

## ORIGINAL ARTICLE

## DIAGNOSTIC ACCURACY OF ANTI-CYCLIC CITRULLINATED PEPTIDE ANTIBODIES IN RHEUMATOID ARTHRITIS

Humaira Ali, Junaid Mahmood Alam, Ali Nadeem\*, Shazia Naureen

Department of Chemical Pathology/Biochemistry Laboratory Services, Liaquat National Hospital and Medical College,

\*Department of Microbiology, Sindh Institute of Urology and Transplantation, Karachi, Pakistan

**Objective:** To determine the diagnostic accuracy of anti-cyclic citrullinated peptide antibodies (Anti-CCP) in the diagnosis of rheumatoid arthritis (RA) in patients of joint pain keeping rheumatoid factor (RF) analysis, clinical and radiological features as the gold standard. **Methods:** A cross sectional study was performed at the Department of Chemical Pathology/Biochemical Laboratory Services, Liaquat National Hospital and Medical College, Karachi from January 2018 to June 2018. Over a period of six months, 363 samples of patients in the age group of 20–60 years having joint pain and suspected of suffering from rheumatoid arthritis were included after they fulfilled the inclusion and exclusion criteria. Basic information that is, patient demographics, age, sex, Anti-CCP results and RF levels were recorded. **Results:** There were 87 males and 283 females in the study. The results showed that sensitivity, specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV) and diagnostic accuracy were 69.2%, 98.5%, 97.8%, 77% and 84.2% respectively. Likelihood Ratio (LR)+ and LR- were 47 and 0.31 respectively. As per Receiver Operator Characteristic (ROC) curve analysis, the Area Under the Curve (AUC) is 0.839. The two-tailed test showed  $p < 0.0001$ . The diagnostic accuracy of Anti-CCP antibodies in the diagnosis of Rheumatoid Arthritis in patients of joint pain was found to be 89.4%. The cutoff of anti-CCP was calculated by the Youden's J. formula and was found to be 14 U/ml. **Conclusion:** Anti-CCP has a high sensitivity and specificity so it can be used for the diagnosis of rheumatoid arthritis in RF positive patients, which are common in Pakistan due to the high prevalence of Hepatitis C.

**Keywords:** Rheumatoid Arthritis, Anti-Cyclic Citrullinated Peptide, Rheumatoid Factor

Pak J Physiol 2019;15(2):3–6

## INTRODUCTION

Rheumatoid arthritis (RA) is the most common form of polyarticular inflammatory arthritis characterized by persistent synovial inflammation, bony erosions and progressive articular destruction leading to varying degrees of physical disability and reduction in the quality of life.<sup>1,2</sup> There is a growing need to recognize RA quickly and reliably before there is permanent joint damage and significant morbidity.<sup>3</sup> In a study done on adults from Northern Pakistan, a high overall burden of rheumatic disease has been shown, and the prevalence of RA was calculated to be 5.5 per 1000.<sup>4</sup> A recent report from a rheumatology clinic in Karachi showed 12.9% of adult patients to be suffering from RA and of these, 3.5% had extra-articular complications.<sup>2</sup> A survey of joint symptoms among 4232 adults, evenly distributed between poor and affluent areas of Karachi, Pakistan reported the prevalence of RA as 0.9 and 1.98 per 1000 in the two areas, respectively.<sup>5</sup>

Rheumatoid factor (RF) alone, if used as a marker for the diagnosis of RA lacks sensitivity and specificity.<sup>6–8</sup> High serum values of RF have also been reported in chronic viral infections, such as Hepatitis C infection and bacterial endocarditis.<sup>6</sup> Therefore, it had become necessary to identify other diagnostic and prognostic markers of RA that would be characterized by high sensitivity and specificity and would be more

appropriate for the diagnosis of new-onset disease. Antibodies against cyclic citrullinated peptides (Anti-CCP) seem to fulfill these requirements. Anti-CCP antibodies are produced locally at sites of inflammation, in the synovium of RA but also in other non-RA diseases.<sup>9</sup> Various studies have shown a discrepancy in the sensitivity and specificity of anti-CCP which may be due to detection techniques, genetic background and differences in the false positive rates of the selected controls.<sup>9</sup> Anti-CCP is the second serological marker (apart from RF) to have recently been included in the 2010 American College of Rheumatism/European League Against Rheumatism (ACR/EULAR) classification criteria for RA, which is focused on early diagnosis and therapy of RA.<sup>10</sup>

Keeping in mind the high prevalence of hepatitis C in Pakistan, and the presence of high serum values of RF in this condition, it became important to evaluate the diagnostic importance of anti-CCP in our setting. A few studies have been done in this regard, but this is believed to be the largest sample size which has been used for any study.

