



STUDY ON SEROPREVALENCE OF ANTI-CYCLIC CITRULLINATED PEPTIDE (ANTI-CCP) ANTIBODIES IN RHEUMATOID ARTHRITIS PATIENTS AT A TERTIARY LEVEL HOSPITAL

Immunology

Ravindra Singh Rathore

Medical Officer, Department of Microbiology, Dr. S.N. Medical College Jodhpur

Sanju Pannu

Assistant Professor, Department of Microbiology, Dr. S.N. Medical College Jodhpur

Richa Agarwal

Assistant Professor, Department of Microbiology, Dr. S.N. Medical College Jodhpur

Mukesh Sharma

Assistant Professor, Department of Microbiology, Dr. S.N. Medical College Jodhpur

Govind Prasad Meena

Senior Demonstrator, Department of Microbiology, Dr. S.N. Medical College Jodhpur

Prabhu Prakash

Senior Professor & HOD Department of Microbiology, Dr. S.N. Medical College Jodhpur

ABSTRACT

Background: Rheumatoid arthritis (RA) is a chronic, systemic autoimmune disease characterized by inflammation of the joints, leading to pain, swelling, stiffness, and potential joint damage and disability. Early diagnosis and assessment of disease activity are crucial for effective management and preventing irreversible joint destruction. Anti-cyclic citrullinated peptide (anti-CCP) antibodies are highly specific biomarkers for RA, often appearing in the preclinical phase and correlating with more aggressive disease courses. This study aimed to determine the seroprevalence of anti-CCP antibodies and their correlation with disease activity in RA patients attending a tertiary care hospital. **Materials and Methods:** This cross-sectional hospital-based study was conducted over a period of 3 months, 500 consecutive patients diagnosed with rheumatoid arthritis attending the outpatient department were included. All samples were processed for Anti-CCP test by Chemiluminescence, titers more than 17 U/ml were reported positive, all positive samples were further substituted for Confirmation of RA and CRP by latex agglutination test. **Results:** Out of 500 RA patients, 90 (18%) tested positive for anti-CCP antibodies, maximum cases were in age group 41-60 (46.66%) in females (77.77%) and by occupation in housewives (44.44%). Among the 90 Anti-CCP positive samples 74 (82.2%) positive for Rheumatoid factor (RF) and 63 (70%) tested positive for C-reactive protein (CRP). **Conclusion:** The seroprevalence of anti-CCP antibodies in RA patients at this tertiary care hospital is substantial, and their presence is significantly associated with higher disease activity. These findings underscore the importance of routine anti-CCP antibody testing in RA diagnosis and prognosis, suggesting its utility as a marker for assessing disease severity and guiding therapeutic decisions in a clinical setting.

KEYWORDS

Autoimmune Disease, Rheumatoid Arthritis (RA), Anti-CCP Antibodies, C-reactive protein (CRP), SLE

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic autoimmune disease that affects many organs and systems and has a frequency of 0.5%-1% in the population. The disease is characterized by chronic inflammation of the synovial joints, and the chronic inflammation in RA causes erosions and deformities of the articular cartilage and bones¹. Recently, cyclic citrullinated peptide antibodies (anti-CCP) have come into use for the diagnosis of RA. It has been reported that anti-CCP has quite a high specificity for RA (98%), together with a sensitivity similar to that for rheumatoid factor (RF). Although different studies reported variable results, it is known that anti-CCP antibodies are associated with active and erosive disease, like high RF titers (4-6)^{2,3}.

Historically, the diagnosis of RA has relied on clinical presentation, imaging, and the detection of rheumatoid factor (RF). While RF has been a long-standing serological marker, its relatively low specificity, particularly in early disease and in the presence of other autoimmune conditions or chronic infections, can limit its diagnostic utility⁴. The need for more specific and sensitive biomarkers for RA, especially in its early, undifferentiated stages, has driven extensive research.

In recent decades, anti-cyclic citrullinated peptide (anti-CCP) antibodies have emerged as highly specific serological markers for RA⁵. These autoantibodies target citrullinated proteins, which are products of post-translational modification of arginine residues, and are strongly implicated in the pathogenesis of RA⁶. The presence of anti-CCP antibodies is not only highly specific for RA but also often precedes the onset of clinical symptoms and is associated with a more aggressive disease course and greater radiographic progression. Consequently, anti-CCP antibody testing has been incorporated into the 2010 American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) classification criteria for RA, enhancing diagnostic capabilities^{7,8}.

