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## **Assessment of Auto-antibodies (RF, Anti-CCP, and Anti-RA33) in Rheumatoid Arthritis Patients: Comparative study**

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**Abstract**--Rheumatoid arthritis is a chronic systemic autoimmune disease which is typically present in adulthood, more common in women than men and affecting 0.5- 1% of the adult population. Many auto-antibodies could be implicated in the pathogenesis of the disease of these Anti-RA33 antibodies and anti-CCP antibodies in addition to rheumatic factor (RF). This case control study was done during the period from January 2021 to August 2021. It was conducted on (105) patients with RA matched with (45) healthy control. RF, anti-CCP and RA33 were positive in 84( 80% ), 89(84.7 %), and 100 ( 96.1 %) patients with RA, respectively. The anti-CCP antibody showed 100% sensitivity for the detection of RA, and the anti-RA33 antibody showed a sensitivity of 96.1%. Anti-CCP demonstrated more favorable predictive values for RA than anti-RA33 and RF. There is a highly significant difference ( $P \leq 0.05$ ) when compared between RA patient groups and control group. In this study the mean levels of all auto-antibodies ( RF, anti-CCP, RA33 ) were increased significantly ( $P < 0.05$ ) in RA patients ( $113 \pm 81$  ,  $92.3 \pm 87.5$ ,  $336 \pm 110$ ), respectively compared with control group. The age group (61- $\geq$ 70) group appeared significantly higher mean level of RF, anti-CCP, RA33 than other age groups. When the Anti-RA33 is used as a diagnostic test according to ROC curve, it appeared beneficial for the diagnosis of RA when compared with Rheumatoid Factor (RF) and anti-CCP.

**Keywords**--Rheumatoid arthritis, RF, anti-CCP, anti-RA33, Sensitivity, Specificity.

## Introduction

Rheumatoid arthritis (RA) is a chronic, systemic autoimmune disease that primarily affect small joints, characterized by inflammation and cellular proliferation in the synovial lining of joints that can ultimately result in cartilage and bone destruction. Many auto-antibodies could be implicated in the pathogenesis of the disease; of these auto-antibodies are RF is detected in 70-80% of patients with established disease, and is an integral part of the definition of this disorder (Wu et al. 2017). Anti-citrullinated protein antibodies (ACPA) are directed against one or more of an individual post translationally modified proteins, and frequently detected in the blood of RA patients. Anti-CCP assays are the most widely used methods to study ACPA. Anti-CCP have been evaluated in patients with early synovitis, and were found to be more specific than RF for early RA, while having comparable sensitivity. Interestingly, RF and anti-CCP have both been found in blood samples taken several years before disease onset in a subset of patients (Plenge et al., 2005). Testing for the presence of Rheumatoid Factor (RF) and Anti-Cyclic Citrullinated Peptide Antibodies (ACPAs) that measured as anti-CCP antibodies are required when rheumatoid arthritis is clinically suspected by physicians. A negative RF or CCP antibody is not excluded as RA; rather, the arthritis is called seronegative (Alm *et al.* 2018). anti-RA33 antibodies which are directed to the heterogeneous nuclear ribonucleoprotein A2 (hnRNP-A2), the (hnRNP-A2) is over expressed in inflamed synovial tissues, but its expression in normal joints is very low, also they were present in 20-25% of systemic lupus erythematosus (SLE) and in 35-40% of patients with mixed connective diseases (Al-Ubaidi et al. 2013). This study aims to assess some diagnostic autoantibodies (RF, Anti-CCP, and Anti-RA33) in RA compared with healthy control individuals.

## Materials and Methods

The case control study was carried out on 105 patients that were attending the Rheumatology out patient's clinic in Al-Sadder Medical City and other hospitals in Najaf/Iraq, during the period between December, 2021 to August, 2022. All patients diagnosed according to 2010 American college of Rheumatology/ European league Against Rheumatism (ACR/EULAR). Forty five (45) age and gender matched healthy subjects taken as control. Patients with other inflammatory autoimmune disease were excluded from the study. From each subject 5ml of venous blood was aspirated, sera were separated by centrifugation and were stored at -20 centigrade and tested. RF was detected by RF latex slide agglutination (Solarbio/china). Anti-CCP antibodies and anti-RA33 antibodies were assessed by enzyme-linked immunosorbent assay (ELISA) kit from Genius / USA protocol.

