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## RESEARCH ARTICLE

# A Comparative Assessment of the Diagnostic Value of Anti-cyclic Citrullinated Peptide Antibodies and Rheumatoid Factor in Rheumatoid Arthritis

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### Abstract

**Objective:** The present study was designed to compare the diagnostic value of anti-cyclic citrullinated peptide antibodies and rheumatoid factor in patients with rheumatoid arthritis.

**Material and methods:** The comparison of (60) sample with RA patients (41 females and 19 males), was done with control groups of (40) sample (25 females and 15 males). Serum levels of anti-cyclic citrullinated peptide antibodies were determined by particle-enhanced (latex) Immunonephelometry on genius analyser (CO,Ltd.china) by analytical kits from Imtec-ccp-Antibodies and levels of rheumatoid factor were determined by turbidimetry on a latex-enhanced agglutination assay. **Results:**The results of this study showed a significant increase of Anti-CCP (p value=0.00021) in rheumatoid arthritis (RA) disease than control groups, as well as significant increase was recorded with age. The present study also exposed a sensitivity of anti-cyclic citrullinated Peptide (anti-CCP) was (68.33 %) for rheumatoid arthritis patients in comparison with (58.03%) for rheumatoid factor RF in same study groups, while the specificity of anti-CCP for RA disease (95.5%) with comparable specificity to the rheumatoid factors (92.5%).

**Conclusion:** There are so many recommendations to include this powerful diagnostic tool in the criteria for the diagnosis of rheumatoid arthritis (RA).

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## INTRODUCTION

The arthritis of joints is known as synovitis, which is inflammation of the membrane that lines joints and tendon sheaths. Joints become swollen, tender and warm, and stiffness limits their movement. Rheumatoid arthritis typically manifests with signs of inflammation (Schueller 2012). Some RA patients show evidence of autoimmunity long before the appearance of clinical arthritis such as autoantibodies (e.g. anti-cyclic citrullinated peptides) can be detected in the blood of the patients many years before the onset of disease, which creates by the chemical conversion of arginine to citrulline, by method known as citrullination or deamination. The conversion of arginine into citrulline can have important consequences for the structure and function of proteins, since arginine is positively charged at a neutral pH, whereas citrulline is uncharged. This increases the hydrophobicity of the protein, leading to changes in protein folding during inflammation (Mor-Vaknin et al 2003). Recently, these Anti-citrullinated protein antibodies (ACPAs) have rotated up as powerful biomarkers, which are accepted as a major diagnostic tool in diagnosing rheumatoid arthritis (RA) in a very early stage of disease (Raptopoulou et al 2007).

## MATERIAL AND METHODS

The studied group includes (100) samples, (60) sample with RA patients (41 were female and 19 male) compared with (40) sample normal control (25 females and 15 males). This study performed during the period from 2014 to 2015. The age ranged from (25-68) years, were divided into four categories groups:

G1: 25-35 years 10.0 % of patients study groups. G2: 36-45 years 23.3% of patients study groups. G3: 46-55 year 48.3 % of patients study groups. G4: 56 and more up to 68years 18.3 % of patients study groups. These subjects were selected from patients attending the outpatient clinic in Baquba Teaching Hospital–Rheumatology Department. Detection of serum Anti-CCP was measured using particle-enhanced (latex) Immunonephelometry on genius analyser (CO,Ltd.china) by analytical kits from Imtec-ccp-Antibodies and levels of rheumatoid factor were determined by turbidimetry on a latex-enhanced agglutination assay. Data were entered to computer and analyzed using computer facility of SPSS (Statistical Package for Social Science), the results were expressed as numbers, percentage and mean  $\pm$  S.D (standard deviation of the mean). ANOVA was used to assess the significance of differences between groups and simple correlation for relationship between two qualitative variables, taking  $p \leq 0.05$  lowest limit of significance.

## 3. Results

In our study, it was seen that sample with RA patients were found to have significantly higher anti-ccp levels compared to normal control groups and with  $p = 0.00021$ , as shown in Table (1) and Fig.(1).The highly levels of anti-CCP that occurs among studied groups was  $(39.02 \pm 12.24)$  and 95% CI  $(33.09; 44.94)$  for RA patients at G4 with (56 and more years old). The levels of anti-ccp among studied groups were (34%) in patients and (17%) in controls individuals as illustrated in Fig. (2).

