arthritis (RA) in patients.

Table 6. Area under the curve of ROC curve for using of serum ESR, CRP, and RF to diagnose the RA in patients.

Test Result Variable(s)	Area Under the Curve				
	Area	Std. Error ^a	Asymptotic Sig. ^b	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
ESR	.777	.042	.000	.696	.858
CRP	.993	.004	.000	.985	1.000
RF	.980	.011	.000	.959	1.000

a. Under the nonparametric assumption
 b. Null hypothesis: true area = 0.5

Discussion

The results are shown in Table.2 suggest that COMP, ESR, CRP, and RF may be useful as biomarkers for RA, and this is especially true for cancerous lesions. Earlier studies indicated relevant findings, and these chemokines have been reported to be associated with the progression of RA. A few years ago, the serum COMP test was introduced as the latest diagnostic tool for rheumatoid arthritis.¹² According to our research, serum COMP levels were greater in RA patients than in controls. A mild, non-destructive form of RA can progress to a severe, rapidly degenerative joint disease. To determine the most effective course of treatment and forecast the

prognosis of RA, several novels have suggested biomarkers be used. These biomarkers could be used to control therapy response and the course of joint degeneration and turnover in clinical practice and research settings. COMP was present in the sample of RA patients. Specificity and sensitivity were sufficient to identify late-stage RA patients' illnesses. The length of the erosive joint injury in RA is correlated with COMP production. Although there is evidence that COMP activates the complement system, which aids in the advancement of the disease, the primary effect of COMP on the course of RA is yet unknown. According to Idriss, Naglaa, et

al.,¹¹ there was a highly significant difference in the patient group compared to the control group ($P < 0.01$). According to a study by Hamid et al¹⁹ the result indicated a significant change between the two groups in the CRP ($P < 0.01$). and according to another study by Qabulio et al²⁰ there was a significant increase in variances in the patient's group compared to the control group ($P < 0.01$). However, according to our findings, serum COMP can also be used to determine the presence of rheumatoid arthritis. Our research found a strong positive connection between this biomarker and joint damage in advanced RA. When compared to healthy controls, its larger quantity denotes more severe joint cartilage destruction. In light of this, in addition to certain additional clinical options, serum cartilage

oligomeric matrix protein is another option. In addition to the Activity Score-28, radiologic imaging is utilized to follow patients, as are other metrics that are now extensively accustomed to tracking the development of illness. The ROC analysis demonstrated that COMP, CRP, and RF may be used for this with good sensitivity and specificity. This demonstrates that COMP was related to disease severity and joint pain in addition to reflecting cartilage damage. It is possible to use this biomarker to diagnose, prognosticate, and predict joint cartilage breakdown in RA patients. This can help doctors figure out which patients may respond well to a certain treatment, which can lower drug side effects and costs⁸.

Conclusion

Serum COMP concentration and CRP, RF, ESR, and CBC were non-significantly correlated with RA. The putative function in the etiology of rheumatoid arthritis cannot be attributed to this. Consequently, it is unlikely that this approach would yield any therapeutic benefits for the treatment of rheumatoid arthritis (RA). The COMP test has exceptional

diagnostic performance and achieves a high level of accuracy in distinguishing individuals with rheumatoid arthritis (RA) from control subjects. This observation suggests the existence of a potential biomarker that may be useful in the diagnosis of rheumatoid arthritis (RA).

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Authors' contributions statement.

T. S. I. has gathered the specimens, conducted the study experiment, then analyzed and explained the

findings. B. F. H. was responsible for the design and oversight of the project.

Authors' Declaration

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are ours. Besides, the Figures and Images, which are not ours, have been given the permission for re-publication attached with the manuscript.

- Authors sign on ethical consideration's approval.
- Ethical Clearance: The project was approved by the local ethical committee at University of Basrah.
- No animal studies are present in the manuscript.
- No potentially identified images or data are present in the manuscript.

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تقييم مصفوفة القسيمات القليلة الغضروفية للبروتين في أمصال المرضى العراقيين المصابين بالتهاب المفاصل الروماتويدي

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الخلاصة

تم تقييم أهمية العلامات الحيوية للكيمويات (بروتين مصفوفة قليل القسيم الغضروفي) في مصل 80 من التهاب المفاصل الرثوي (RA) و 40 من الرجال والنساء الأصحاء، تم استخدام اختبار ELISA Kit في جهاز Human Reader HS لقياس المصل COMP و CRP و RF و Nihon، تم استخدام جهاز تحليل Kohden Europe (MEK-6550K) لاختبار تعداد الدم الكامل، ومعدل ترسيب كرات الدم الحمراء (ESR) في جهاز Westergren في هذا التقييم. أظهرت النتائج أن COMP و CRP و RF و ESR أظهرت زيادة كبيرة في الوسيط في مرضى RA مقارنة بمجموعة الأصحاء (نانوغرام / مل 47.79 ± 1.34 مقابل 104.79 ± 1.95 COMP؛ جزء من الغرام. / مل 1271.41 ± 35.14 مقابل 2442.09 ± 172.32 CRP؛ نانوغرام / مل 0.34 ± 0.024 مقابل RF: 0.92 ± 0.03 ؛ مم / ساعة 15.53 ± 1.13 مقابل 33.81 ± 2.52 ESR). كشف تحليل الانحدار اللوجستي الثنائي أن COMP كانت مؤشرا هاما على RA. كشف تحليل منحنى ROC أيضًا أن COMP سجلت منطقة تحت منحنى 1.00 في مرضى RA. في الختام، يعد COMP في المصل معلمة تشخيصية موثوقة قد تميز بدقة بين أولئك الذين يعانون من التهاب المفاصل الرثوي النشط والأفراد الأصحاء.

الكلمات المفتاحية: تعداد الدم الكامل، بروتين مصفوفة قليل القسيم الغضروفي، بروتين سي التفاعلي، عامل الروماتويد، التهاب المفاصل الرثوي.