Despite the established diagnostic and prognostic significance of anti-CCP antibodies, their precise seroprevalence and correlation with various measures of disease activity can vary across different

populations due to genetic, environmental, and methodological factors^{9,10,11}. Understanding these correlations is vital for optimizing patient management, predicting disease trajectory, and tailoring therapeutic strategies.

This study aims to investigate the seroprevalence of anti-CCP antibodies in a cohort of rheumatoid arthritis patients and to determine its correlation with established measures of disease activity. By shedding further light on these relationships, we hope to contribute to a more comprehensive understanding of anti-CCP antibodies as valuable tools in the diagnosis, prognosis, and management of rheumatoid arthritis.

MATERIALS AND METHODS

Study Design and Setting: This was a cross-sectional, hospital-based study conducted over a period of three months, from August 2024 to October 2024. The study was carried out in the Department of Microbiology at a tertiary care hospital. The primary objective was to determine the seroprevalence of anti-cyclic citrullinated peptide (Anti-CCP) antibodies in a cohort of patients diagnosed with Rheumatoid Arthritis (RA) and to analyze their demographic distribution.

Study Population and Sample Collection: A total of 500 consecutive patients with a clinical diagnosis of Rheumatoid Arthritis were included in the study. These patients were attending the outpatient department at the tertiary care hospital. Exclusion criteria included patients with other autoimmune diseases that could potentially interfere with the results, such as Systemic Lupus Erythematosus (SLE) or Psoriatic Arthritis, as well as patients who were unwilling to provide consent.

Upon enrollment, a detailed case history was recorded for each patient, including demographic information such as age, gender, place of residence (rural/urban), and occupation. A single venous blood sample (approximately 3-5 mL) was collected from each participant under aseptic conditions. The blood samples were collected in plain vacutainer tubes and allowed to clot at room temperature. The serum

was then separated by centrifugation at 3000 rpm for 10 minutes and stored at -20°C until further analysis.

Laboratory Analysis: The collected serum samples were analyzed for the presence of three key serological markers: Anti-CCP antibodies, Rheumatoid Factor (RF), and C-Reactive Protein (CRP).

Anti-CCP Antibody Testing: The presence of Anti-CCP antibodies was determined using a commercially available Enzyme-Linked Immunosorbent Assay (ELISA) kit. The test was performed according to the manufacturer's instructions. A cutoff value was established based on the manufacturer's specifications, and samples with values above 17U/ml were considered positive.

Rheumatoid Factor (RF) Testing: RF was tested using a qualitative or semi-quantitative latex agglutination test [Insert Manufacturer and Kit Name, e.g., Omega Diagnostics Latex Agglutination Kit]. The test was performed on undiluted serum, and positive results were confirmed by testing serially diluted samples. A titer of 1:80 or greater was considered positive.

C-Reactive Protein (CRP) Testing: CRP levels were measured using a qualitative or semi-quantitative latex agglutination test [Insert Manufacturer and Kit Name, e.g., Omega Diagnostics Latex Agglutination Kit]. A value above 6.0 mg/L was considered a positive result, indicative of inflammation.

RESULT

A total of 500 samples were received, out of which 90 (18%) tested positive for Anti-Cyclic Citrullinated Peptide (Anti-CCP). Among these 90 Anti-CCP positive samples, 74 (82.2%) also tested positive for Rheumatoid Factor (RF), and 63 (70%) tested positive for C-Reactive Protein (CRP). The age-wise distribution of these 90 Anti-CCP positive cases is detailed in Table 1. The largest proportion of positive cases was observed in the 41-60 years age group, accounting for 42 cases, which is 46.66% of the total positive samples. This indicates that nearly half of the Anti-CCP positive cases fall within this middle-aged demographic.

The 21-40 years age group followed, contributing 29 cases, or 32% of the total. The elderly population, defined as >60 years, had 15 positive cases, representing 17% of the total. The youngest age group, <20 years, showed the lowest number of positive cases, with only 4 individuals, making up 4.44% of the total. This distribution suggests a higher prevalence of Anti-CCP positivity in older adult and middle-aged populations compared to younger individuals.