## Results and Discussion

In the current study, there is a highly significant difference ( $P < 0.05$ ) when compared between RF, CCP, and RA33 values between RA patient and healthy control group. RF, anti-CCP and RA33 were positive in 84 (80%), 89 (84.7%), and in 100 (96.1%) patients with RA, respectively, as shown in table in table 1.

Table 1: RF and Anti-CCP and Anti-RA33 in patients and control groups.

Parameter	Result	RA patients No (%)	Healthy control No (%)	P-value
RF	Positive	84(80%)	6 (13.3%)	P<0.05
	Negative	21 (20%)	39 (86.7 %)	
Anti-CCP	Positive	89 (84.7%)	0	P<0.05
	Negative	16 (15.3%)	45 (100%)	
Anti-RA33	Positive	100 (96.1%)	0 (0%)	P<0.05
	Negative	5 (3.9%)	45 (100%)	

Table 2 and figure 1 shown the Risk of having RA in the presence of selected positive test results. 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. There is a significant difference when compared between cases and controls according to positive tests. There are limited researches about the RA associated with specific markers which are RF, RA33 and CCP. Our results have been shown in figure 4-1, the RF (IgM) was positive in 84 (80%), the results showed also Anti-CCP antibodies were positive in 89 patients (84.7%). It is good indicator to explore a new sensitive marker. These results were in agreement with the study of Al-Ubaidi et al. (2013) have demonstrate the same values. It seems that the CCP is more sensitive marker than anti-RA33 because they remained positive even when the patient had treatment for long time while anti-RA33 usually positive in the initial phase of the disease.

Table 2: Risk of having RA in the presence of selected positive test results.

Positive tests	Healthy controls (n=45)		Cases with RA(n=105)		P value
	No.	%	No.	%	
CRP	10	22.2	70	66.7	P<0.05
RF	6	13.3	84	80	P<0.05
ACCP	0	0.0	89	84.7	P<0.05

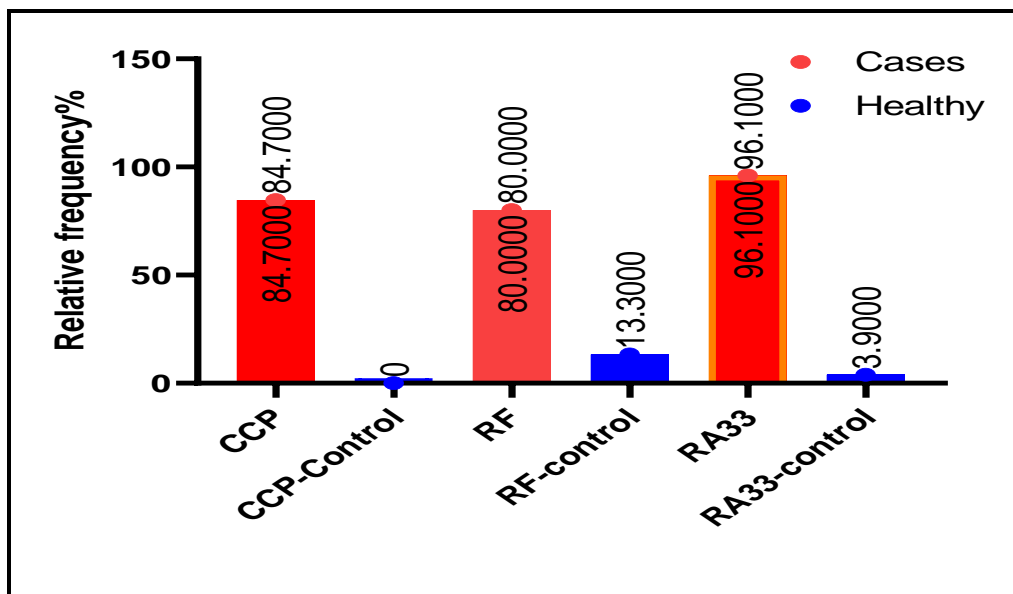


Figure 1: Bar chart showing the case-control difference in relative frequency of positive tests.