## MATERIAL AND METHODS

This cross-sectional diagnostic accuracy study was performed in the Department of Chemical Pathology/Biochemistry Laboratory Services, Liaquat National Hospital and Medical College, Karachi from 1<sup>st</sup>

January 2018 to 30<sup>th</sup> June 2018 after approval from the ethical review committee. Sample size was calculated<sup>11</sup> and a total of 363 samples were included in our study, by non-probability consecutive sampling, using Prevalence= 6%<sup>12</sup>, Sensitivity= 81.6%<sup>9</sup>, Specificity= 87.5%<sup>9</sup> and desired precision= 10% for specificity and 14% for sensitivity with 95% confidence level. Patients of either gender, between 20–60 years of age complaining of joint pain presenting to the rheumatology clinic and suspected of having RA were included. Samples with hypergammaglobulinaemia, hemolyzed, lipaemic, icteric samples were excluded as these interfere with the assay.

Blood sample was taken for the analysis of anti-CCP antibodies and RF by peripheral venipuncture done by expert phlebotomist after aseptic measures. Six ml of venous blood was collected in a tube containing silica particles, which acted as clot activator (red top) and allowed to clot. All samples marked for Anti-CCP antibody by the phlebotomist were received in the well-equipped and skilled Chemical Pathology Lab of Liaquat National Hospital by the principal investigator for chemical pathology and allotted sample number. Patient demographics e.g. age, gender were recorded by the principal investigator. Samples were centrifuged and the supernatant collected for analysis. To perform anti-CCP assay, the serum was analyzed by Electrochemiluminescence Immunoassay using e411 analyzer (Roche Diagnostics). Optimal cut-off of 17 U/ml was used. Samples with a concentration of >17 U/ml were considered positive for anti-CCP antibodies. For RA factor analysis the same supernatant was analyzed using Immunoturbidimetry by c501 analyzer (Roche Diagnostics). Optimal cut-off of 14 IU/ml was used. Samples with a concentration of >14 IU/ml were considered positive for RF. All samples were screened by the principal investigator for initial diagnosis. Final diagnosis was made by experienced senior chemical pathology consultant. Confounding variables as well as biasness was controlled by strictly following the inclusion and exclusion criteria. All the data including patient demographics and laboratory findings of Anti-CCP and RA factors were recorded in a pre-designed Proforma by the principal investigator.

Data was collected and analyzed using Statistical Package for Social Sciences version-19. Mean and Standard Deviation (SD) were calculated for the quantitative variables, i.e., age, Anti-CCP, and RA factor. Frequency and percentages were calculated for

qualitative variables, i.e., gender, Anti-CCP (Positive/Negative) and RA Factor (Positive/Negative). Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), Likelihood ratio (LR) positive (+), LR negative (-) and diagnostic accuracy were calculated taking clinical, imaging and RA factor as gold standard, diagnosed by a consultant chemical pathologist and/or rheumatologist. To overcome the effect modifier sensitivity, specificity, PPV, and NPV were calculated after stratification of age and gender using  $\chi^2$  test, and  $p \leq 0.05$  was taken as significant.

## RESULTS

A total of 370 samples brought to the laboratory for the diagnosis of rheumatoid arthritis were tested using non-probability consecutive sampling, to determine the diagnostic accuracy of anti-CCP for the diagnosis of RA using RF, clinical and radiological features as the gold standard, diagnosed by chemical pathologist/rheumatologist. Sensitivity, specificity, PPV, NPV, LR+, LR- and diagnostic accuracy of anti-CCP were calculated. Stratification was done to see the effect of modifiers on outcome. Post stratification chi square test was applied considering  $p \leq 0.05$  as significant.

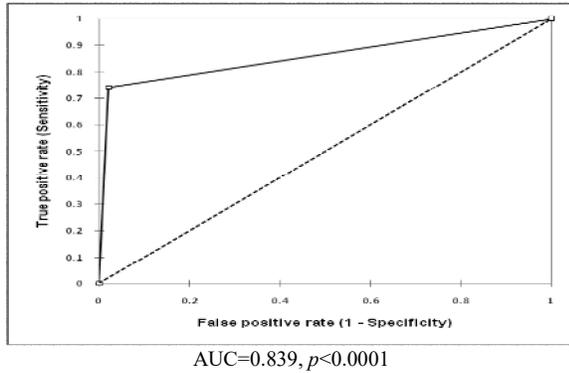
Overall there were 87 (24%) males and 283 (76%) females. Age was stratified in groups. Patients belonged to the age group of 20–34 years were 95 (25.7%), 141 (38.1%) patients belonged to the age group of 35–49 years and 134 (36.2%) belonged to the age group of 50–60 years. Rheumatoid Arthritis was assessed and frequency of normal and abnormal RA with respect to Anti CCP and RF was evaluated. Positive RA was observed with Anti CCP in 103 (27.8%) patients and positive RA was observed with RF in 132 (35.7%) patients.