Further analysis of the 90 Anti-CCP positive samples reveals a notable disparity in distribution based on gender, as presented in Table 2. The data indicates a significantly higher prevalence of positive cases among females compared to males. Out of 70 were female, accounting for a substantial 77.77% of the positive cohort. In contrast, male individuals represented only 20 cases, making up 22.22% of the total. This striking difference suggests that females are disproportionately affected by Anti-CCP positivity in this sample population, with a ratio of 2.3:1 female to male. This finding aligns with known epidemiological patterns of certain autoimmune conditions, where females often exhibit a higher incidence.

Table1:Age Wise Distribution

S.no.	Age group	No.of cases	Percentage
1	<20Years	4	4.44%
2	21-40Years	29	32%
3	41-60Years	42	46.66%
4	>60Years	15	17%
	Total	90	100%

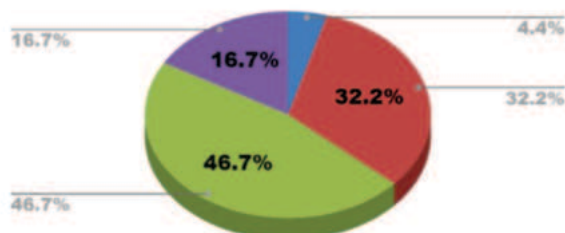
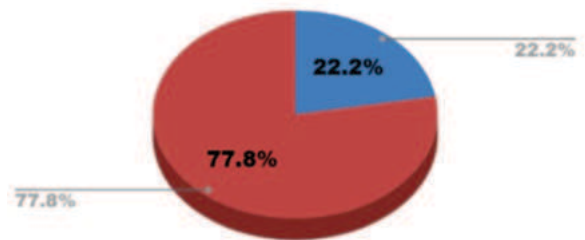


Table2: Gender Wise Distribution

Gender	No.of cases	Percentage
Male	20	22.22%
Female	70	77.77%
Total	90	100%

Gender Wise Distribution



An analysis of the 90 Anti-CCP positive samples, categorized by their demographic, reveals a distinct distribution pattern. As observed in the data, the majority of Anti-CCP positive cases originate from rural areas, with 58(64.44%) individuals. In contrast, individuals residing in urban areas represent a smaller proportion, with 32(35.55%) cases.

This distribution indicates a higher prevalence of Anti-CCP positivity among individuals from rural settings compared to those from urban areas in this study. This finding could suggest potential environmental, lifestyle, or access-to-healthcare factors that differ between rural and urban populations and may influence the incidence of Anti-CCP positivity.

An examination of the 90 Anti-CCP positive samples, as detailed in Table 3, reveals their distribution across various occupational categories. This analysis provides insight into which occupational groups are predominantly represented among individuals testing positive for Anti-CCP in this study. The largest segment of Anti-CCP positive cases was identified among housewives, comprising 40 individuals, which accounts for a substantial 44.44% of the total positive cohort. This finding indicates that nearly half of the Anti-CCP positive cases in this particular study population fall within this occupational group.

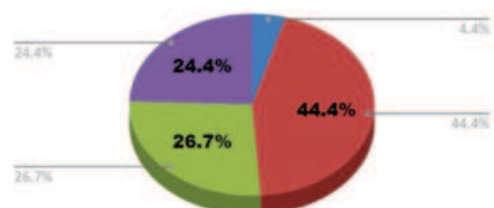
Following housewives, farmers represent the second-largest group, with 24 positive cases, making up 26.66% of the total. Individuals classified under "Job" (implying those employed in various other professions) are closely behind, with 22 cases, contributing 24.44% to the total. The student demographic showed the lowest number of positive cases, with only 4 individuals, which is 4.44% of the overall positive samples.

This occupational breakdown suggests a higher prevalence of Anti-CCP positivity among housewives and farmers within this sample, with students exhibiting the lowest number of cases. Such data can be valuable for further research into potential links between occupational factors, lifestyle, and the incidence of Anti-CCP positivity.

