

## Cartilage Oligomeric Matrix Protein as New Marker in Diagnosis of Rheumatoid Arthritis

Mohamed I Aref<sup>1</sup> and Hamdy Ahmed<sup>2\*</sup>

<sup>1</sup>Clinical pathology Department, Al Azhar University, Cairo, Egypt

<sup>2</sup>Biochemistry of National Research Center (NRC), Al Azhar University, Egypt

### Abstract

Rheumatoid arthritis (RA) is the most common systemic inflammatory autoimmune disease of unknown etiology. The early in diagnosis of RA is crucial. To facilitate diagnosis during the early stages of RA, when often not all clinical symptoms are manifest, a good serological marker is needed. Among serological markers are rheumatoid factor (RF), erythrocyte sedimentation rate (ESR) C-reactive protein (CRP), anti-cyclic citrullinated peptides (anti-CCP) and cartilage oligomeric matrix protein (COMP). A comparison between those markers in respect to the accuracy was the aim of this study.

### Patient and methods

Sixty patients with RA and auto-immune non-RA were selected for this study compared with 20 normal healthy persons. The results showed both COMP and anti-CCP can be help for diagnostic value than other selected parameter.

**Keywords:** Rheumatic arthritis; Serological markers; Cartilage; Oligomeric matrix protein

### Introduction

Rheumatoid arthritis (RA) is a chronic systemic autoimmune inflammatory disorder characterized by inflammation and destruction of articular structures in disorder in association with extra-articular manifestation [1] such as: nodules [2], muscle weakness, nervous system [3], vasculitis, hematological abnormalities [4], skin disease e.g neutrophil dermatitis, ocular [5], lung [6], cardiac [7] and other organs could be involved. Joint destruction in RA results from the invasion of the cartilage and subchondral bone by the hyperplastic synovium, with synovial fibroblasts and inflammatory cells such as macrophages and T cells key rules in this process [8]. Proliferation of synovial membrane following infiltration by immune cells is thought to results in degradation of articular cartilage and bone, causing irreversible damage [9].

Diagnosis of RA depends on a constellation of signs and symptoms that can be supported by serology and radiographs, where involvement of small joints of the hands and feet is often the key of diagnosis [10]. But there's a difficulty in making an early diagnosis for RA, as inflammatory arthritis is a common manifestation of many conditions. Moreover, the classical clinical pattern of RA tend to emerge over time, or incomplete pattern often present in the first few months or even years. Additional, symptoms and signs may be masked by empirical treatment with anti-inflammatory drugs or corticosteroids [11]. Moreover, the damage may be progress in spite of decreased inflammatory activity and erosion may develop in patients without clinical signs of inflammation [12].

Laboratory tests such as ESR and CRP provide useful information for disease activity but are not specific to joint inflammation and correlate poorly with cartilage damage [13]. The presence of RF was used before as a diagnostic marker but now RF titer used as diagnostic and prognostic value in the evaluation of RA. The positive RF test can occur with other diseases such as systemic lupus erythematosus (SLE), Sjogren's syndrome, cryoglobulinemia, polymyositis, psoriatic arthritis, scleroderma, polymyaglia rheumatic, viral infections, active tuberculosis, tumor, Lyme disease, autoimmune thyroid disease [14].

During the last years a variety of circulating non-RA antibodies have been discovered and reported to be potential diagnostic value. Most of them neither could nor demonstrate to have adequate sensitivity and

specificity to form a basic for clinical and therapeutic decisions [15].

Based on the knowledge that mature filaggrin is the target of the AFP and AKA antibodies, synthetic citrulline-containing peptides were developed and tested for their reactivity with RA sera [15]. Citrulline is a nonstandard amino acid, as it is not incorporated into proteins during protein translation. It can be generated by post-translational modification of arginine residues by peptidyl-arginine deiminase enzymes. Antibodies against citrulline-containing peptide which was derived from filaggrin sequences can be detected in up 48% of RA sera with 98 specificity [15,16].

