# ANTI-CYCLIC CITRULLINATED PEPTIDE ANTIBODIES IN RHEUMATOID ARTHRITIS PATIENTS

**Amer Fakhr, Farzana Hakim*, Mushtaq Ahmed, Mukarram Bashir, Rabia Basri****

Military Hospital / National University of Medical Sciences (NUMS) Rawalpindi Pakistan, *Army Medical College / National University of Medical Sciences (NUMS) Rawalpindi Pakistan, **Combined Military Hospital / National University of Medical Sciences (NUMS) Rawalpindi Pakistan

## ABSTRACT

***Objective:*** To detect the anti cyclic citrullinated peptide (Anti-CCP) antibody in rheumatoid arthritis (RA) patients to determine its diagnostic value in Pakistani patients.

***Study Design:*** Cross sectional study.

***Place and Duration of Study:*** Military Hospital (MH) Rawalpindi, from January 2013 to June 2015.

***Material and Methods:*** A total of 58 patients with complications of rheumatoid arthritis were recruited in the study using convenient sampling technique after their informed consent. Age and gender of the patients were recorded. Blood was collected from the patients subjected to ELISA based detection of anti-CCP and latex agglutination test for detection of Rheumatoid Factor (RF). Data obtained were analyzed using Microsoft excel 2010.

***Results:*** Among the fifty eight RA patients, 40% were males and 60% were females. Age ranged between 12 to 80 years (mean age 49.74 ± 16.81 years) of the males RA patients and was higher as compared to females (mean age 43.2 ±16.70 years). ELISA based detection of anti-CCP antibody showed that about 91 percent of RA patients were positive for anti CCP antibody. About 72% were positive for anti CCP antibody alone, 19 percent were positive for both anti-CCP and RF and 9 percent were positive for RF.

***Conclusion:*** The results concluded that a higher percentage of the RA patients are positive for anti-CCP antibody marking its importance as a diagnostic marker. Anti-CCP has more sensitivity as compared to RF in RA patients.

**Keywords:** Antibodies, cyclic citrullinated peptide (CCP peptide), Diagnostic test, Enzyme assay, ELISA, Rheumatoid arthritis, Rheumatoid factor (RF).



## INTRODUCTION

Rheumatoid arthritis (RA), is a chronic inflammatory and autoimmune disease. It has great impacts on physical as well as psychosocial health[1]. The reasons for developing RA root from interaction of genetic along with environmental factors that is a series of immune reactions eventually result in development of synovitis, damage to joints and structural bone. Altogether these complications results in pain and disability along with challenges that a patient facesemotionally, socially and economically. Together with RA, the patient suffers from extra-articular manifestations as well as co morbidities leading to increased mortality[2].

RA has victimized approximately 1% people around the world[2]. RA prevalence is variable in developing countries[3]. The old research articles published indicate that RA prevalence is 0.142% in urban residents of southern Pakistan[4] and 0.55% in northern Pakistan[5]. According to report by Khurram, A. in the express tribune under the title "Arthritis - A debilitating condition" states that 14 million Pakistanis are affected with arthritis. No definite figures for RA are available at national level.

RA costs significantly by affecting multiple organ systems. Hence understanding RA pathophysiology is beneficial with respect to development of therapies. Success of therapy depends on early diagnosis of RA so that treatment can be started as soon as possible. The

**Correspondence: Dr Amer Fakhr,** Medical Specialist & Rheumatologist, MH Rawalpindi Pakistan
*Email: amerfakhr@yahoo.com*
*Received: 09 Mar 2016; revised received: 14 Apr 2016; accepted: 20 Apr 2016*

anti-cyclic citrullinated peptide (anti-CCP) antibody detection is particularly beneficial in RA diagnosis being highly specific more than that of rheumatoid factor (RF)[6].