The demographic data on RA are shown in table (2). There were more females than males. Anti-CCP has been described for male, about (21.1 %) from 25 while anti-CCP antibodies for female (75.6 %) from 35 were positive. Anti-CCP antibodies levels have been created on the cutoff value suggested by the manufacturer for anti-CCP at  $>18$  Unit/ml reflect as positive

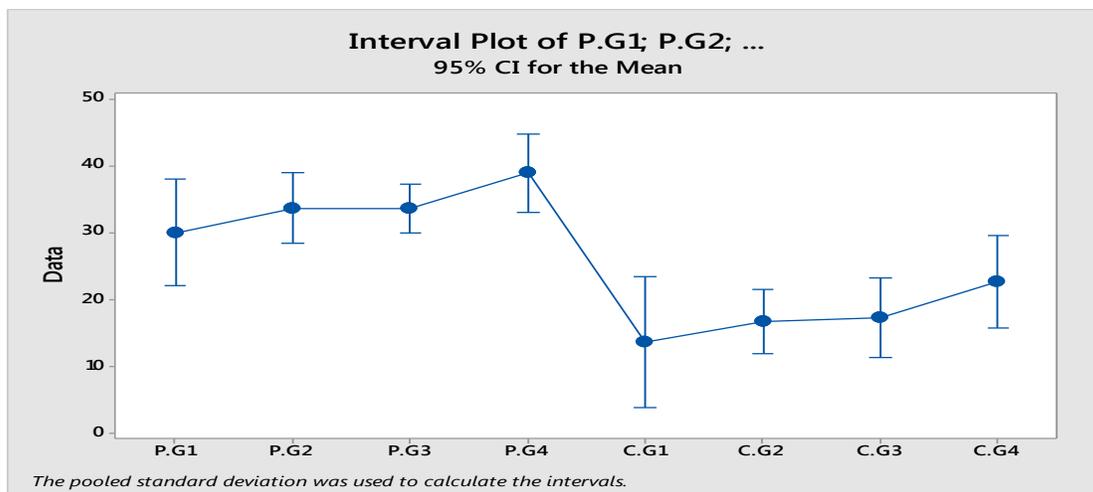
Table(3) is a comparative table of age effect in RA patients and controls, comparatively with (25-55) years group the percentage of positive anti -ccp antibodies were (50-51.7%) ,While highly frequency increase with (56 and more years old) the percentage of positive anti -ccp antibodies were (81.8%)

The positive analytical value of rheumatoid factor RF regarding the diagnosis of RA was (70%) and the negative analytical value was (30) % using the rheumatoid factor test (RF).While the positive analytical value of anti-cyclic citrullinated Peptide (anti-CCP) regarding the diagnosis of RA was (56.6%) and the negative analytical value was (43.3) % using the anti citrullinated Peptide test as appearance in table (4). Sensitivity of anti-cyclic citrullinated Peptide anti-ccp was (68.33 %) for RA studied groups in comparison with (58.03%) for rheumatoid factor RF in RA studied group. These results give a good diagnostic value because of specificity of anti-ccp for RA disease (95.5%) while the specificity of RF for RA disease (92.5%). Due to its high positive predictive value and negative predictive value, it is an important diagnostic test and gives accurate diagnosis of the disease. However, this does not mean that anti-cyclic citrullinated peptide antibodies can replace rheumatoid factor in the diagnosis of rheumatoid arthritis, because not all rheumatoid arthritis patients have anti-cyclic citrullinated peptide antibodies. The two tests therefore appear to be complementary concerning the previous findings; it seems that anti-ccp is a good diagnostic marker since it elevated significantly in the sera of RA patients as exposed in fig (3). The two diagnostic tests have linear relationships.

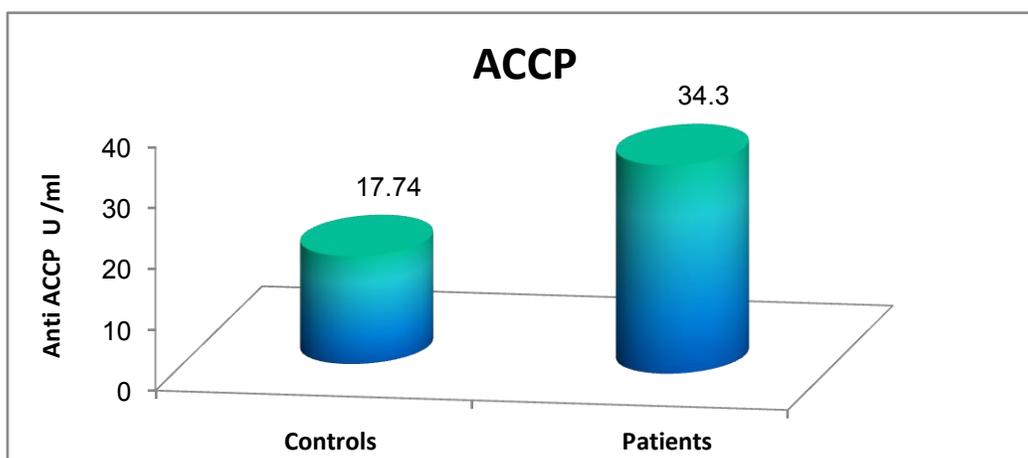
**Table (1): Distribution of Anti-CCP (U/ml) among studied groups**