Table:3 Occupational Distribution

Occupation	Study subject	
	Number	Percentage
Student	4	4.44%
Housewife	40	44.44%
Farmer	24	26.66%
Job	22	24.44%
Total	90	100%

Occupation wise Distribution



DISCUSSION

This study aimed to investigate the prevalence and demographic distribution of Anti-Cyclic Citrullinated Peptide (Anti-CCP) positivity within a sample of 500 individuals. Our findings reveal an overall Anti-CCP positivity rate of 18% (90 out of 500 samples), which underscores the clinical relevance of this biomarker in the studied population. A notable association was observed with other inflammatory markers, as 82.2% of Anti-CCP positive samples were also positive for Rheumatoid Factor (RF) and 70% for C-Reactive Protein (CRP). This high rate of co-positivity aligns with established literature, which frequently notes the presence of multiple autoantibodies in individuals with inflammatory conditions, particularly Rheumatoid Arthritis (RA) (Martinez-Prat et al., 2018; Raza K et al., 2005)^{12,13}. The combined presence of these markers is often indicative of a more severe disease state and may be used to predict disease progression (Shankar et al., 2006)¹⁴.

Our analysis of the age-wise distribution of Anti-CCP positive cases revealed a distinct pattern. The highest prevalence was found in the 41-60 years age group, accounting for 46.66% of the positive cases. This is consistent with the known epidemiology of RA, where onset often occurs in middle age (Pramod et al., 2022)¹. The decreasing prevalence in both younger (<20 years) and older (>60 years) populations mirrors typical disease onset patterns, suggesting that Anti-CCP positivity may be a strong indicator of an active or recent disease process rather than a long-standing one, although some studies have also found a strong correlation in long-standing cases (Korkmaz et al., 2006)⁴.

A striking finding of our study is the significant gender disparity in Anti-CCP positivity. Females accounted for a substantial 77.77% of all positive cases, compared to only 22.22% in males. This aligns with the well-documented higher prevalence of autoimmune diseases, including RA, in females (Klareskog et al., 2008)⁹. The underlying reasons for this gender bias are complex and are thought to involve a combination of hormonal, genetic, and environmental factors. Further research into these factors could provide valuable insights into the pathophysiology of autoimmune conditions.

The geographical and occupational distribution of our Anti-CCP positive cases presents a unique aspect of our findings. We observed a higher prevalence of Anti-CCP positivity in individuals from rural areas (64.44%) compared to urban areas (35.55%). This finding is particularly interesting and suggests that environmental or lifestyle factors unique to rural settings may play a role in the development of Anti-CCP positivity. Previous studies have linked certain environmental exposures, such as cigarette smoke, to RA and Anti-CCP positivity (Fatima et al., 2013; Klareskog et al., 2008)^{8,9}. The higher prevalence in rural populations could be linked to a different set of environmental exposures, such as agricultural chemicals, or could be a reflection of disparities in healthcare access and diagnostic delays, which may be more pronounced in these areas.

Our occupational analysis further supports this notion, with housewives (44.44%) and farmers (26.66%) representing the largest occupational groups among the positive cases. The high number of positive cases among farmers could be related to specific environmental exposures in agricultural settings. Similarly, the high prevalence among housewives, when considered in the context of the strong female predominance and the rural-urban divide, points towards potential lifestyle, dietary, or domestic environmental factors that warrant further investigation. The lowest prevalence was found in students, which is consistent with their younger age and suggests that the development of Anti-CCP positivity is more common in adult populations with specific occupational and environmental exposures.

While our findings provide valuable insights, the study has certain limitations. The sample size, while adequate for this preliminary analysis, may not be representative of the entire population. The study's cross-sectional design does not allow for a determination of causality between the demographic factors and Anti-CCP positivity. Furthermore, while Anti-CCP is a key biomarker for RA, our study did not confirm a formal RA diagnosis for all Anti-CCP positive individuals, as some may have other rheumatic diseases or even be asymptomatic (Eker et al., 2014; Saigal et al., 2018)^{2,7}.

CONCLUSION

Present study highlights the high seroprevalence of Anti-CCP antibodies in rheumatoid arthritis patients and demonstrates their

significant correlation with disease activity. These findings underscore the critical role of Anti-CCP antibodies as valuable biomarkers for the diagnosis and management of RA, supporting their continued use in clinical practice. Further research, particularly longitudinal studies, is warranted to fully elucidate the dynamic changes in anti-CCP levels and their precise role in predicting disease progression and response to therapy.

In summary, this research underscores the continued importance of Anti-CCP antibodies as critical biomarkers in the comprehensive assessment and management of rheumatoid arthritis. These insights reinforce their value in clinical practice for accurate diagnosis, prognosis, and potentially for guiding therapeutic strategies. Further longitudinal studies are encouraged to fully understand the dynamic changes of anti-CCP levels and their long-term predictive power in RA.