RA sera showed a remarkable variety in the reactivity pattern towered different citrulline-containing peptides, indicating that the amino acids flanking the citrulline residue are important for the antigenicity of the epitope and that anti-citrullinated protein activities such as AFP, AKA and anti-CCP are strongly polyclonal responses. It has been established that these antibodies are produced locally in the synovium of RA patients. However, anti-CCP is now a golden test for diagnosis of RA [17].

A valuable approach to monitor RA would be measuring biological markers of cartilage degradation and repair to reflect variations in joint remodeling. One such potential biological marker of arthritis is cartilage oligomeric matrix protein (COMP). This marker is released into the synovial fluid and other body fluids such as blood. In various studies, COMP has shown promise as a diagnostic and prognostic indicator as a marker of disease severity and the effect of treatment. The present study aimed to evaluate a laboratory marker, COMP in diagnosis of RA and comparing it with other laboratory markers.

**\*Corresponding author:** Hamdy A Ahmed, Biochemistry of National Research Center (NRC), Al Azhar University, Cairo, Egypt, Tel: 02-115-400-055; E-mail: drhamdyahmed@yahoo.com

Received March 23, 2015; Accepted May 01, 2015; Published May 05, 2015

**Citation:** Aref MI, Ahmed H (2015) Cartilage Oligomeric Matrix Protein as New Marker in Diagnosis of Rheumatoid Arthritis. Mod Chem appl 3: 151. doi:10.4172/2329-6798.1000151

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## Material and Methods

This study was conducted on 80 patients divided into three groups:

The 1<sup>st</sup> group included 40 patients (33 females and 7 males) with RA, fulfilling the American College of Rheumatology diagnostic criteria [18], as a test group.

**Inclusion criteria:** Patients reserved non-steroidal anti-inflammatory drugs.

Other diseases must be considered which appear similar to RA include: [19],

- Spondyloarthropathies: Ankylosing spondylitis, enteric infections, inflammatory bowel disease, psoriatic arthritis, Reiter's arthritis, Whipple's disease.
- Infections cause acute rheumatic fever, bacterial endocarditis, gonococcal arthritis, Lyme disease. Viral infections (HIV, HBC)
- Metabolic and endocrine causes: arthritis of thyroid disease, Gout, hemochromatosis, Pseudogout
- Connective tissue diseases: dermatomyositis, polymyalgia rheumatic, polymyositis, scleroderma, Still's disease, systemic lupus erythematosus
- Other diseases that can mimic RA: amyloidosis, angioimmunoblastic lymphadenopathy, arthritis associated with oral contraceptives, malignancy, sarcoidosis.

**Exclusion criteria:** Patient under treatment of cortisone or biological treatment.

The 2<sup>nd</sup> group included 20 patients (13 females and 7 males) with other rheumatic diseases as SLE, vacuities, dermatomyositis, systemic sclerosis, mixed connective tissue disease and reactive arthritis, as a pathological diseases; a control diseases group with other autoimmune diseases.

The 3<sup>rd</sup> group included 20 apparently healthy subjects (11 female and 9 male) as a normal control group.

All groups were matched as regard age and sex.

Morning blood samples were collected. CPC, ESR, liver and kidney function tests, CRP and RF were done in the same day of blood collection while the rest of blood left to clot and centrifuge at 3000.

rpm for 15 minutes. Serum was separated and kept in refrigerator at -C20 for other parameters (anti-CCP and COMP).

Complete blood count was done by Sysmes XT2000i series. Liver and kidney function tests (ALT, AST, and Urea creatinine were done by ADVIA 1800 chemistry system.

ESR was done by Wester's method using Westergren's tube. CRP assay is designed for the quantitative measurement by nephelometry using Hs-CRP reagent on BN-ProSpec Nephelometer.

Qualitative determination of rheumatoid factor (RF) was determined by latex agglutination test provided by SPINREACT, Spain, for research and diagnostic products. Measurement of anti-CCP 2 IgG using ELISA kit provided by the Binding Site Ltd, for research and diagnostic products.