Anti-CCP				
Study groups	Number	Mean	StDev	95% CI
P.G1	6	30.07 bc	15.11	( 22.06; 38.09)
P.G2	14	33.76 ab	11.10	( 28.51; 39.01)
P.G3	29	33.72 ab	12.42	( 30.08; 37.37)
P.G4	11	39.02 a	10.24	( 33.09; 44.94)
C.G1	4	13.57 e	6.36	( 3.75; 23.39)
C.G2	17	16.74 de	5.87	( 11.98; 21.50)
C.G3	11	17.28 d	3.06	(11.360;23.202)
C.G4	8	22.60 cd	4.11	( 15.66; 29.54)
Total P.Titer	60	34.3	12.0	-----
Total C.Titer	40	17.74	5.46	-----

\*\* F-Value = 10.59 P-Value = 0.00021 \*a= significance, U/ml (b, c d) slightly significance \*\*e= Insignificance



**Figure (1): Distribution of Anti-ccp patients among studied groups**



**Figure (2): levels of Anti-ccp patients among studied groups**

**Table (2): Percentage of positive and negative ACCP in RA according to the sex**

SEX	+VE	%	-VE	%	TOTAL
MALE	4	21.1	15	78.9	19
FEMALE	31	75.6	10	24.4	41
TOTAL	35	58.33	25	41.6	60

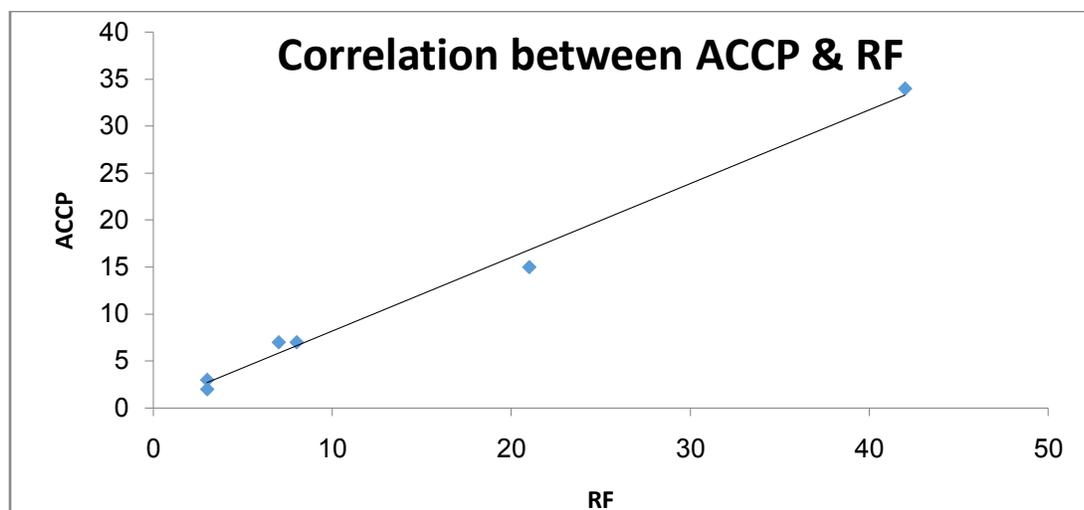
Chi- Sq =20.8286 \*\*      P-Value =0.00052

**Table (3): Percentage of positive and negative Anti-ccp according to the age**

AGE	+VE	%	-VE	%	TOTAL
25 – 35	3	50	3	50	6
36 – 45	7	50	7	50	14
46 – 55	15	51.7	14	48.3	29
56 >	9	81.8	2	18.2	11
TOTAL	34		26		60
Chi-Sq* =8.82353      P-Value =0.032					