REFERENCES

1. Pramod GR, Dihingia P, Jha AK, Gadgade A, Agarwal D. Rheumatoid Arthritis Correlation with Anti-CCP Antibodies with special reference to its Prevalence in Asymptomatic First-Degree Relatives. *Mediterr J Rheumatol* 2022;33(1):42-7.
2. Eker YÖ, Pamuk ÖN, Pamuk GE, Dönmez S, Çakır N. The Frequency of anti-CCP antibodies in patients with rheumatoid arthritis and psoriatic arthritis and their relationship with clinical features and parameters of angiogenesis: A comparative study. *Eur J Rheumatol*. 2014 Jun;1(2):67-71. doi: 10.5152/eurjrheumatol.2014.022. Epub 2014 Jun 1.
3. Shankar S, Grover R, Handa R. Role of anti cycliccitrullinated peptide antibodies in erosive disease in patients with rheumatoid arthritis. *Indian J Med Res* 2006;124:689-96.
4. Korkmaz C, Us T, Kaşifoğlu T, Akgün Y. Anti-cyclic citrullinated peptide (CCP) antibodies in patients with long-standing rheumatoid arthritis and their relationship with extra-articular manifestations. *Clin Biochem*. 2006 Oct;39(10):961-5.
5. Gr P, Dihingia P, Jha AK, Gadgade A, Agarwal D. Rheumatoid Arthritis Co-relation with Anti-CCP Antibodies with special reference to its Prevalence in Asymptomatic First-Degree Relatives. *Mediterr J Rheumatol*. 2022 Mar 31;33(1):42-47.
6. Martinez-Prat L, Nissen MJ, Lamacchia C, Bentow C, Cesana L, Roux-Lombard P, Gabay C, Mahler M. Comparison of Serological Biomarkers in Rheumatoid Arthritis and Their Combination to Improve Diagnostic Performance. *Front Immunol*. 2018 Jun 6;9:1113.
7. Saigal R, Bhakal SS, Goyal L, Goel AD. Seroprevalence of Anti-Citrullinated Protein Antibodies (ACPA) in Patients with Rheumatic Diseases other than Rheumatoid Arthritis. *J Assoc Physicians India*. 2018 Apr;66(4):26-8.
8. Fatima N, Shameem M, Malik A, Khan PA, Shujatullah F, et al. A Study on the Pulmonary Manifestations of Rheumatoid Arthritis from a North Indian Town. *Open J Respir Dis* 2013;3:128-31.
9. Klareskog L, Widhe M, Hermansson M, Rönnelid J. Antibodies to citrullinated proteins in arthritis: pathology and promise. *Curr Opin Rheumatol* 2008. May;20(3):300-5.
10. Greiner A, Plischke H, Kellner H, Gruber R. Association of anticyclic citrullinated peptide antibodies, anticitrullin antibodies, and IgM and IgA rheumatoid factors with serological parameters of disease activity in rheumatoid arthritis. *Ann N Y Acad Sci* 2005;1050:295303.
11. Vinithra S, Sethumadhavan K, Bhaskaran K, Pradeep J, Suresh M, Nachammai SM, (2025) Diagnostic Value of Rheumatoid Factor and Anti-CCP in Seropositive and Seronegative Rheumatoid arthritis patients at a Tertiary Hospital in Tamil Nadu. *Journal of Neonatal Surgery*, 14 (26s), 272-275.
12. Jarlborg M, Courvoisier DS, Lamacchia C, Martinez Prat L, Mahler M, Bentow C, Finckh A, Gabay C, Nissen MJ: physicians of the Swiss Clinical Quality Management (SCQM) registry. Serum calprotectin: a promising biomarker in rheumatoid arthritis and axial spondyloarthritis. *Arthritis Res Ther*. 2020 May 6;22(1):105.
13. Raza K, Breese M, Nightingale P, Kumar K, Potter T, Carruthers DM, Situnayake D, Gordon C, Buckley CD, Salmon M, Kitas GD. Predictive value of antibodies to cyclic citrullinated peptide in patients with very early inflammatory arthritis. *J Rheumatol*. 2005 Feb;32(2):23.
14. Shankar, Grover, Handa Role of Anti-Cyclic citrullinated peptide antibodies in erosive disease in patients with rheumatoid arthritis. *Indian J Med Res*. 2006 Dec;689-96.