**ORIGINAL ARTICLE**

# Serum Anti-CCP Antibody and its Correlation with Disease Activity in Local Pakistani Rheumatoid Arthritis Patients

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## ABSTRACT

**Aims:** To investigate the correlation of anti-Cyclic Citrullinated Peptide (anti-CCP) with clinical manifestations and disease activity in a cohort of patients with established diagnosis of rheumatoid arthritis.

**Methods:** A total of 58 patients attending the Rheumatology outpatient department of Fatima Memorial Hospital, Lahore were recruited in the study. Data of disease related parameters such as disease duration, medications, degree of pain (visual analog scale, VAS), disease activity score 28 (DAS 28), and health assessment questionnaire (HAQ) were recorded. Laboratory workup included erythrocyte sedimentation rate (ESR>20mm/hr considered positive), rheumatoid factor (determined by ELISA technique) and anti-CCP antibody (ELISA Immco Diagnostics, USA).

**Results:** The anti-CCP antibody levels did not correlate with the number of tender joints (p=0.282), swollen joints (p=0.705), VAS (p=0.487), ESR (p=0.128), HAQ scores (p=0.317) or DAS28 scores (p=0.348). However, anti-CCP levels correlated significantly (p=0.000) with the rheumatoid factor titers.

**Conclusion:** No correlation existed between the serum anti-CCP antibody titers and DAS-28 and other disease activity parameters besides RF titers.

**Key words**: Rheumatoid arthritis, Anti-Cyclic Citrullinated Peptide Antibody (anti-CCP)

## INTRODUCTION

Rheumatoid arthritis is an autoimmune disorder of multifactorial etiology characterized by symmetric and erosive synovitis. It causes progressive joint destruction and disability. It is the most common inflammatory arthritis, affecting 0.5 to 1% of the general population worldwide, with a female to male ratio of 2.5:1. The disease may appear at any age, but it is most common among those aged from 40-70 years[1]. Many studies have shown that the disease progresses rapidly within first two years of onset and can lead to irreversible erosive joint destruction. Early diagnosis and the ability to predict who will develop erosive disease are important as these patients can be treated early and deformities can be prevented[2].

Early diagnosis of rheumatoid arthritis rests mainly on clinical symptoms which are usually mild and nonspecific, and patients usually do not fulfill the American College of Rheumatology (ACR) criteria for the diagnosis[3]. By the time clinical diagnosis of RA is made, irreversible joint erosions usually have occurred. Ongoing research has shown that early therapeutic intervention results in earlier disease control and consequently less joint damage[4]. There is no single test or finding that can diagnose rheumatoid arthritis. Rheumatoid factor is the only serological test included in the ACR criteria. RF is an antibody directed against Fc region of IgG. It has been used widely as a diagnostic marker of RA for decades. However, this auto-antibody lacks specificity. It may be found in patients with other autoimmune diseases and infectious disorders. It may also be present in sera of apparently healthy elderly individuals. Upto 25% of patients with rheumatoid arthritis have negative rheumatoid factor test (seronegative)[5]. Therefore, detection of disease-specific auto antibodies is needed for early diagnosis. The RF assay in its current form remains suboptimal as a diagnostic tool. It lacks sensitivity (54-88%) and specificity (40-92%). However, it has been established that high titers of RF indicate aggressive disease[6].

Other RA associated antibodies which have been described are anti-perinuclearfactor (APF), anti-keratin antibody (AKA), anti-fillagrin antibody and anti-cyclic citrullinated peptide antibody. These all belong to the family of anti-citrullinated protein/ peptide antibody (ACPA)[7]. All these antibodies recognize the antigenic epitope containing citrulline[8], which is generated by post-translational modification of naturally occurring amino acid arginine by the activity of enzyme peptidylargininedeiminase (PAD)[9]. Several citrullinated proteins proposed as antigens

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include fibrin, Ebstein-Barr virus nuclear antigen, alpha-enolase and vimentin[10]. Process of citrullination is augmented in inflammatory conditions. Citrullinated peptides have been synthesized as antigens for diagnostic immunoassays[7]. Several assays for detecting anti-citrullinated peptide antibodies (ACPA´s) have been developed employing filaggrin derived peptides (CCP-assay), viral citrullinated peptides (VCP-assay), mutated citrullinatedvimentin (MCV-assay)[6].

