

# Frequency of Positive Anti-CCP Antibodies in Rheumatoid Arthritis Patients with Negative Rheumatoid Factor

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## ABSTRACT

**Objective:** Rheumatoid arthritis (RA) is a polyarticular autoimmune disease that affects about 1% of the adult population. The disease is characterized by synovioocyte hyperplasia, mainly synovial fibroblasts, resulting in bone and joint destruction. Recent studies have shown that cytokines and other systemic inflammation mediators have a key role in the development of rheumatoid arthritis. Recently, Anti-Citrullinated Protein Antibodies (anti-CCP) have come into use for the diagnosis of rheumatoid arthritis (RA). It has been reported that anti-CCP has quite a high specificity for RA (98%), together with sensitivity similar to that for rheumatoid factor (RF).

The study aimed to determine the frequency of positive anti-CCP antibodies in rheumatoid arthritis patients with negative rheumatoid factor.

**Methodology:** 91 patients (both male and females) aged 40-70 years with negative RF clinically diagnosed with rheumatoid arthritis were included. Patients with positive rheumatoid factor and joint injuries were disclaimed. The consultant pathologist used a second-generation enzyme-linked immunosorbent assay (ELISA) to assess the serum levels of anti-CCP antibodies.

Anti-CCP level >20IU/ml was considered positive, p-value of 0.05 was considered statistically significant.

**Results:** In the study, the age range was 40 to 70 years, averaging  $53.44 \pm 7.16$  years. 59 (64.84%) of the patients were between the ages of 40 and 55. Of 91 patients, 56 (61.54%) were women, and 35 (38.46%) were men with a ratio of 1.6:1 man to women. In patients with negative rheumatoid factors, 27 (40.66%) patients were found to have high levels of positive anti-CCP antibodies.

**Conclusion:** Patients with negative rheumatoid factor showed the high frequency of positive anti-CCP.

**Keywords:** Antibodies, Rheumatoid arthritis, Rheumatoid factor.

### Authors' Contribution:

<sup>1,2</sup>Conception; Literature research; manuscript design and drafting; <sup>1,2</sup> Critical analysis and manuscript review; <sup>2</sup>Data analysis; Manuscript Editing.

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## Introduction

Cytokines and other inflammatory mediators have been shown to play important roles in the production of articular rheumatism and other

articular diseases.<sup>1</sup> Synovitis is one form of joint disease with symptoms of pain and swelling of the joints.<sup>2</sup> RA exists in all racial and ethnic groups, but is most prominent in some demographic groups (for example, Black individuals in the Caribbean

region). RA affects the general population. The family members of people with RA are 2 to 3 times more likely to have it than any other person. Monozygotic twins display a disease concordance of up to 20%. Since RA is considered a universal disease, it is suspected that a constant infectious agent is at work.<sup>3</sup>The pathway of creation of RA is not very well understood. It can potentially cause various symptoms in people with such genetic conditions (e.g., cigarette smoking, infection or trauma). There are pre-inflammation events that lead to tissue changes leading to inflammation or uncontrolled inflammation. Genetically, defects in the immune system may result in the creation of certain illnesses.<sup>4</sup> Rheumatoid arthritis (RA) is a chronic auto-immune disease that affects many organs and structures in the body.<sup>5</sup> This situation includes inflammation of the synovial joints. Serological testing is widely used in RA to determine the autotherapy, Serum and joint fluids from RA patients contain unique autoantibodies. Rheumatoid factor (RF) is antibodies against the Fc component of IgG autoantibodies.<sup>6</sup> Rheumatoid factor is a well-established diagnostic method for the diagnosis of rheumatoid arthritis.<sup>7</sup>

Recently, cyclic citrullinated peptide (CCP) antibodies have been found useful in diagnosing rheumatoid arthritis. Anti-CCP patients have got confirmed with RA and RF sensitivity (98% and 97% respectively). Test findings have shown that antibodies against the CCP are also believed to be correlated with active and erosive disease.<sup>8</sup> More than 37% of patients with RF negative rheumatoid arthritis had anti-CCP antibodies in their blood supply.<sup>9</sup> This study revealed that among 100 people with RA, 92% were with positive Anti-CCP antibodies and 89% with negative rheumatoid factors. We will obtain different results from foreign studies due to our ethnic, geographical and genetic heterogeneity. Through this study, we will analyse and determine how to effectively fix dysfunctional character in our culture. The aim of this study was to determine the frequency of

positive anti-CCP antibodies in rheumatoid arthritis patients with negative rheumatoid factor.