The sensitivity and specificity of these three parameters (RF, anti-CCP, RA33) used in this study were assessed according to (Zhang *et al* 2013) as in table (3). In the present study the anti-CCP antibody showed 100% sensitivity for the detection of RA, and the anti-RA33 antibody showed a sensitivity of 96.1%. Anti-CCP demonstrated more favorable predictive values for RA than anti-RA33 and RF. The reported association between anti-CCP and RA was confirmed in this study. The values of sensitivity and specificity of anti-CCP test vary from one study to another. In a study by Kaptanoglu *et al.* (2010) the sensitivity and specificity were 53% and 79%, while Awwad and Aboukhamis (2010) were reported the same specificity(100%)and 71.9% sensitivity. Al-Mughales *et al.*, (2015) reported that rheumatoid factor Compared to anti-CCP antibodies, and C-reactive proteins, the anti-RA33 autoantibodies seem to be not representing as an important additional immunodiagnostic marker in Saudi patients with established RA. RA33 may have more interest in early RA or less severe RA and other systemic connective tissue disorders, in which anti-CCP specificity appeared in 90.6% with sensitivity in 63.4% in RA patients compared to healthy control. However, the anti-CCP test values alone were significant in correctly identifying patients with RF positivity, as compared to the anti-RA33 test.

Our data has indicated sensitivity of anti-RA33 antibodies about 95.2 % and 100 %specificity. We compared our results with other findings authors. Interestingly, Zahran *et al* and Al-Mughales (2015) have reported that the sensitivity is about 6–58% and specificity is about 69–96% (Zahran *et al.*,2013; Al-Mughales *et al.*2015). However, others resarchers findings were in disagreement with ours including 98% sensitivity and 20% specificity for anti-RA33 in RA patients. Al-Ubaidi *et al.*(2013) showed that anti-CCP antibodies have the highest specificity and rheumatoid factor has the highest sensitivity than anti-RA33 antibodies. These

different results than ours, it could be; because there was a different condition to collect the samples, genders, ages and other diseases.

Lashkari et al. (2014) and Al-Mughales et al. (2015) have reported the similar findings. The significant linear relation between RA33 and CRP suggests that the few patients with positive RA33 have less severe RA. In addition to less sensitivity of anti-RA33 they reported that anti-RA33 is not exclusively present in RA. Although the current findings were in agreement with most studies, but differences between these studies reported above might be attributed to either RA severity or ethnic origin This is supported by Maslyanskiy et al. (2014) when used hnRNP B1 (RA33) as autoantigens and also suggested the influence of genetic involvement. Moreover, they reported that anti-hnRNP B1 autoantibodies are significantly more prevalent in RA patient with combined systemic sclerosis and hypertension.

Table 3: Comparison of sensitivity and specificity between RF, anti-CCP, and anti-RA33 in patients with RA

Parameter	Sensitivity	Specificity
RF	80.0	86.6
Anti-CCP	84.7	100.0
Anti-RA33	95.2	100.0

### **Rheumatoid Factor (RF), anti-CCP, and anti-RA33**

In the current study, there is a highly significant difference ( $P \leq 0.05$ ) when compared between RA patient groups and control group as shown in figure 2. According to age group, Group 60- $\geq$ 70 appeared the high mean of RF concentration followed by groups (31-40), (41-50), and (51-60) as in figure 3. The mean of anti-ccp observed highly significant in ( $P < 0.05$ ) (RA patients compared with control group as in figure 4. The mean of anti-CCP positivity also highly significant ( $P < 0.05$ ) in age (61- $\geq$ 70)group. Anti-RA33 antibodies were a highly significant difference ( $P \leq 0.05$ ) when compared between RA patient groups and control group as shown in figure 5. The positivity of RF for RA patients was 80% and the results observe that of Abdullah (2010) and Chiad (2015) studies who reported positively in 47% & 78.6%, respectively of RA cases. Rheumatoid factor had been used as a marker for RA and was included into RA classification criteria for more than half a century (Sokka, et al., 2009).