**Table (4): Relation of rheumatoid factor (RF) and ACCP results among studied group**

AGE	ACCP			RF		
	+VE	-VE	Total	Positive	Negative	Total
25 – 35	3 (50%)	3 (50%)	6	3 (50%)	3 (50%)	6 (10%)
36 – 45	7 (50%)	7 (50%)	14	8 (57.1%)	6 (42.9%)	14(23.33%)
46 – 55	15 (51.7%)	14 (48.3%)	29	21(72.4%)	8 (27.6%)	29(48.33%)
56 >	9(81.8%)	2 (18.2%)	11	10 (90%)	1 (9.1%)	11(18.33%)
TOTAL	34(56.6%)	26(43.3%)	60	42(70%)	18(30%)	60
ACCP Sensitivity=68.33% Specificity=95.5%				RF Sensitivity=58.0 3% Specificity=92.5%		



**Figure (3):- correlation between anti-CCP & RF among studied groups.**

## DISCUSSION

In the present study, it was seen that sample with RA patients were found to have significantly higher anti-ccp levels compared to normal control groups and with  $p = 0.00021$ , as shown in table (1) and fig.(1). The highly levels of anti-CCP that occurs among studied groups was  $(39.02 \pm 12.24)$  and 95% CI  $(33.09; 44.94)$  for RA patients at G4 with (56 and more years old), The levels of anti-ccp among studied groups were  $(34.3 \pm 12.0)$  in patients and  $(17.75 \pm 5.46)$  in controls individuals as illustrated in Fig.(2). Rheumatoid arthritis is more common in female than in male as presented in table (2). This is consistent with many studies (Gabriel et al 2009) who stated that RA is more common in females and (Masi, 2000) represent the ratio of rheumatoid arthritis 3:1 or 4:1. The explanation is that Androgens are believed to be more protective of RA risk than estrogens. While (Cutolo et al 2002) found that estrogen (which act as enhancer to humeral immunity) may decrease incidence and enhance improvement of RA by mechanism away from their action as sex hormone this is supported by the fact that RA is more among post-menopausal women as stated by (Panayi 2003) and by study of (Masi, et al 2006) who found that pre menapausal women with RA have low estrogen level and men with RA have high cortisol level. The results that previously mentioned denoted that-high frequency among females rather than males which may be due to the hormonal differences (stimulatory effects of estrogen) between them that will affect the immune responses. Those make women tend to mount more Th 2 responses which are proinflammatory, so may enhance the development of autoimmunity (Kindt et al 2007).

Table(3) is a comparative table of age effect in RA patients and controls, comparatively with (25 -68) years old groups the percentage of positive anti -ccp antibodies were (50-51.7%) ,While highly frequency increase with (56 and more up to 68 years old) the percentage of positive anti -ccp antibodies were (81.8%) . which stated that RA is most common between 40-60 years old. This could be because the diagnosis increases in old age due to decrease physical ability and increase joint damage in older age group. The present study compared diagnostic performances of two tests in RA patients. Markers such as RF and anti-CCP antibodies were selected from among other markers. RF has been widely used as a screening test for patients with arthritis. The positive analytical value of rheumatoid factor RF regarding the diagnosis of RA was (70%) and the negative analytical value was (30) % While the positive analytical value of anti-cyclic citrullinated Peptide anti-ccp regarding the diagnosis of RA was (56.6%) and the negative analytical value was (43.3)% using the anti citrullinated Peptide test as appearance in table (4). It seems that it is a good diagnostic marker since it elevated significantly in the sera of RA patients in comparison to control individuals. Specificity was (95.5%) and sensitivity(68.33%). However, other studies reported sensitivity of (41-58%) of RA patients for anti-CCP (Ronnelid et al 2005), which are too low in comparison with the current study. The interpretation of these variations is that the level of anti-CCP decrease during therapy. In addition to the lack of international reference serum, different manufacturing companies with different cut-off values, and different serum dilution, all of these alters the results and necessitates the need for international standardization. High specificity of anti-CCP antibodies had been investigated by other researchers most of them reported specificity of (97%-98%) Institute of Biomedical Science (2006). Regarding the previous findings, it seems that anti-CCP is a good diagnostic marker since it elevated significantly in the sera of RA patients it was observed that Anti-CCP antibodies can be detected years before manifestations of the symptoms, indicating that the initial events that lead to this autoimmune

disease may have taken place before clinical manifestation and definitive diagnosis can be made as have been reported by (Pruijn et al 2005). Figure (3) showed that there is a significant correlation between Anti-CCP and RF and linear relationship between these two tests. There are so many recommendations to include this powerful diagnostic tool in the criteria for the diagnosis of RA; some authors( Khosla 2004) call it (the new rheumatoid factor in the serology of RA); However, The Anti-CCP Ab marker is important in early diagnosis of RA as it was announced by (van Van Venrooij et al 2004)., prediction of severe disease and differentiation of RA from other diseases like Polymyalgia rheumatica OA and erosive SLE as represented by (Khosla et al 2004).

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