## Methodology

This descriptive cross-sectional study was performed at Bahawal Victoria Hospital, Bahawalpur from September 15, 2018 till March 14, 2019. Sample size was 91 calculated with 95% confidence level, 10% margin of error and taking positive anti-CCP antibodies as 37.9% in rheumatoid arthritis patients with negative rheumatoid factor.<sup>10</sup>The sampling technique was non-probability consecutive. The inclusion criteria were all patients with rheumatoid arthritis, rheumatoid factor negative, symptom period > 3 months, 40-70years age in both sexes. The exclusion criteria were positive rheumatoid factor in patients, joint trauma victims (historically assessed) and patients not able to participate.

The study was approved by the ethical committee, a total of 90 rheumatoid arthritis (RA) patients with negative rheumatoid factor met the inclusion criteria for study. For each patient in the group, age, gender and duration of the symptoms were noted. In order to evaluate the level of anti-CCP antibodies in sera from myocardial infarction patients, the pathologist employed second-generation enzyme-like immunosorbent assay to establish serum levels of anti-CCP antibodies and > 20 IU/mL was regarded as positive. All of these data were expressed in a specific format (Annexure I). Statistical analysis was performed using SPSS version 20.0. Results were presented as mean and standard deviation for quantitative variables i.e. age, BMI and duration of symptoms. Frequency and percentage were calculated for qualitative variables like gender and positive anti-CCP antibodies (yes/no).

Effect modifiers like age, gender, BMI and duration of symptoms were controlled through stratifications. Post-stratification chi square was applied to see their effects on frequency of positive

anti-CCP antibodies and p value  $\leq 0.05$  was considered as significant.

## Results

The age range was 40 to 70 years in this study, with an average age of  $53.44 \pm 7.16$ . Most patients were between the ages of 40-55, as shown in Table I. (64.84 % n=59).

Of the 91 patients, 56 were women (61.54%) and 35 were men (38.46%), with a ratio of male to female 1.6:1. The mean RA period amounted to  $7.92 \pm 2.70$  months. The mean MMDI was  $29.68 \pm 2.64$  kg / m<sup>2</sup>. (Table 1)

The frequency of positive and negative anti-CCP antibodies in rheumatoid arthritis patients was 37 (40.66 %) and 54 (59.34 %) respectively.

Table II demonstrates the stratification of positive anti-CCP antibodies with respect to age, gender, duration of RA and BMI.

**Table-1: Distribution of patients according to age, duration of RA and BMI (n=91)**

Age (in years)	No. of Patients	%age
40-55	59	64.84
56-70	32	35.16
<b>Total</b>	91	100.0
Duration (months)	No. of Patients	%age
$\leq 6$	38	41.76
$> 6$	53	58.24
<b>Total</b>	91	100.0
BMI (kg/m <sup>2</sup> )	No. of Patients	%age
$\leq 27$	19	20.88
$> 27$	72	79.12
<b>Total</b>	91	100.0

Age: Mean  $\pm$  SD =  $53.44 \pm 7.16$  years

RA: Mean  $\pm$  SD =  $7.92 \pm 2.70$  months

BMI: Mean  $\pm$  SD =  $29.68 \pm 2.64$  kg/m<sup>2</sup>

**Table 2: Stratification of positive anti-CCP antibodies concerning age, gender, duration of RA and BMI.**

Age (years)	positive anti-CCP antibodies		p-value
	Positive	Negative	
40-55	21	38	<b>0.182</b>
56-70	16	16	
Gender			p-value
	Positive	Negative	
Male	12	23	<b>0.328</b>
Female	25	31	
Duration (months)			p-value
	Positive	Negative	
$\leq 6$	18	20	<b>0.270</b>
$> 6$	19	34	
BMI (kg/m <sup>2</sup> )			p-value
	Positive	Negative	
$\leq 27$	08	$\leq 27$	<b>0.885</b>
$> 27$	29	$> 27$	

