

## **DETECTION OF CYCLIC CITRULLINATED PEPTIDE ANTIBODIES IN PATIENTS WITH AUTOIMMUNE DISEASE AND BACTERIAL INFECTION**

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### **Abstract**

**Background:** Autoantibodies, commonly known as CCP antibodies or anti-CCP antibodies, are a kind of antibody. The immune system produces proteins called antibodies and autoantibodies. Your immune system can defend you against disease by battling external agents like viruses and bacteria. By mistakenly targeting the body's healthy cells, autoantibodies can lead to illness.

**Methods:** Between the first of September 2022 and the first of November 2022, 50 venous blood samples (5 ml) from rheumatoid arthritis patients in Baghdad were obtained. With a history of autoimmune illnesses, the patients ranged in age from 19 to 79 and included both sexes—11 men and 39 women. Up to usage, serum samples were kept at -20°C. Private laboratories in Baghdad were contacted for blood samples. RF-LATEX - Linear Chemicals kit was used to identify rheumatoid factor - RF isotypes (IgG, IgM), while Snibe Company China's completely automated system was used to detect anti-CCP antibodies and ANA.

**Results:** There were fifty individuals with rheumatoid arthritis. Of them, 11 (22%) are male and 39 (78%) are female. Additionally, some of these patients have chronic tonsillitis, middle ear infections, and recurrent gingivitis. All of these patients also have high blood pressure despite their young age, and some of them have a family history of the disease in one of their parents, suggesting that genetic factors may have contributed to the infection. As a result, their RA is categorized as severe. The highest incidence of RA disease in females was in the age group (49 – 40) years with a percentage of 11(28.20%). In men, the highest incidence of the disease was in the two age groups (40-49) years and (60-69) years with a percentage of 3(27.27%) for the two age groups. 7 (14%) male patients had positive RF test results, 4 (8%) had positive ANA test within ranges ranging from 45.1 IU/ml to 101.8 IU/ml, and 7(11%) of the patients were positive for the anti-CCP antibody test. While it was found that 30 (69%) of the female patients had positive RF test results, 25 (50%) of the female

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patients had a positive ANA test within ranges of 45.1 to 101.8 IU/ml as in males, and 36 (72%) of the female were positive for an anti-CCP antibody test.

Conclusion: Although not everyone with a positive anti-CCP Ab will have RA, this test is a little more specific than the RF for RA. Anti-CCP antibody levels that are greater are more suggestive of RA.

**Keywords:** *Rheumatoid Factor, Anti-CCP Antibody, Bacterial Infection.*

## Introduction

A chronic, systemic inflammatory condition known as rheumatoid arthritis (RA) causes gradual joint deterioration and inflammation of the synovial membrane surrounding the joints. Although the exact cause of RA is still unknown, it is becoming more and more obvious that altered cellular immune responses are a factor. The primary features of RA are autoreactive T cells and autoantibodies, both of which may be identified in the early stages of the illness (Smolen *et al.*, 2016). Patients with RA might test positive for autoantibodies, such as rheumatoid factor (RF), in blood and synovial fluid (SF) tests. As of 2015, 24.5 million individuals worldwide suffer from RA (Vos *et al.*, 2015). Under the age of 15, onset is rare, and after that, the prevalence increases with age until the age of eighty. Women are affected three to five times as frequently than men (Shah, 2012). Autoantibodies that target citrullinated peptides and proteins are known as anti-citrullinated protein antibodies (ACPAs). The majority of people with rheumatoid arthritis have them. Clinically detecting these antibodies in patient serum or plasma typically involves using the use of cyclic citrullinated peptides (CCP) (Puszczewicz and Iwaszkiewicz, 2011). In joints and other organs where it presents, RA generally begins as a condition of prolonged cellular activity that causes autoimmunity and immune complexes. The primary synovial membrane inflammation and joint injury are the disease's clinical symptoms, and the fibroblast-like synoviocytes are important players in these pathogenic processes. An initiation phase, which is brought on by non-specific inflammation, an amplification phase, which is brought on by T cell activation, and a chronic inflammatory phase, which is marked by tissue injury brought on by the cytokines IL-1, TNF-alpha, and IL-6, are the three stages of the progression of RA (Shah, 2012; Nygaard and Firestein, 2020). A doctor may do RF and ACPA tests when RA is clinically suspected. A negative RF or CCP antibody does not rule out RA; instead, the arthritis is referred to as seronegative, which is present in roughly 15–25% of RA patients. It is positive in 75–85% of cases. RF is more frequently negative during the first year of the disease, with some people later turning seropositive. A non-specific antibody called RF is present in many different chronic illnesses, including hepatitis C, and is found in roughly 10% of healthy individuals. Consequently, the test is not particular to RA. New serological assays thus look for ACPAs. In 61–75% of all RA patients, these tests are once more positive, but with a specificity of about 95%. Similar to RF, ACPAs frequently exist before the onset of symptoms. The anti-cyclic citrullinated peptide (anti-CCP) ELISA is by far the most widely used clinical test for ACPAs (van Venrooij *et al.*, 2011). Antinuclear antibody (ANA) testing can be used to detect antibodies that are present in RF patients' serum and