Sensitivity, specificity, predictive values and diagnostic accuracy of anti-CCP for the diagnosis of rheumatoid arthritis taking clinical, radiological features and RA Factor as the gold standard were calculated. The results showed that sensitivity, specificity, PPV, NPV, LR+, LR- and diagnostic accuracy were 69.2%, 98.5%, 97.8%, 77%, 47, 0.31 and 84.2% respectively. These results are presented in the Table-1.

As per Receiver Operator Characteristic (ROC) curve analysis (Figure-1), the area under the curve (AUC) is 0.839. The two-tailed test showed a  $p < 0.0001$ . The cut-off of anti-CCP was calculated by the Youden's J formula<sup>13</sup> and was found to be 14 U/ml.

**Table-1: Diagnostic Utility of Anti-CCP antibodies in Rheumatoid Arthritis**

	Sensitivity	Specificity	PPV	NPV	LR+	LR-	TP	TN	FP	FN	Accuracy
<b>Anti-ccp</b>	0.692	0.985	0.978	0.770	47.077	0.312	45	67	1	20	0.842
<b>RF</b>	1.000	0.000	0.489		1.000		65	0	68	0	0.489



**Figure-1: Receiver Operating Characteristic Curve of Anti-CCP levels**

## DISCUSSION

The purpose of this study is to evaluate the sensitivity and specificity of anti-CCP antibodies in diagnosing rheumatoid arthritis. A study by Khan *et al*<sup>6</sup> showed the sensitivity, specificity, PPV, NPV of Anti-CCP to be 54.7%, 95.5%, 93.5% and 64.1% respectively. Another study by Dogan *et al*<sup>14</sup> shows the sensitivity, specificity, PPV and NPV as 69%, 95%, 97% and 59% respectively. Shafiaa *et al*<sup>15</sup> demonstrated the sensitivity, specificity, PPV and NPV as 78.7%, 100%, 100% and 48.4% respectively. All these results are similar to our study which shows sensitivity, specificity, PPV and NPV of anti-CCP as 69.2%, 98.5%, 97.8% and 77.0% respectively.

There is an association between the presence of anti-CCP and structural damage in early RA. In case of established RA, this association is not so conclusive.<sup>16</sup> Anti-CCP and insulin resistance has been shown to be helpful in the early detection of sub-clinical atherosclerosis in RA patients.<sup>17</sup> This study showed increased insulin resistance and anti-CCP levels in RA as compared to controls, and they were positively correlated with intima-mediated thickness in the carotid arteries.<sup>18</sup> This emphasizes the importance of anti-CCP as a diagnostic and prognostic marker in all complications of RA.

In a study conducted in Malaysia, the sensitivity and specificity of Anti-CCP was found to be 35% and 100% and for RF, 43% and 85% respectively.<sup>18</sup> On combining both the assays, the sensitivity and specificity became 50% and 85% respectively.<sup>18</sup> This study also found an association of anti-CCP antibody with involvement of multiple joints, pain in the joints of the hand, symmetrical joint involvement, raise in CRP and RF.<sup>18</sup>

Smoking has also been found to have a strong relationship to RA especially in HLA-DRB1 positive patients and substances in tobacco also induce citrullination.<sup>16</sup> The role of smoking in RA has not been addressed in this study. Further work needs to be done in this regard.

A study performed in Egypt gave the sensitivity and specificity of RF as 66.67% and 22.22% and of anti-CCP of 72.22% and 100%.<sup>19</sup> This is similar to our study. They also found that anti-CCP antibodies are a very strong serum marker to differentiate RA from HCV patients.<sup>19</sup> Khan *et al* have also observed Hepatitis C positive patients presenting with arthralgia may have positive RF which can be confusing, so in these patients anti-CCP can be used to distinguish between the two, especially in areas like Pakistan where Hepatitis C is highly prevalent.<sup>6</sup> Further testing is needed in HCV patients in this regard.