Research has shown that ACPA have a higher specificity than rheumatoid factor in diagnosing rheumatoid arthritis[11]. Studies have shown that ACPA´s can be detected years before the appearance of first symptoms of RA[12]. Although the presence of anti-CCP is accepted to be a reliable diagnostic and prognostic tool in RA, it association with disease activity and severity remains unclear.

In the present study, we have investigated the correlation of the presence of anti-CCP antibody with clinical manifestations and disease activity in a cohort of established cases of rheumatoid arthritis.

## SUBJECTS& METHODS

A total of 58 patients attending the Rheumatology outpatient department of Fatima Memorial Hospital, Lahore were recruited in the study. All the patients fulfilled the American College of Rheumatology criteria for RA and were diagnosed by the rheumatologist. The study was approved by the Ethical and Review Committee of University of Health Sciences, Lahore. Informed written consent was taken from each study participant. Data regarding disease variables was collected during clinical evaluation of the patients. Patients with other connective disease, acute or chronic infectious diseases or malignancy were excluded from the study to avoid positive anti-CCP results associated with other conditions[11].

Purposefully designed proforma was used to record the data of the subjects including age, gender, duration of disease, medications, degree of pain (visual analog scale, VAS) and clinical characteristics. Stanford Health Assessment Questionnaire-Disability Index (HAQ-DI) was used to get a score so as to access the functional disability of RA[13]. Disease activity was estimated in all patients using the disease activity score of 28 joints and for four variables, with the help of a preprogrammed calculator (DAS-28)[14].

Laboratory workup included erythrocyte sedimentation rate (ESR>20mm/hr considered positive), rheumatoid factor (determined by ELISA technique) and anti-CCP antibody (ELISA Immco Diagnostics, USA).

At the end of study, patients were divided into anti-CCP positive and anti-CCP negative and comparisons between the two groups were performed in all the above mentioned characteristics. Spearman's rho correlation was used to observe correlation between non-normally distributed quantitative variables. The level of two-sided statistical significance was set at 0.05. All data was analyzed using SPSS version 17.

## RESULTS

The socio-demographic and disease related characteristics of the patient with rheumatoid arthritis are summarized in table-1. Among the58 rheumatoid arthritis patients (47 were females and 11 were males),34 were found to be anti-CCP positive (58.5%) and 24(41.4%) were anti-CCP negative. Mean age±SEM of the RA group was 44±1.2 years. All the patients (n=58) were using methotrexate, while 35 were using steroids.

Different characteristics of the aCCP positive and aCCP negative RA patients were compared to see if their means differ significantly (Table-2). Significant (p=0.001) difference was found in the RF titer of aCCP+ive (Md=30.1, n=34) and aCCP-ive group (Md=2.03, n=24). Significant (p=0.001) difference was observed in the aCCP titer of aCCP+ive (Md=451, n=34) and aCCP-ive group (Md=0.00, n=24).

Correlation of serum anti-CCP titers with various disease variables like TJC, SJC, VAS, DAS-28 was determined. There was a significant (rho=0.665, n=58, p=0.000) correlation between serum anti-CCP titers and serum RF titers (Figure 3.7). There was no significant correlation between serum anti-CCP titers and TJC (rho= -0.144, n=80, p=0.282), SJC (rho= -0.051, n=80, p=0.705), VAS (rho=0.093, n=58, p=0.487), DAS-28 (rho= -0.126, n=58, p=0.348), HAQ-DI (rho= -0.134, n=58, p=0.317), ESR (rho= -0.202, n=58, p=0.128) shown in table-3.

