

# Comparison Between RF, CRP And CCP in Diagnosis of Rheumatoid Arthritis in an Iraqi Population

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## Abstract

A total of 60 patients with RA and 36 apparently healthy control individuals without other autoimmune disease non-diabetic, non-pregnant were included in this study. Patient and control groups were matched in age and gender (P value is 0.37). C-reactive protein (CRP), rheumatoid factors (RF) were detected in all participants. Antibodies directed to cyclic citrullinated peptide (ACPA), in the human serum samples were measured by enzyme-linked immunosorbent assay (ELISA). The results of this study showed that the RA disease is more frequent in female (51/60, 85%) than male (9/60, 15%). The mean age was  $44.6 \pm 12.5$  years and female to male ratio was 5.6:1. Patients with RA had recorded a higher CRP positivity (66.7) compared with control group (36.1) with highly significant difference. In conclusions, most RA patients were females, there were many laboratories diagnostic tests to the diagnosis of RA like CRP, RF and ACCP but the ACCP antibodies test was more specific for predicting and/or diagnosing RA.

## 1. Introduction

Rheumatoid Arthritis (RA): is a chronic systemic autoimmune disease that primarily affects small joints. It typically results in warm, swollen and painful joints, with fever and low energy. Most commonly, the wrist and hands are involved symmetrically and may also affect other parts and organs of the body. RA results in the inflammation and thickening of the synovial tissue. It also affects the underlying bone and cartilage. Often, the symptoms of disease come on gradually over weeks to month.<sup>1</sup>

In some patients RA primarily affect joints and other organs such as lungs, kidneys, pericardium, skin, eyes, heart and blood vessels. RA Patients have an increased mortality compared to the general population, especially due to cardiovascular disease.<sup>2</sup>

When rheumatoid arthritis is clinically suspected by physicians, testing for the presence of Rheumatoid Factor (RF) and Anti-Cyclic Citrullinated Peptide Antibodies (ACPAs that measured as anti-CCP antibodies) are required.<sup>3</sup> A negative RF or CCP antibody is not excluded as RA; rather, the arthritis is called seronegative. This is the case that occur in about 15-25% of people with RA.<sup>4</sup>

The autoimmune diseases are multifactorial and caused by an interaction of many factors genetic and environmental factors, and share a number of characteristics that suggest common etiological pathways or mechanisms.<sup>5</sup>

### Anti CCP test

can detect some citrullinated self proteins such as

fibrinogen, keratin, collagen, fibronectin, vimentin and  $\alpha$ -enolase. These subsets of seropositive patients elicit true disease. About 43% to 63% of patients with Rheumatoid arthritis are seropositive for citrullinated  $\alpha$ -enolase that is strongly related with PTPN22, HLA-DRB1 (04) and smoking.<sup>6</sup>

Also, the age and smoking have a role where the prevalence of Rheumatoid Arthritis in Iraq is around 1% in adult population<sup>7</sup> and the smoking concurrently increases the production of ACPAs and pro-inflammatory cytokines responsible for the progress of RA<sup>8 9</sup>

## 2. Materials and Methods

### Patients

The study population included patients and control (case control study). Patients, which were diagnosed with Rheumatoid arthritis (according to rheumatologist physicians and serological tests). Blood samples were collected from 60 cases at Al-Sadder Medical City and other hospitals in Najaf/Iraq, in the period between January 2016 to August 2016. From 60 cases, there were 51 females and 9 males, and the patients age was 9-70 years.

### Control

thirty-six apparently healthy controls who had no history or clinical evidence of rheumatoid arthritis or any other disease

### Exclusion Criteria

all patients with any acute or chronic disease and patients with rheumatoid arthritis and other autoimmune disease were excluded from this study

in addition excluding diabetic individuals and pregnant women.

### Immunological Studies, including

Anti-CCP antibody by ELISA.

### Serological Tests

#### Rheumatoid Factors (RF) Assay

The Rheumatoid factor RF latex test is a slide agglutination test for the qualitative and semi-quantitative detection of rheumatoid factor in human serum. Latex particles coated with human gamma globulin are agglutinated when mixed with serum samples that are contain RF.

#### Reactive Protein Assay

This test is performed by using CRP- Latex slide agglutination.

### Immunological Test

Enzyme Immunoassay for Quantitative Detection of Antibodies against Cyclic Citrullinated Peptide (ACCPs):

### 3. Results

#### Distribution of the Studied Groups according to Gender

This case control study includes (60) patients with RA matched with (36) healthy controls. Table shows the gender distribution and reveals that most patients with RA were females (85%) (females at more risk than male). (With no significant difference in the distribution according to gender among RA cases and controls group).