The serum concentration of RF has been found to correlate with disease severity, RFs are detected in 60-80% of RA patients (Smolen, et al., 2010). Rheumatoid arthritis may be difficult to diagnose in the early stages of the disease. The laboratory markers are helpful. RF has been used since many years as a diagnostic marker. RF has some limitations: it is the immunologic marker of Rheumatoid arthritis included in the ARA-criteria. In addition to Rheumatoid arthritis (RA), it is also present in other inflammatory rheumatic diseases such as Sjogren's syndrome, SLE and mixed connective tissue disease, nonrheumatic disease and even in healthy persons aged over 60 years (Nowak et al., 2005). 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).

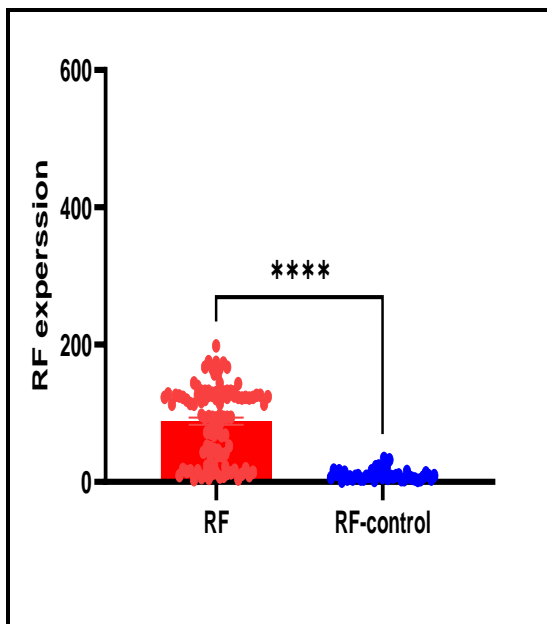


Figure 2: the mean serum concentration level of RF in RA patients & control

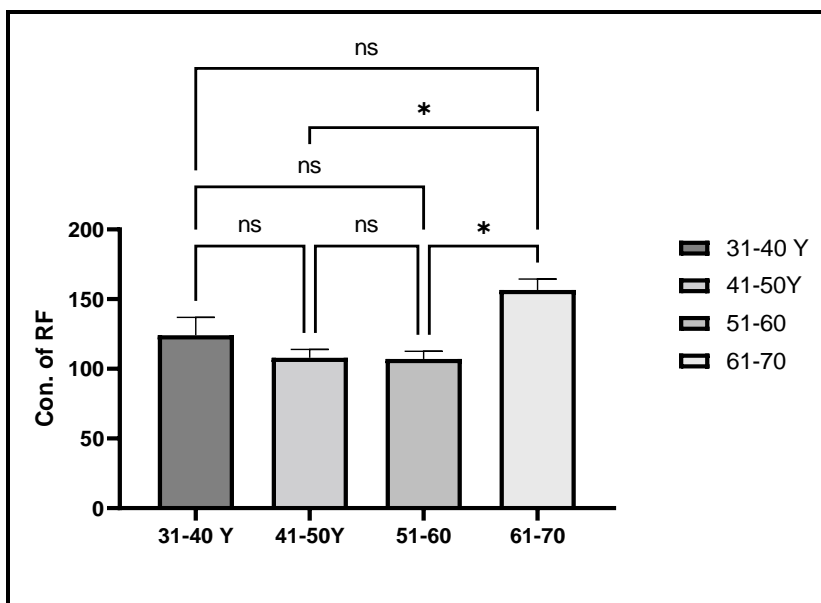


Figure 3: Distribution of RF (IU /ml) among different age groups in RA patients

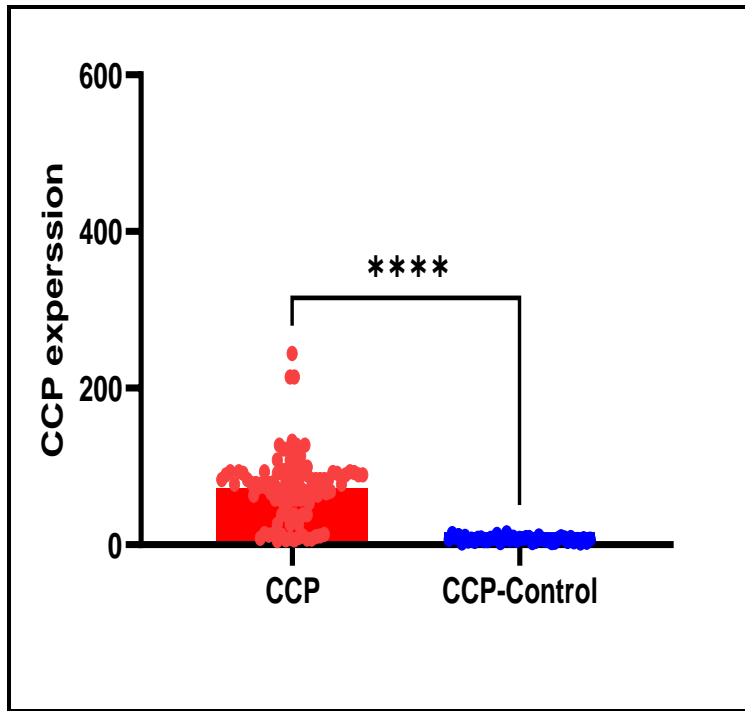


Figure 4: the mean serum concentration level of anti-ccp in RA patients & control

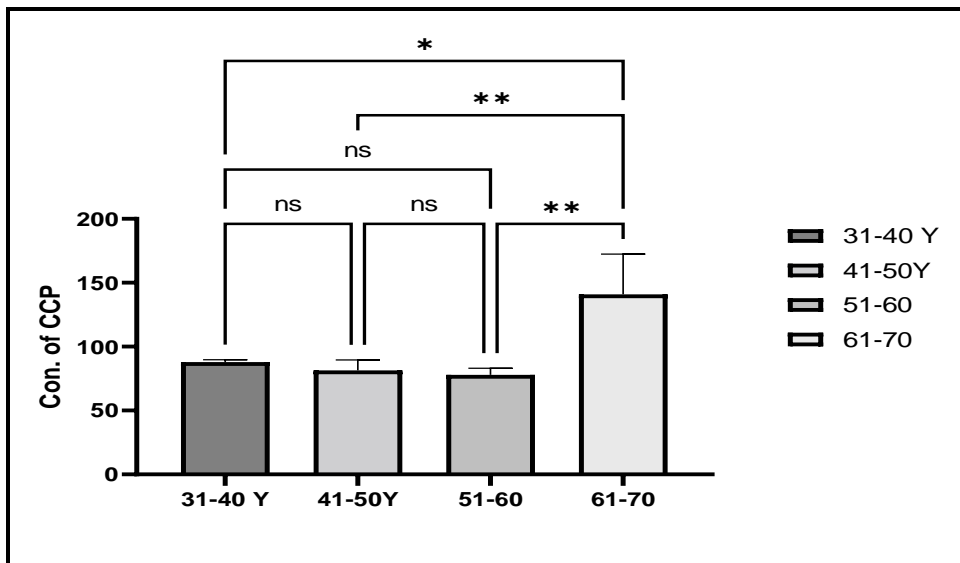


Figure 5: Distribution of anti-CCP (IU /ml) among different age groups in RA patients