that bind to autoantigens found in the mammalian cells' nuclei. Although IgG antibodies predominate, IgM and IgA antibodies have also been seen in RF patients (Al-Zougbi, 2022). Therefore, the aim of the current study is to investigate CCP antibody, ANA and RF in serum samples of patients with rheumatoid arthritis.

**Materials and Methods:**

**A. Blood sample collection:** 50 venous blood samples (5 ml) were collected from patients suffering from rheumatoid arthritis in the city of Baghdad for the period from the first of September 2022 to the first of November 2022. The patients' ages were of both sexes (19-79) years; 39 were female and 11 were male with a history of autoimmune diseases. Serum samples were stored at -20°C until use. Blood samples were collected from private laboratories in Baghdad

**B. Serological tests:** Rheumatoid factor - RF isotypes (IgG, IgM) were detected using RF-LATEX - Linear Chemicals kit (Spain), Anti-CCP antibodies and ANA were detected by Snibe company China for fully automated (Maglumi). The CCP antibody and ANA test are considered positive when the test results are up to 17 IU/ml and up to 40 IU/ml, respectively.

**C. Statistical Methods:** The mean and standard deviation were provided. Sensitivity and specificity were used to establish diagnostic features. Using an Excel application and the SPSS program (version 10), all statistical analysis was completed on a Pentium-4 machine. Any test's significant difference (P-value) was significant if (P≤ 0.005).

**Results and Discussion**

From table 1, it was found that 50 patients were suffering from Rheumatoid Arthritis (RA), of both sexes. 39 (78%) of them are female and 11 (22%) are male. These patients also had previous bacterial infections, some of them suffer from recurring tonsillitis and middle ear infection and recurrent gingivitis, and all patients also suffer from high blood pressure despite their young age some of them, as well as have a family history of one of their parents being infected with the disease and so the cause of their infection may be due to genetic factors, their RA is classified as severe.

**Table 1: Distribution of the incidence of RA disease in females and males**

Male (%)	Female (%)	Total (%)
11 (22)	39 (78)	50 (100)

It was found that the highest incidence of RA disease in females was in the age group (49 – 40) years with a percentage of 11(28.20%), followed by the age group (60 – 69) years, with a percentage of 8(20.51%). In men, the highest incidence of the disease was in the two age groups (40-49) years and (60-69) years with a percentage of 3(27.27%) for the two age

groups mentioned, respectively (Table 2). It was found that 7 (14%) of male patients had positive RF test results, 4 (8%) had positive ANA test within ranges ranging from 45.1 IU/ml to 101.8 IU/ml, and 7(11%) of the patients were positive for the anti-CCP antibody test (Table 3). While it was found that 30 (69%) of the female patients had positive RF test results, 25 (50%) of the female patients had a positive ANA test within ranges of 45.1 to 101.8 IU/ml as in males, and 36 (72%) of the female were positive for an anti-CCP antibody test. So, the significant differences between female and male infections with the disease were clearly observed within  $P < 0.05$  (Table 3).