**Table 1: Differences in gender distribution among cases with RA and controls. P (Chi-square) =0.37[NS]**

Gender	Healthy controls	Healthy controls	Cases with RA	Cases with RA
	N	%	N	%
Female	28	77.8	51	85

#### Distribution of the Studied Groups According to Age

Sixty patients of RA are selected with the age ranging

from (9 to 70) compared with 36 healthy controls with age ranging from (15 to 63) This table shows no significant difference between patients when compared to the controls.

**Mean age ±SD of RA patients and controls were (44.6±12.5 , 42.3±10.8).**

Age (year)	Healthy controls	Cases with RA	P value
Range	(15 to 63)	(9 to 70)	0.37 (NS)
Mean	42.3	44.6	
SD	10.8	12.5	

#### Detection of (CRP), (RF) and (ACCP) in the Sera of the Studied Groups

This table shows that 85% from all cases of RA(n=60) in this study have ACCP antibody in their sera, 80%

from the cases have RF in their sera while 66.7% have CRP in their sera while no one from controls have ACCP in their sera. So, there is a significant difference when compared between cases and controls according to positive tests.

**Table 3: Risk of having RA in the presence of selected positive test results**

Positive tests	Healthy controls (n= 36)		Cases with RA (n=60)		OR	95%CI OR	P value
	N	%	N	%			
CRP	13	36.1	40	66.7	3.54	(1.49-8.41)	0.004
RF	5	13.9	48	80.8	24.8	(7.96-77.31)	< 0.001

#### Specificity and Sensitivity of CRP, RF and ACCP

This table shows the ACCP test is highly specific

100% and sensitive 85%. The RF test has good specificity 86.1% and 80% sensitivity while CRP has less sensitivity 66.7 and specificity 63.9 than RF and ACCP tests.

**Table: Validity parameters for the selected tests used to diagnose RA differentiating it from healthy controls**

Positive if > cut-off value	Sensitivity	Specificity	Accuracy
CRP	66.7	63.9	65.6
RF	80	86.1	82.3
ACCP	85.5	100	90.6

## 4. Discussion

This study showed that most rheumatoid arthritis patients (n=60) were females (51/60) (85%) while males were (9/60) (15%). So, the female: male ratio is (5.6:1).

The female to male ratio among RA patients in this study was higher if compared with the previous local studies recorded in Iraq which showed that the ratio of female: male is (3.3:1) (Al-Haidary, 2003)<sup>10</sup>, (3.6:1) (Chiad et al., 2015)<sup>11</sup> and (4:1) (Al-Safar 2008), (2.9: 1) while it was less than (6.1:1 and 6.2:1) (Al-Timimi et al., 2014)<sup>12</sup>. Rheumatoid arthritis is higher in women than in men due to the changes in hormone levels which may affect the level of proteins in the blood that contribute to inflammation. The estrogen hormone plays a role in affecting the B and T cells which are involved in the immune response and environmental factor could explain the reverse in the trend for women. (Gabriel et al., 2008).

Most epidemiological studies suggested an age of RA disease onset during or after the fifth decade of life<sup>13</sup>

Regarding the mean age of patients, this study revealed that the mean of age  $44.6 \pm 12.5$  years was observed in RA patients. Other studies nearly have the same results ( $45.6 \pm 12.245$  and  $45.5 \pm 14.6$ ) (Saleh et al., 2014; Hasan et al., 2016).

The lower mean of age between Iraqi patients probably was because of the fact that the life spans of Iraqi were lower than that of European populations that was may be because of accumulative effects of environmental, psychological, and chemical agents of the last war in Iraq.<sup>14</sup>

The statistical result of this study refers to the high frequency of positive serum CRP test which was significantly found among RA patients 66.7% compared with 36.1% in control group (P value = 0.004). and OR (3.54). This result was nearly compatible with Al-Obeidy et al, who reported that 72.4% of RA patients were positive for CRP compared with 8.0% for control group<sup>15</sup>

While results of (Akeel, 2014). showed that 90% of RA patients were positive for CRP compared with 5.0% CRP positivity in control group.

The results of the present study demonstrated a high frequency of RF positivity among RA patients 80.0% compared with 13.9% for control group with (P value < 0.001) and OR (24.80). This present study demonstrated a high positivity of antibody against citrullinated protein in patients with RA (85.0% ) compared with control group which does not report any positivity. These findings correlate with an Iraqi study carried by Al-Yasiri et al. who reported relatively similar results with a positive serum ACCP 80% among RA patients, and none of the healthy controls' sera were positive for anti-CCP.<sup>14</sup>

When individuals are negative for CCP antibody test but have a positive RF test, then the clinical signs and symptoms are more vital in determining whether they have RA or some other inflammatory condition.

When someone is negative for both CCP antibody and RF, then it is less likely that the person has RA disease. It must be emphasized, however, that RA is a clinical diagnosis and may be made in the absence of positive tests for autoantibodies this based on the physicians.<sup>16</sup>

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