Variable measurement of cartilage oligomeric matrix protein (COMP) using ELISA kit provided Bio Vender-laboratories medicine for research and diagnostic products. Radiological investigations were done on both hands and feet and assessed for erosions and for joint space narrowing in both hand and feet joints.

Results were tabulated and statistical analysis was performed with statistical package for social science (SPSS version 13). All data are expressed as mean  $\pm$  SD.

The Tests used were :- X mean, SD, Student's T test for testing statistical significant difference between means of two samples. Specificity, sensitivity, positive and negative predictive value accuracy and Pearson correlation test were used. Significant result is considered if  $P < 0.05$ . Highly significant result is considered if  $P < 0.01$ .

## Results

The comparison between the RA group and the other autoimmune diseases group for CRP and ESR showed no statistical significant difference while those 2 groups showed a significant difference when compared to the control healthy group (Tables 1-3).

The comparison between the RA group and the other autoimmune diseases group for RF, Anti-CCP and COMP showed a statistical significant difference moreover those 2 groups showed significant differences when compared to the control healthy group (Tables 4-6).

There was high significant positive correlation in group I (RA patient) between anti-CCP versus COMP, CRP, and ESR. While anti-

Variable	ESR group I	ESR group II	CRP group1	CRP group II
Mean $\pm$ SD	45.7 $\pm$ 23.4	57.4 $\pm$ 31.5	49.7 $\pm$ 44.8	45.5 $\pm$ 43.6
T test	1.632		0.437	
P value	>0.5		>0.5	

Table 1: The comparison between group 1 and group II for ESR and CRP.

Variable	ESR group I	ESR group III	CRP group1	CRP group III
Mean $\pm$ SD	45.7 $\pm$ 23.4	7.4 $\pm$ 4.3	49.7 $\pm$ 44.8	2.7 $\pm$ 1.4
T test	4.2201		8.3231	
P value	<0.1		<0.1	

Table 2: The comparison between group 1 and group III for both ESR and CRP.

Variable	ESR group II	ESR group III	CRP group1I	CRP group III
Mean $\pm$ SD	57.4 $\pm$ 31.5	7.4 $\pm$ 4.3	45.5 $\pm$ 43.6	2.7 $\pm$ 1.4
T test	8.333		4.2663	
P value	<0.1		<0.1	

Table 3: The comparison between group II and group III.

CCP had no correlation with other variable in the other autoimmune diseases group II.

There was a high significant positive correlation between COMP versus ESR in RA patients, while there were no significant correlations between COMP and all variables in the other autoimmune disease group (Tables 7-9).

## Discussion

Diagnosis of RA depends on a constellation of signs and symptoms that can be supported by serology and radiographs, where involvement of small joints of the hands and feet is often the key of the diagnosis [10]. But there's a difficulty in making an early diagnosis for RA, as inflammatory arthritis is a common manifestation of many conditions. Moreover, the classical clinical pattern of RA tends to emerge over time, or incomplete pattern often present in the first few months or even years. Additionally, symptoms and signs may be masked by empirical treatment with anti-inflammatory drugs or corticosteroids [11].

Recently COMP test was introduced as new marker for diagnosis and prognosis of RA. A comparison between COMP and the well-

known markers such as ESR, CRP, RF and anti-CCP was the aim. The present work showed a significant increase of ESR and CRP in RA group compared to control healthy group while there is no significant difference between group I and group II, that is to say those markers cannot be used for diagnosis.

As regard to RF, the finding concluded a high positive percentage between RA group compared to either non-RA group or healthy control group. On the other hand a lack of accuracy make RF is out of choice as a marker for diagnosis.

A comparison between RF and COMP were concordance with a cross-sectional study by Andrade et al. and Heidari et al. [20,21]. The average levels of COMP and anti-CCP was superior than RF. Skoumal et al. [22] suggested that this marker can be used for prediction in diagnosis of RA in addition to joint destruction.

A comparison between COMP and anti-CCP showed that COMP is more or less in accuracy with anti-CCP. All available data indicate variation in sensitivity and specificity of COMP and anti-CCP across different studies [23,24].