**Table 2: The rate of infection with RA disease within the different age groups for both sexes and all these patients suffered from gingivitis and bacterial dental caries**

<b>Age groups (years)</b>	<b>Male (%)</b>	<b>Female (%)</b>	<b>Total (%)</b>
<b>10-19</b>	0 (0)	1 (2.56)	1 (2)
<b>20-29</b>	1 (9.09)	6 (15.38)	7 (14)
<b>30-39</b>	2 (18.18)	5 (12.82)	7 (14)
<b>40-49</b>	3 (27.27)	11 (28.20)	14 (28)
<b>50-59</b>	1 (9.09)	7 (17.94)	8 (16)
<b>60-69</b>	3 (27.27)	8 (20.51)	11 (22)
<b>70-79</b>	1 (9.09)	1 (2.56)	2 (4)
<b>Total (%)</b>	11 (100)	39 (100)	50 (100)

**Table 3: Positive and Negative Percentages of RF, ANA, and Anti-CCP Ab Tests in Patients with Rheumatoid Arthritis**

Test	Male no. (11)			Female no. (39)			Total (%)
	+ve (%)	-ve (%)	Total (%)	+ve (%)	-ve (%)	Total (%)	
<b>RF</b>	7 (14)	4 (8)	11 (22)	30 (60)	9 (18)	39 (78)	50 (100)
<b>ANA</b>	4 (8)	7 (14)	11 (22)	25 (50)	14 (28)	39 (78)	50 (100)
<b>Anti-CCP Ab</b>	7 (14)	4 (8)	11 (22)	36 (72)	3 (6)	39 (78)	50 (100)

(+ve) Anti-CCP Ab = (> 17 IU/ml)

(+ve) ANA= (> 40 IU/ml)

Many researchers in Iraq and other countries of the world have indicated the seriousness of RA and its future effects on the health of the individual, in which the patient may reach the extent of bone deformation and his inability to move and touch and perform his daily activities normally. In 2014, a research team in Iran conducted a study on the relationship between anti-CCP antibodies and RA, and the researchers concluded that the frequency and titer were high in patients. It appeared that 258 (61.87%) of the patients had high RF, and anti-CCP Ab was also positive in 222 (53.1%) of the patients. The study also showed that RF-IgM was higher in patients' sera compared with RF-IgG. And CRP was positive in 3.4% of patients who had anti-CCP Ab positive and 4% of patients who had RF positive, whose ages ranged from 20 to 80 years (Shakiba *et al.*, 2014). These results were somewhat consistent with the results of the current study.

In a similar study, researcher Abbas concluded in 2015 that early diagnosis of the disease may help the doctors in Al-Nassyria hospital to help the patient follow up on his disease and not reach the stage of serious complications, and this is done by conducting anti-CCP and CRP analysis, and the results of the analysis showed that 50% of patients appeared Positive for anti-CCP and 37% for CRP. The researcher linked the results of the study with clinical diagnosis, bone radiography, and vitamin D levels in the body (Abbas, 2015). The current study of the RF and Anti-CC tests was much higher (74% and 86%, respectively) than the results of the study of researcher Abbas, and this may be due to the small samples of the researcher compared to the samples of the current study.

The result of the study by Gassid *et al.* showed that the majority of RA patients are female, with a percentage (of 80%), and this percentage was four times that of males who suffer from the same disease. Furthermore, the mean age was  $48.03 \pm 12.95$  years. It was

found that there is a high positivity for anti-CCP in patients' sera (70%) and for both sexes (Gassid *et al.*, 2012).