## Discussion

In patients with rheumatoid arthritis and negative rheumatoid causes, anti-CCP antibodies were tested. This research study included patients over 40 years with an average age of  $53.44 \pm 7.16$  years. Among all, 64 patients were between the ages of 40 and 55 years same as the study done by Shankar S *et al.*<sup>11</sup> Of the 91 patients, 56 (61.54%) were women, while 35 (38.46%) were men with a 1.6:1 ratio of men to women as same reported in Gabriel, S, et al study.<sup>12</sup> According to this study, for 37 (50 %) patients with rheumatoid arthritis and negative rheumatoid factor, anti-CCP antibodies were found. Around 11% of patients tested positive for rheumatoid arthritis with positive anti-CCP antibodies. 37.9% of rheumatoid arthritis (RA) patients reported to have positive anti-CCP antibody.<sup>13, 14</sup>

Rheumatoid Arthritis (RA) can affect 0.5 percent to 1 percent of the population.<sup>15, 16</sup> It reduces the life expectancy of an individual by an average of about

ten years as well as causing years of pain and disability. Over the last 2-decades, effective antimicrobial drugs like methotrexate have been used early and intensively.

Anti-CCP or anti-PRC testing were conducted in several surveys among different ethnically determined communities. The findings published in anti-CCP trial in RF negative RA patients showed that antibody positivity was higher.

In the same research conducted in Turkey, positivity for anti-CCP was greater in RF-positive RA (81%) than in RF-negative RA (20%). Korkmaz *et al.* found 60% and 32% less prevalence in the beginning and long-term cases and principles that are lower than Western countries. In early- and late-stage RA patients, we have a substantial difference in degree of anti-CCP antibodies. In this study, anti-CCP and RF were positive in 50% and negative in 30% of the cases. In this total sample, the double positive incidence (46.9 percent) is essentially equivalent to Inanc *et al* factor's however, the double negative proportion (11.7 percent) is much smaller.<sup>17, 18</sup>

According to a meta-analysis by Nishimura *et al.* of 37 studies of anti-CCP and 50 studies of RF, the anti-CCP is unique to RA than RF. In IgM RF, there were 69 percent, 85 and 4.86, respectively. Among many other factors that explain sensitivity and specificity differently, the probability of a false positive test in non-RA sample is worth more co-existent. For the anti-CCP antibody, the pooling of sensitivity, specificity and positive likelihood was 67 percent, 95 percent, and 12.46 respectively. Compared with other trials, the smaller specificities of anti-CCP against fake positive non-RA arthritis can be explained by the involvement in the tests of a large proportion. In comparison, the specificity was higher in studies in which healthy subjects or non-inflammatory arthritis patients were chosen as controls, as a result in healthy subjects or non-inflammatory arthritis anti-CCP positivity was lower than that for patients with inflammatory arthritis.<sup>19,20</sup>

There was a positive correlation between anti-CCP and higher ESR, CRP, and between RF and increased ARR and CRP.<sup>21</sup> Anti-CCP and anti-RF are closely linked to one another. In this test of 50% of patients had anti-CCP and CRP with increased ARR. Anti-CCP and CRP were said to be optimistic. Most (56%) of 199 SARS patients had no anti-CCP antibodies in their sera after SARS. Only 65%(n=22) had RF antibody in their sera. In a study Anti-CCP was constructive and impacts on discourse.<sup>23</sup> Eighty-one percent of patients who are anti-CCP test positive (59 percent). In research, there are higher immunity rates between RA patients who are first-grade family members and RA patients. In RF and anti-CCP, seropositivity has been found to be 14.4 and 5.0, respectively, 97.8 and 98.5 percent the anti-CCP group had a substantially increased RF rate as compared to the other groups.<sup>24</sup>

In 90% and 40% of positive and negative anti-CCP patients, the risk of RF and CRP are compared in that the former is associated with anti-CCP. They also found that 50% of the patients were positive for both RF-positive and RF-negative antibody tests. This study indicates that antibodies to CC are the same as agglutinating RF and that seropositivity to CC and agglutinating RF are identical.<sup>25</sup>

The study showed that approximately 40 percent of patients had RF-positive and baseline-positive anti-CCP.<sup>26</sup> Another study found that approximately 80% of RF positive RA patients were seronegative for CCP and the percentage was even higher in RF negative RA patients (16, 18). Both RF and CCP antibody tests were found to be positive in 40.4% CP and negative in 28.1% of cases(5, 13).A study of the clinical progression of RA showed that the proportion of anti-CCP positive antibody positive patients with the number of ACR requirements already met was 21 percent.

## Conclusion

In patients with rheumatoid arthritis with negative rheumatoid factor, the level of positive anti-CCP

antibodies is very high. We are also advocating the use of anti-CCP antibodies to enhance patients' quality of life in the early detection and treatment of rheumatoid arthritis.

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