Variable	RF group I	RF group II	Anti-CCP group I	Anti-CCP group II	COMP group I	COMP group II
Mean ± SD	60%	20%	874 ± 741.9	8.2 ± 6.9	1110.1 ± 536.4	44.8 ± 233.7
T test			91.5411		98.41341	
P value			<0.1		<0.1	

Table 4: The comparison between group 1 and group II for RF, anti CCP and COMP.

Variable	RF group I	RF group III	Anti-CCP group I	Anti-CCP group III	COMP group I	COMP group III
Mean ± SD	60%	0%	874 ± 741.9	6.7 ± 5.9	1110.1 ± 536.4	100.3 ± 1.4
T test			31.1461		43.3684	
P value			<0.1		<0.1	

Table 5: The comparison between groups 1 and group III for RF, CCP and COMP.

Variable	RF group II	RF group III	Anti-CCP group II	Anti-CCP group III	COMP group II	COMP group III
Mean ± SD	20%	0%	8.2 ± 6.9	6.7 ± 5.9	44.8 ± 233.7	100.3 ± 1.4
T test						
P value			<0.5		<0.5	

Table 6: The comparison between group II and group III for RF, anti-CCP and COMP.

Variable	Anti-CCP	COMP	RF
Sensitivity	92.5%	90%	60
Specificity	95%	92.5%	90
PVP	94.9%	92.3%	85.7
NPV	92.7%	90.2%	69.2
Accuracy	93.8%	91.3%	75

The results showed that the accuracy was towered anti-CCP and COMP.

Table 7: A summary for accuracy between anti-CCP and COMP.

Correlation	Group I (RA) Coefficient correlation r	Group II (other autoimmune disease) Coefficient correlation r
Anti-CCP with ESR	0.44**	0.11
Anti-CCP with CRP	0.41**	0.33
Anti-CCP with COMP	0.8**	-0.01

\*p<0.05 \*\*p<0.01

Table 8: Pearson correlation test between anti-CCP and other studied variables in group I (RA) and group II (other autoimmune diseases).

Correlation	Group I (RA) Coefficient correlation r	Group II (other autoimmune disease) Coefficient correlation r
COMP with ESR	0.4**	0.31
COMP with CRP	0.17	0.14

\*p<0.05 \*\*p<0.01

Table 9: Pearson correlation test between COMP and other studied variables in group I (RA) and group II (other autoimmune diseases).

Among several factors that could explain the discrepancy in accuracy between diverse studies including the present study is the presence of high proportion of false positive non-RA among controls. However, other factors such as genetic background may be also responsible for these variations. Also, the difference in scale size of various studies may contribute in this discrepancy.

The correlation analysis between both anti-CCCP and COMP with the clinical signs of RA was significant while not significant with other autoimmune diseases. A prospective study by Lindqvist et al. [25], radiographic changes in hands and feet at 5 and 10 years after inclusion were evaluated and compared with several laboratory markers. The markers analyzed were: ESR, CRP, COMP, RF and anti-CCP. Multiple linear regressions with backward elimination were used to determine the prognostic value of the variables. After 5 years, the presence of IgA RF, serum COMP and anti-CCP were significant associated with more severe damage. Baseline COMP and anti-CCP predicted radiographic outcome after 10 years. A stronger prediction was obtained by combining the prognostic factors. A combination of these measures reflecting different aspects of disease process should be useful for evaluating prognosis in individual patients with early RA.

Feyertag et al. [26] evaluated the changes in a local biomarker, the COMP was better correlated to changes in different clinical measurements in RA than those biomarkers other autoimmune diseases. So, COMP is better in assessment of joint status than other markers which may be masked by the treatment. Indeed, the previous conclusion was also supported by Vilim et al. [27] and Skoumal et al. [28]. Furthermore, Tseng et al. [29] described COMP to be specific marker for the cartilage degradation in RA and not related to the nonspecific inflammatory process.

## Conclusion

In this study concluded that anti-CCP is not now the sole specific marker for RA patients. The addition of COPM can enhance the diagnosis especially in very early disease.

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