

## Determination of Anti-Cyclic Citrullinated Peptide (ACCP) and C-Reactive Protein (C-RP) Concentrations in Serum of Patients with Fibromyalgia Syndrome (FMS)

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### ABSTRACT:

#### BACKGROUND:

Fibromyalgia syndrome (FMS) is a chronic musculoskeletal syndrome; almost invariably, symptoms persist at 5- and 10-year follow-ups. The degree of functional impairment is similar to that seen in patients with moderate to severe rheumatoid arthritis. Anti-cyclic citrullinated peptide (anti-CCP) antibody testing is particularly useful in the diagnosis of rheumatoid arthritis, with high specificity, in the early disease process, with the ability to identify patients who are likely to have severe disease and irreversible damage. CRP is a member of the class of acute-phase reactants that its levels rise dramatically during inflammatory processes occurring in the body.

#### OBJECTIVE:

the main objective of this study is to determine the frequency of anti CCP- in patients with FM and the association of anti-CCP level with C-RP.

#### PATIENTS & METHODS:

This study included 60 patients with FMS according to the ACR 1990 criteria. Their age range was 20-60 years. These patients were then matched by age and sex to 30 healthy control persons with mean age  $42.81 \pm 2.16$  years.

#### RESULTS:

The mean values of ACCP antibodies concentration in serum of patients with FMS were no significant difference as compared to the level in serum of healthy controls ( $p > 0.05$ ), while the mean values of C-RP concentration in serum of patients with FMS were significantly increased than the level in serum of healthy control ( $p < 0.05$ ).

#### CONCLUSION:

It is clear from this study that there is no relationship between the levels of ACCP concentration and FMS while C-RP concentration in patients with FMS may be a good indicator to evaluate this disease.

**KEY WORD:** ACCP, C-RP, FMS

### INTRODUCTION:

Fibromyalgia syndrome (FMS) is a chronic musculoskeletal syndrome; almost invariably, symptoms persist at 5- and 10-yr follow-ups. The degree of functional impairment is similar to that seen in patients with moderate to severe rheumatoid arthritis<sup>(1, 2)</sup>. Fibromyalgia is characterized by chronic widespread pain and also

allodynia, a heightened and painful response to pressure.<sup>(3)</sup> Other core symptoms include debilitating fatigue, sleep disturbance, and joint stiffness. Some patients<sup>(4)</sup> may also report difficulty with swallowing,<sup>(5)</sup> bowel and bladder abnormalities<sup>(6)</sup>. FMS affects (2-4) % of general population, and affects more females than males, with a ratio of 9:1 by ACR criteria<sup>(7)</sup>. People with FMS also may have imbalances in any of the following brain chemicals: Serotonin, Melatonin, Stress hormones, IGF-1 growth hormone all showed low level, while Substance P shows high levels and there is Abnormal pain perception<sup>(8)</sup>. The important sign of FMS is a pain in "tender

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points" on physical examination <sup>(9)</sup>. During diagnosis, four kilograms-force is exerted at each of the 18 points; the patient must feel pain at 11 or more of these points for fibromyalgia to be considered <sup>(10)</sup>.

The discovery of anti-CCP antibodies evolved from previous work examining autoantibodies in sera from RA patients that were distinct from rheumatoid factor. The first citrulline-binding autoantibodies in RA sera were discovered by Nienhuis *et al.* in 1964 <sup>(11)</sup>. Antibodies to cyclic citrullinated peptide (ACCP) can be detected by enzyme-linked immunosorbent assay (ELISA) and have been found to be a more specific serum test for RA than the Rheumatoid factor (RF) titer. Citrulline is a typical amino acid formed by the deimination of arginine that catalyzes by peptidylarginine deiminase, in certain proteins <sup>(12)</sup>. C-reactive protein (C-RP) is a protein found in the blood, the levels of which rise in response to inflammation (an acute-phase protein). C-RP is synthesized by the liver in response to factors released by fat cells (adipocytes). C-RP is a member of the class of acute-phase reactants as its levels rise dramatically during inflammatory processes occurring in the body. It is also believed to play another important role in innate immunity, as an early defense system against infections <sup>(13)</sup>.

### PATIENTS & METHODS:

The prospective study comprised 60 Iraqi patients (50 female, 10 male) fulfilling the ACR criteria for the diagnosis of FMS.

Another 30 (22 female, 8 male) healthy individuals matched for age and sex were collected serving as a control group.