For identification of disease development as well as erosive or non- erosive advancement of disease, anti-CCP antibody detection can be a good choice. The anti-CCP specificity for RA diagnosis can be valuedto distinguish it from diseases with similar clinical symptoms[7]. RA, a severe chronic systemic inflammation with unknown etiology, leads to morbidity and mortality because of local as well as systemic inflammatory routes that destroy cartilage, bone, soft tissue, along with blood vessels and viscera[8]. Hence based on the previous data, this study was designed to detect the anti-CCP antibody in RA patients to evaluate its importance as diagnostic marker in Pakistani RA affected subjects. For this purpose, the anti CCP was compared for its efficacy in diagnosis as compared to RF.

**Table-I: Evaluation of RA diagnostic and prognostic marker.**

| Anti Cyclic Citrullinated Peptide | Rheumatoid factor | | Total |
|---|---|---|---|
| | Positive | Negative | |
| Positive | 11 | 42 | 53 |
| Negative | 5 | 0 | 5 |
| Total | 16 | 42 | 58 |

## MATERIAL AND METHODS

The study design was a cross sectional study conducted from January 2013 to June 2015 at Military Hospital (MH) Rawalpindi. A total of 58 already diagnosed rheumatoid arthritis patients were recruited in the study after their informed consent. The details of the patients were kept confidential and an identity number was given to each patient for their identification. The patients were included in the study using convenient sampling technique. Age and gender of the patients were recorded. Blood was collected from the patients in 5 cc syringe under sterile conditions and was analyzed for Rheumatoid Factor (RF) and anti-CCP detection. RF was evaluated using latex agglutination and anti CCP was determined using the ELISA based tests. Descriptive data was compared using microsoft excel 2007. The figures were reported in frequencies and percentages.

## RESULTS

The study was conducted on 58 subjects affected with RA including 23 males (i.e. about 40 percent) and 35 females (i.e. about 60 percent). The male patients had age ranged between 12 to 80 years (mean age 49.74 ± 16.81years)and was higher as compared to females (mean age 43.2 ±16.70 years). ELISA based detection of anti-CCP antibody showed that about 91 percent of RA patients were positive for anti CCP antibody. About 72% were positive for anti CCP antibody alone, about 19 percent were positive for both

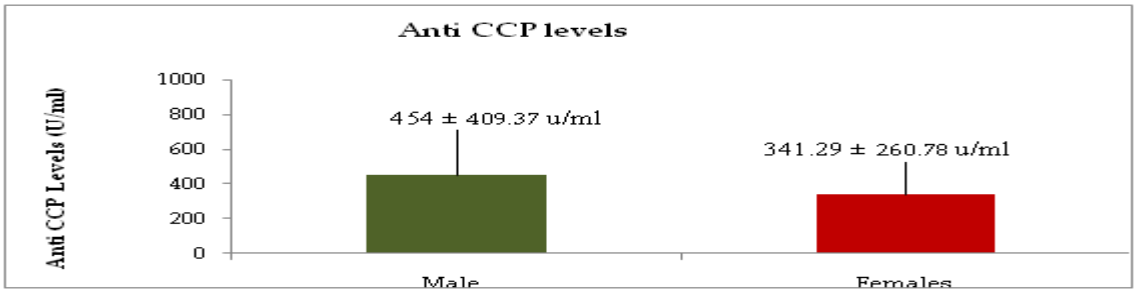


**Figure-1: Anti-CCP levels of male and female rheumatoid arthritis patients.**

anti-CCP and RF (Rheumatoid factor) and about 9 percent were positive for RF aloneas shown in table-I. Only in five patients, anti-CCP was not detected showing that a higher percentage of the RA patients have antibodies against CCP in their blood marking its importance as a diagnostic marker. The results showed that anti-CCP levels were higher in males as compared to females as shown in fig-1.The age of the RA patients positive for anti-CCP was also compared with that of RA patients negative for anti-CCP. The age was lower in the RA patients positive for anti-CCP (mean age 44.78 ± 16.76 years) as compared to RA patients negative for anti-CCP (mean age 53 ± 19.03 years). The anti-CCP positive group included both males (n=22) as well as females (n=31) where as anti-CCP negative group comprised of 4 female and one male(fig-2). The sensitivity of the anti-CCP was calculated to be 91.4% whereas the sensitivity of RF was found to be 27.6%.