The concentration of anti-CCP antibodies increases as the disease becomes more severe, it predicts the course of the disease, and patients with positive anti-CCP antibodies show more joint destruction than RA patients without anti-CCP antibody positivity.<sup>20</sup>

Swart *et al*<sup>21</sup> showed the sensitivity and specificity of anti-CCP of 77.3% and 98.1% for 2<sup>nd</sup> generation anti-CCP assay with area under the curve (AUC) of 0.891, these findings are similar to our study which showed an AUC of 0.839. Similar results were found in the study by Manivelavan *et al*<sup>22</sup> which showed 83% sensitivity and 96% specificity for Anti-CCP, the findings of which are much similar to our study. Further, this study also showed that the sensitivity and specificity of anti-CCP was much higher compared to that of RF. Due to this reason anti-CCP antibody test is more useful for the diagnosis of Rheumatoid Arthritis as compared to RF.<sup>22</sup>

Anti-CCP assays are useful in the early diagnosis of RA, in cases where there is RF-negative RA and in cases of hepatitis C related joint involvement in which RF may be positive.<sup>23</sup> In undifferentiated arthritis, anti-CCP positivity seems to have value in the diagnosis, prognosis and prediction of the disease course.<sup>23</sup>

Anti-CCP positivity was seen in 23% patients with early RA, in 50% of patients at diagnosis of the disease and in 53–70% of patients 2 years after diagnosis.<sup>24</sup> In the healthy population, less than 1.5% have positive anti-CCP, and with other rheumatic disease the incidence is about 10%.<sup>24</sup> So a positive anti-CCP is more reliable than a positive RF for the diagnosis of RA.<sup>24</sup>

Earlier studies have used the 1987 American College of Rheumatology (ACR) criteria for the diagnosis of RA. These criteria have been criticized for their lack of sensitivity and the newer 2010 ACR-EULAR criteria has now incorporated anti-CCP in the diagnostic criteria. These criteria use the signs and symptoms in the early stages of the disease rather than the late-stage features for diagnosing RA.<sup>10</sup> High serum values of anti-CCP were present in more severe disease as seen by the scoring of the symptoms in the ACR-EULAR criteria.<sup>6</sup> The positivity of anti-CCP antibodies

was associated with more severe radiological joint damage and the development of more erosive disease than those without anti-CCP.<sup>6</sup>

It is also seen that anti-CCP positive patients remain positive even after long term drug therapy, with a slight reduction or even an increase in titres of the antibody.<sup>25</sup> Due to this reason it is not possible to observe the effectiveness of medications on the disease process by the stability or reduction of the titres of the antibody.<sup>25</sup>

## CONCLUSION

Anti-CCP antibodies have a high sensitivity and specificity and they can be used for the diagnosis of RA, especially in cases where RF is negative, in the early course of the disease, in cases of severe joint destruction, or very active disease.