Blood samples were taken from individuals in both groups for estimating ACCP conc. and C-RP conc. Laboratory investigation which include: Hemoglobin (Hb), Erythrocyte sedimentation rate (ESR) was done in Laboratory Teaching center of Baghdad Hospital.

The study was based on the immobilization of cyclic Citrullinated peptides (CCP) to the surface of microtiter plate and subsequent binding of anti-CCP antibodies from human serum.

IMTEC-CCP-Antibodies is an indirect solid-phase enzyme immunoassay (ELISA) for the quantitative measurements of IgG class autoantibodies against Cyclic Citrullinated Peptide (CCP) in human serum.

The hsC-RP ELISA is based on the principle of a solid phase enzyme – liked immunosorbent assay. The assay system utilizes a unique monoclonal anti C-RP antibody directed against a distinct antigenic determinant on the C-RP molecule <sup>(14)</sup>.

### Statistical analysis:

Descriptive statistics for all data of each set were expressed as a mean  $\pm$  SD. And compared using independent sample (t) test  $p < 0.05$  were considered statistically significant. The overall productive values for the results in studied groups were performed according to program of office xp.

### RESULTS:

The characteristics of 60 FMS patients and 30 controls are shown in table(1).

Table 1: Characteristics of FMS patients and controls

Parameters	FMS (N = 60) Mean $\pm$ SEM	Healthy Control (N= 30) Mean $\pm$ SEM	P-value	Sig.
Age (y)	39.95 $\pm$ 1.10	42.81 $\pm$ 2.16	0.42	NS
BMI (kg/m <sup>2</sup> )	28.53 $\pm$ 0.56	27.64 $\pm$ 0.89	0.21	NS

Values are the (mean $\pm$ SD), BMI=body mass index, FMS=Fibromyalgia Syndrome and NS=not significant, N=number of samples.

The mean values of ACCP antibodies conc. in serum of patients with FMS were no significant difference as compared to the level in serum of health control group ( $P > 0.05$ ), while the mean value of C-reactive protein

concentration was significantly elevated as compared to the level in serum of health control group ( $p < 0.05$ ) as shown in table (2).

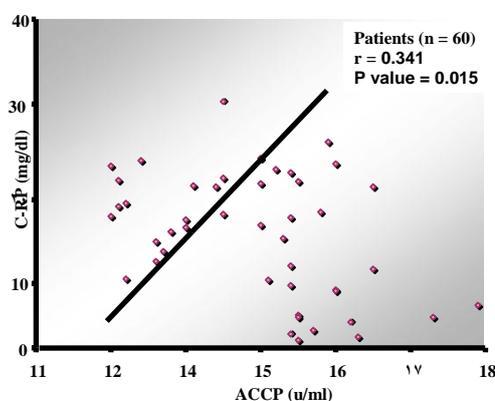
By using a correlation analysis, there was a weak positive relationship found between ACCP and C-RP ( $r = 0.341$ ) as shown in figure (1).

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**Table 2: Serum ACCP, C-RP levels (mean±SD) in patients with FMS (n=60) and controls (n=30).**

Parameters	N	Mean	SD	t-test (P-value)	Sig.
ACCP (u/ml)					
Healthy Controls	30	9.6788	0.9891	0.201	NS
Patients	60	9.5735	0.8650		
Total	90				
C-RP (mg/dl)					
Healthy Controls	30	4.933	1.049	0.031	S
Patients	60	8.780	6.045		
Total	90				

SD = standard deviation, N = number of samples, NS= no significant, S= significant



**Figure 1: Correlation between C-RP and ACCP.**

### DISCUSSION:

In this study we showed no significant difference in serum ACCP concentration and significant increases in C-RP concentration in patients with FMS.

No considerable Change in ACCP concentration agree with the result of a study demonstrated that the determination of ACCP was specific test to RA, which agree with other study<sup>(15)</sup> suggests that in the joints of patients with RA, proteins may be changed to citrulline as part of the process that leads to inflammation of the rheumatoid joint. The results of the present study correlation between ACCP and C-RP have agreed with a previous study were done on sera from 242 RA patients who were followed for 3 years. Anti-CCP antibodies were positively correlated with higher erythrocyte sedimentation rate (ESR), C-reactive protein (CRP)<sup>(16)</sup>.

Another study to determine the frequency of antibodies to cyclic citrullinated peptides (CCPs) in patients with RA and the association of anti-CCP antibodies with disease activity, which forty patients with RA and 38 patients with fibromyalgia were

### CONCLUSION:

It is clear from this study that there is no relationship between the levels of ACCP concentration and FMS while C-RP concentration in patients with FMS may be a good indicator to evaluate this disease.

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