## DISCUSSION

The treatment options for RA are limited. Effective treatment at early stage can change disease course, increase life and improvement in the function[8] but in order to identify early the patients with erosive RA, superior diagnostic and prognostic markers are needed[9]. This is the reason why anti-CCP antibodies were tested for RA diagnosis and prognosis.

Rheumatoid factor (RF) has moderate sensitivity as well as specificity for RA diagnosis, and is found to be at high rates in other inflammatory as well as infectious diseases[9]. Anti-CCP antibodies are promising for early and accurate erosive RA diagnosis withbetter prognostic information and thus have implication in RA pathogenesis. Use of such biomarker along with other diagnostic features can be very useful for physician to prevent the patient from irreversible damage[6].

In order to treat RA patients at early stage, markers more reliable than RF are required. A study was conducted to evaluate the accuracy of anti-CCP antibody along with other newer biomarkers (i.e. IgA-RF and IgG-RF along with levels of cartilage oligomeric matrix protein (COMP)) for diagnosis of RA. The results showed that anti-CCP Antibody was most accurate marker for diagnosis of RA as compared to all of the other markers tested[10]. Anti-CCP antibodies are found to be highly specific for diagnosis of RA[11]. The 2008 guideline for RA treatment, a publication by American College of Rheumatology (ACR), has included anti-CCP positivity to measure patient prognosis and for treatment selection[12]. ACR and European League Against Rheumatism (EULAR) together developed new set of classification criteria for RA which included that anti CCP antibodies detection be used for RA diagnosis[13].The RF and anti CCP antibodies as tools for RA diagnosis have been evaluated by a number of studies and it was found that anti CCP antibodies have higher specificity as compared to RF for RA diagnosis[14].

In the present study, RF and anti-CCP antibodies were detected in RA patients to check their diagnostic value. The results showed that anti-CCP antibodies were more specific marker for RA diagnosis as compared to RF in our population. But at the same time RF can also be used in combination with anti CCP antibody detection to increase the accuracy of RA diagnosis.

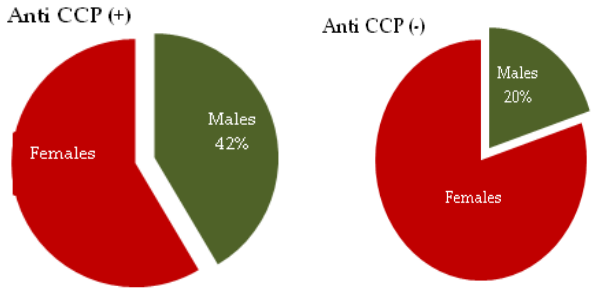


**Figure-2: Gender ratio of rheumatoid arthritis patients between Anti CCP (positive) and (negative) groups.**

All around the world, studies have been conducted to determine the application of anti-CCP testing as well RF testing in RA diagnostics.

A number of studies support that anti CCP antibody detection is helpful to detect RA[15-18]. Niewold et al also discussed in detail the implication of anti CCP antibody in diagnosis and prognosis. Anti-CCP antibodies are marked specifically for RA but its low sensitivity in test does not rule out disease, but its positive test markedly increases the chance of patient having RA. The higher percentage of patients with anti-CCP antibodies does not have RF. The anti-CCP antibodies detection can be helpful to predict RA as well as disease pathogenesis[6]. We also have found that anti-CCP antibodies are important for diagnosis of RA along with RF. This is mentioned in the new criteria set in 2010 to diagnose the RA. According to the new criteria, RF along with anti-CCP antibodies detection is important for efficient diagnosis of RA[13].

## CONCLUSION

Results showed that a higher percentage of the RA patients are positive for anti-CCP antibody as compared to RF hence marking its importance as a diagnostic marker for RA.

Thus it is concluded that anti CCP antibody detection is more specific marker for RA diagnosis as compared to RF.

## CONFLICT OF INTEREST

This study has no conflict of interest to declare by any author.