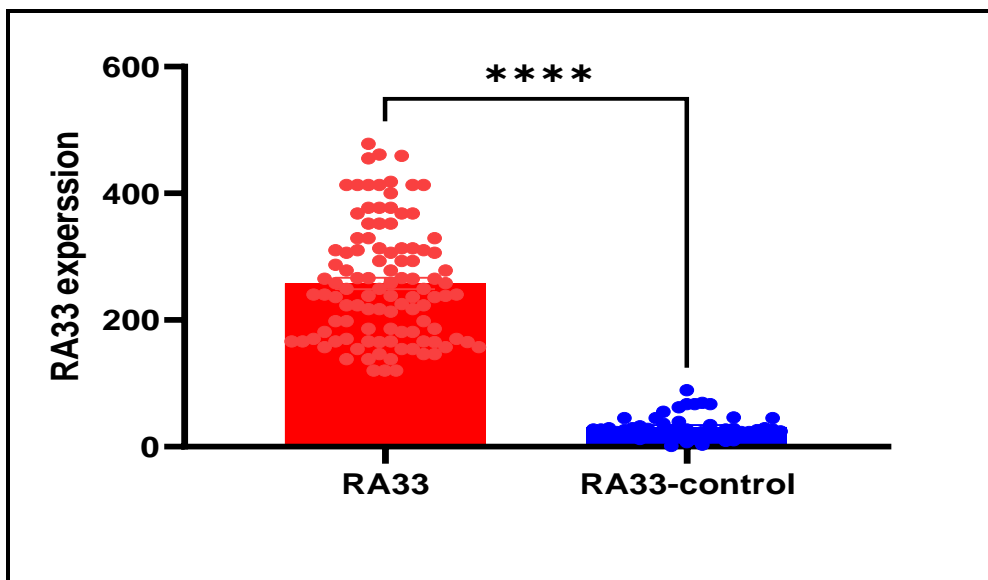


Figure 6: The mean serum concentration level of RA33 in RA patients & control

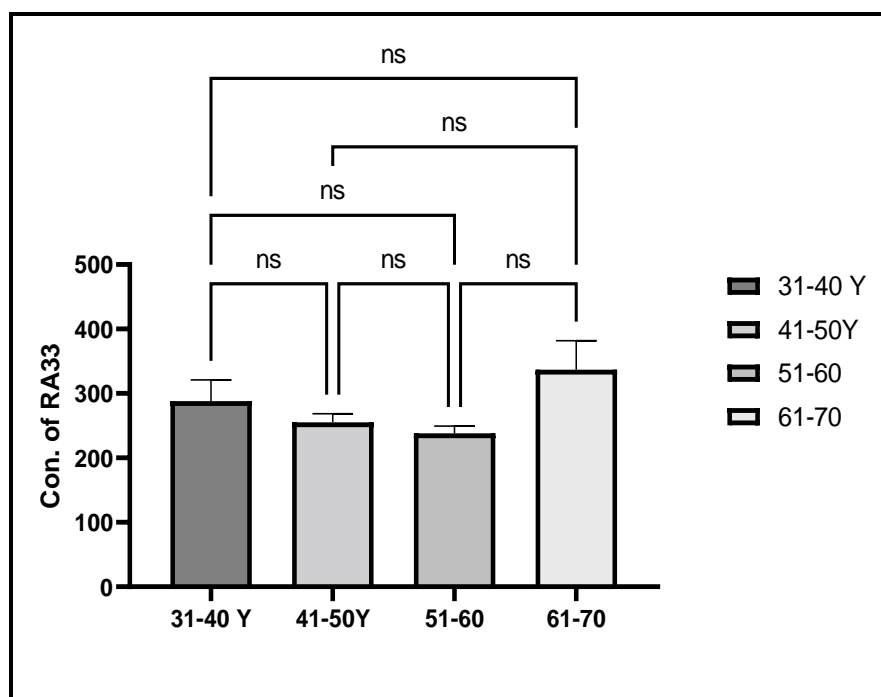


Figure 7: Distribution of RA33 (IU /ml) among different age groups in RA patients

The anti-RA33 autoantibodies seem to be not representing as an important additional immunodiagnostic marker in Saudi patients with established RA. RA33 may have more interest in early RA or less severe RA and other systemic

connective tissue disorders, in which anti-CCP specificity appeared in 90.6% with sensitivity in 63.4% in RA patients compared to healthy control. However, the anti-CCP test values alone were significant in correctly identifying patients with RF positivity, as compared to the anti-RA33 test.

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## Conclusion

The mean levels of diagnostic markers (RF, anti-CCP, RA33) were higher significantly ( $P \leq 0.05$ ) in RA patient groups compared with healthy control. Anti-ccp and RA33 can be used as a good diagnostic marker in RA due to their presence in healthy control other than conventional marker of inflammation (RF, CRP, ESR).

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