Researchers in Baghdad found that patients suffering from Rheumatoid Arthritis disease during the period from 2014 to 2015 and the ages 20 to 68 years of both sexes, were also suffering from acute bacterial periodontal infections at 41.9%, and the incidence of RA disease was in females. With a rate of 22 (78.5%) higher than in males, whose percentage was 6 (21.4%) (Hachin *et al.*, 2015), and this is consistent with the results of the current study. In another study in 2018, researchers found that anti-CCP and CRP levels were high in RA patients (mean of age  $62 \pm 14$ ) who had been infected with *Escherichia coli*, *Porphyromonas gingivalis*, and *Streptococcus pyogenes*. In addition, these two tests are considered serological biomarkers to diagnose and investigate RA (Terato *et al.*, 2017). In 2022, Horino *et al.* found that a 58-year-old Japanese woman who did not smoke had bacterial pneumonia infections that were *Staph. aureus*, *Pseudomonas aeruginosa*, and *Streptococcus pyogenes* in sputum. The severity and symptoms of RA increased, and the rates and levels of both ACCP and CRP tests were high (Horino *et al.*, 2022). Another study was conducted in Erbil during the period from April to November 2015 and included the study 55 females with RA and five males with the same disease. Their mean age was  $46 \pm 11.46$  years. 39 (65%) of them had ophthalmic manifestations of rheumatoid arthritis. The researchers found that there was no relationship between RA severity and visual manifestations. While there was a relationship between Anti-CCP antibody titer and the presence of ocular manifestations in rheumatoid arthritis (Fadhil and Al-Dabbagh, 2017). The results of research by Mohamad *et al.* in 2018, showed that the average anti-CCP level was  $180.8 \pm 290.3$  U/ml. The results of the study conducted on patients in Malaysia showed also that 27.3% of RA patients did not take good care of oral and dental hygiene. The positive percentage of the ACCP test reached 60% (Mohamad *et al.*, 2018). Citrulline-containing proteins are located in synovitis. The scientific literature confirmed that gingival bacterial DNA was found in the serum and synovial fluid of RA with PD patients in Italy. *Porphyromonas gingivalis* possesses a unique microbial enzyme, peptidylarginine deiminase (PAD), for which the human equivalent has been identified as having susceptibility to factor RA. Researchers speculate that there are anti-CCP antibodies in the serum of PD with RA patients. Anti-CCP antibody was absent in 21 sera ( $<10$ U/ml), including RA in control groups, whereas only one patient with chronic PD was investigated at a depth of 7.1 mm and was positively determined for anti-CCP Ab (22.2 U/ml) (TETE *et al.*, 2010). ELISA technique was used by some Iraqi researchers in Basra to estimate anti-CCP agglutination assay and slide test for determination of RF, CRP and ANA. Among the RA patients, seropositivity for anti-CCP was 61.2%, 23% with low levels, and 51.3% with high levels  $>60$  units/ml. Long-term survival of CCP antagonists in sera of RA patients can last up to 10 years, with a percentage of 54.7%. However, CCP antagonists are rarely found among rheumatic diseases other than RA (Barakat *et al.*, 2009).

Eighty patients were included in the researcher's study. Age ranged from (20-86) years in RA patients. Females represented the majority of patients (73 patients), most of whom were Malays, followed by Chinese and Indian. Most of the patients (93%) complained of joint pain, especially in the hands and knees. They had a high RF test, as well as 47 and 70 patients, had a high CRP (Wahab *et al.*, 2013). González-Febles *et al.*, in 2020 found that the diagnostic value of Synovial-anti-CCP for RA is similar to that of Synovial-anti-CCP (early and late RA). Therefore, anti-CCP synovial fluid can be important if RA cases are suspected. The study did not show any significant differences in SF-anti-resistance CCP between early and late rheumatoid arthritis (Hashemzadeh *et al.*, 2020).

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