## REFERENCES

- Ahmed I, Ali Z, Taqweem A, Alam I, Mehboob A. The relationship between the clinical manifestations and the presence of anti cyclic citrullinated peptide antibodies in very early rheumatoid arthritis. *J Postgrad Med Inst* 2011;25(4):309–13.
- 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.
- Soroush M, Mahmoudi M, Akhlaghi M. Determination of specificity and sensitivity of rheumatoid factor and anti CCP tests in patients with RA in private clinic in Tehran, Iran. *Biomed Pharmacol J* 2016;9(2):775–80.
- 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.
- 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.
- Khan AH, Jafri L, Hussain MA, Ishaq S. Diagnostic utility of anti-citrullinated protein antibody and its comparison with rheumatoid factor in rheumatoid arthritis. *J Coll Physicians Surg Pak* 2012;22(11):711–5.
- Whittingham SF, Stockman A, Rowley MJ. Collagen auto antibodies and their relationship to CCP antibodies and rheumatoid factor in the progression of early rheumatoid arthritis. *Antibodies* 2017;6(2). doi:10.3390/antib6020006
- Takeuchi T, Miyasaka N, Inui T, Yano T, Yoshinari T, Abe T, *et al.* High titers of both rheumatoid factor and anti-CCP antibodies at baseline in patients with rheumatoid arthritis are associated with increased circulating baseline TNF level, low drug levels, and reduced clinical responses: a post hoc analysis of the RISING study. *Arthritis Res Ther* 2017;19(1):194.
- Heidari B, Firouzjahi A, Heidari P, Hajian K. The prevalence and diagnostic performance of anti-cyclic citrullinated peptide antibody in rheumatoid arthritis: the predictive and discriminative ability of serum antibody level in recognizing rheumatoid arthritis. *Ann Saudi Med* 2009;29(6):467–70.
- Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham CO 3<sup>rd</sup>, *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.
- Zaidi SMH, Waseem HF, Ansari FA, Irfan M, Fahim S. Sample size estimation of diagnostic test studies in health sciences. *Proceedings of 14<sup>th</sup> International Conference on Statistical Sciences*, at Jinnah Sindh Medical University Karachi 2016;29:239–46.
- Mandal A. How many people does rheumatoid arthritis affect? 2018. Available from: <http://www.news-medicalnet/health/Rheumatoid-Arthritis-Epidemiology.aspx>. [Accessed: 15 Apr 2019]
- Unknown. ROC Curve. *Medical Biostatistics & Research*. Available from: <http://www.medicalbiostatistics.com/> [Accessed 15 Apr 2019]
- Dogan M, Kucuksarac S, Tufekci O, Feyzioglu B, Ozdemir M, Baykan M, *et al.* Comparison of the diagnostic values in rheumatoid arthritis: Anti-CCP antibodies and other serological tests. *Biomed Res (India)*2014;25(3):381–6.
- Shafiaa S, Shaha ZA, Sofib FA, Rasoola R, Gulla A. Anti-CCP is associated with greater disease burden in Kashmiri population with rheumatoid arthritis. *Rheumatology (Sunnyvale)* 2016;6(1):190. doi: 10.4172/2161-1149.1000190
- Porto LS, Tavares WC Junior, Costa DA, Lanna CC, Kakehasi AM. Anti-CCP antibodies are not a marker of severity in established rheumatoid arthritis: a magnetic resonance imaging study. *Rev Bras Reumatol* 2017;57(1):15–22.
- Wahab MAKKA, Laban AE, Darweesh AF. The clinical value of anti-cyclic citrullinated peptide (anti-ccp) antibodies and insulin resistance (IR) in detection of early and subclinical atherosclerosis in rheumatoid arthritis (RA). *Egypt Heart J* 2016;68(2):109–16.
- Abdul Wahab A, Mohammad M, Rahman MM, Mohamed Said MS. Anti-cyclic citrullinated peptide antibody is a good indicator for the diagnosis of rheumatoid arthritis. *Pak J Med Sci* 2013;29(3):773–7.
- Hussein MS, Abdel Ghany SE, Elashkar DS, Rabea MY, Nosair NA. Anti-CCP hs (high sensitive) in Egyptian rheumatoid arthritis patients associated with chronic hepatitis C virus infection. *Egypt Rheumatol* 2016;38:15–20.
- Sarah K, Nadeem A. Anti-CCP Antibodies: an emerging marker for the early diagnosis of rheumatoid arthritis. *Int Med J Malaysia* 2010;9(1):63–5.
- Swart A, Burlingame RW, Gurtler I, Mahler M. Third generation anti-citrullinated peptide antibody assay is a sensitive marker in rheumatoid factor negative rheumatoid arthritis. *Clin Chim Acta* 2012;414:266–72.
- Manivelavan D, Vijayasamundeeswari CK. Anti-CCP antibody: an early diagnostic and prognostic biomarker of rheumatoid arthritis. *J Clin Diagn Res* 2012;6(8):1393–6.
- Bizzaro N, Bartoloni E, Morozzi G, Manganelli S, Ricciari V, Sabatini P, *et al.* Anti-cyclic citrullinated peptide antibody titer predicts time to rheumatoid arthritis onset in patients with undifferentiated arthritis: results from a 2-year prospective study. *Arthritis Res Ther* 2013;15(1):R16.
- Braschi E, Shojania K, Allan GM. Anti-CCP: a truly helpful rheumatoid arthritis test? *Can Fam Physician* 2016;62(3):234.
- da Silva Carvalho C, Coelho Andrade LE, Keusseyan SP, Rangel JL, Strixino JF, Martin AA, *et al.* Study of advanced rheumatoid arthritis. *Engenharia Biomed* 2014;30(1):54–63.

## Address for Correspondence:

**Dr. Humaira Ali.** Senior Registrar Chemical Pathology, Department of Chemical Pathology/Biochemistry Laboratory Services, Liaquat National Hospital and Medical College, Karachi, Pakistan, **Cell:** +92-331-2529712  
**Email:** howrali@hotmail.com

Received: 25 Sep 2018

Reviewed: 17 Apr 2019

Accepted: 17 Apr 2019