## REFERENCES


1. Poh LW, He HG, Chan WC, Lee CS, Lahiri M, Mak A, et al. Experiences of Patients With Rheumatoid Arthritis: A Qualitative Study. Clin Nurs Res. 2016. [Epub ahead of print]
2. Gibofsky A. Overview of epidemiology, pathophysiology, and diagnosis of rheumatoid arthritis. Am J Manag Care. 2012;18(13 Suppl):S295-302.
3. Alam SM, Kidwai AA, Jafri SR, Qureshi BM, Sami A, Qureshi HH, et al. Epidemiology of rheumatoid arthritis in a tertiary care unit, Karachi, Pakistan. J Pak Med Assoc. 2011;61(2):123-6.
4. Hameed K, Gibson T, Kadir M, Sultana S, Fatima Z, Syed A. The prevalence of rheumatoid arthritis in affluent and poor urban communities of Pakistan. Br J Rheumatol. 1995;34(3):252-6.
5. Farooqi A, Gibson T. Prevalence of the major rheumatic disorders in the adult population of north Pakistan. Br J Rheumatol. 1998;37(5):491-5.
6. Niewold TB, Harrison MJ, Paget SA. Anti-CCP antibody testing as a diagnostic and prognostic tool in rheumatoid arthritis. QJM. 2007;100(4):193-201.
7. Manivelavan D, C KV. Anti-cyclic citrullinated peptide antibody: an early diagnostic and prognostic biomarker of rheumatoid arthritis. J Clin Diagn Res. 2012;6(8):1393-6.
8. De Vries-Bouwstra JK, Dijkmans BA, Breedveld FC. Biologics in early rheumatoid arthritis. Rheum Dis Clin North Am. 2005;31(4):745-62.
9. Dorner T, Egerer K, Feist E, Burmester GR. Rheumatoid factor revisited. Curr Opin Rheumatol. 2004;16(3):246-53.
10. Nikolaisen C, Rekvig OP, Nossent HC. Diagnostic impact of contemporary biomarker assays for rheumatoid arthritis. Scand J Rheumatol. 2007;36(2):97-100.
11. Quinn MA, Gough AK, Green MJ, Devlin J, Hensor EM, Greenstein A, et al. Anti-CCP antibodies measured at disease onset help identify seronegative rheumatoid arthritis and predict radiological and functional outcome. Rheumatology (Oxford). 2006;45(4):478-80.
12. Saag KG, Teng GG, Patkar NM, Anuntiyo J, Finney C, Curtis JR, et al. American College of Rheumatology 2008 recommendations for the use of nonbiologic and biologic disease-modifying antirheumatic drugs in rheumatoid arthritis. Arthritis Rheum. 2008;59(6):762-84.
13. Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham CO, 3rd, et al. 2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. Arthritis Rheum. 2010;62(9):2569-81.
14. Nishimura K, Sugiyama D, Kogata Y, Tsuji G, Nakazawa T, Kawano S, et al. Meta-analysis: diagnostic accuracy of anti-cyclic citrullinated peptide antibody and rheumatoid factor for rheumatoid arthritis. Ann Intern Med. 2007;146(11):797-808.
15. Russell AS, Devani A, Maksymowych WP. The role of anti-cyclic citrullinated peptide antibodies in predicting progression of palindromic rheumatism to rheumatoid arthritis. J Rheumatol. 2006;33:1240–2.
16. Jansen AL, van der Horst-Bruinsma I, van Schaardenburg D. Rheumatoid factor and antibodies to cyclic citrullinated peptide differentiate rheumatoid arthritis from undifferentiated polyarthritis in patients with early arthritis. J Rheumatol. 2002;29:2074–6.
17. Saraux A, Berthelot JM, Devauchelle V. Value of antibodies to citrulline-containing peptides for diagnosing early rheumatoid arthritis. J Rheumatol. 2003;30:2535–9.
18. Nielen MM, van Schaardenburg D, Reesink Hw. Specific autoantibodies precede the symptoms of rheumatoid arthritis: a study of serial measurements in blood donors. Arthritis, Rheum. 2004;50:380–6.