Table 1: Disease Related Characteristics of RA patients

| **Characteristics** | **Mean±SEM/ Median(IQR)** |
|---|---|
| Drug treatment<br>Methotrexate (MTX)<br>Steroid | <br>58<br>35 |
| Disease duration (years) | 5(4-8) |
| TJC | 10 (5.25-16) |
| SJC | 4 (0.0-7.0) |
| VAS | 50 (31-73.75) |
| ESR (mm/1$^{st}$hr) | 44 (25-63.8) |
| DAS-28 Score | 5.37 (4.35-6.49) |
| HAQ-DI Score | 1.9 (1.0-2.38) |
| Serum RF titer (IU/ml) | 27.76 (2.51-32.9) |
| Serum aCCP titer (IU/ml) | 10.8 (0.00-340.5) |

Table 2.Comparison of different disease related and serological parameters in anti-CCP positive and anti-CCP negative sub-groups of RA patients

| | aCCP positive patients (n=34) | aCCP negative patients (n=24) | P value |
|---|---|---|---|
| | Mean±SEM/Median(IQR) | Mean ±SEM/Median(IQR) | |
| Age ( years) | 44 (34-51) | 40(35-55) | 0.645 |
| Disease duration (years) | 5(4-10) | 5(3.25-8.0) | 0.33 |
| VAS | 55.88±4.1 | 57.92±4.7 | 0.78 |
| TJC | 10.5(3.75-16) | 9.50 (7-14) | 0.76 |
| SJC | 3(0-7) | 4.5(1-6) | 0.96 |
| HAQ-DI Score | 1.44(0.87-2.37) | 1.88(1.12-2.37) | 0.37 |
| DAS-28 Score | 6.06(4.3-6.65) | 5.7(4.99-6.6) | 0.75 |
| ESR (mm/hr) | 44±4.12 | 50.29±5.6 | 0.36 |
| Mean titers RF-IgM (IU/ml) | 30.1(4.6-32) | 2.03(0.05-5.48) | 0.001* |
| aCCP titer (IU/ml) | 632(151-1794) | 0.00(0-3.96) | 0.001* |

*p-value of <0.05 is considered as statistically significant

Table 3.Correlation of serum aCCP antibody titers and other disease variables

| Correlation between aCCP titers | N | Spearman rho correlation coefficient (rho) | p-value |
|---|---|---|---|
| ESR | 58 | -0.202 | 0.128 |
| TJC | 58 | -0.144 | 0.282 |
| SJC | 58 | -0.051 | 0.705 |
| VAS | 58 | 0.093 | 0.487 |
| DAS-28 | 58 | -0.126 | 0.348 |
| HAQ-DI | 58 | -0.134 | 0.317 |
| RF titers | 58 | 0.665 | 0.000* |

*****p-value of <0.05 is considered as statistically significant

## DISCUSSION

The modern trend of RA treatment has been changed to start it as early as possible. Early control of inflammation in RA results in reduced joint damage. It is therefore important to differentiate between RA and other forms of arthritis early after the onset of symptoms, so that the rheumatologists are able to target the use of potentially toxic and expensive drugs to those patients, where the benefits clearly outweigh the risk. Joint erosions and deformities are the major adverse outcomes. Several studies demonstrated the association of anti-CCP positivity and joint destruction in patients with established rheumatoid arthritis[15]. Follow up parameters of disease activity in RA patients are duration of morning stiffness, degree of joint pain, HAQ, DAS-28, ESR and serum RF positivity.

Although the presence of anti-CCP is accepted to be a reliable diagnostic and prognostic tool in RA, its association with disease activity and severity remains unclear. In the present study, we have investigated the correlation of levels of anti-CCP with clinical manifestations and disease activity in a cohort of patients with established diagnosis of RA. The anti-CCP antibody levels did not correlate with the number of tender joints (p=0.282), swollen joints (p=0.705), VAS (p=0.487), ESR (p=0.128), HAQ scores (p=0.317) or DAS28 scores (p=0.348). However, anti-CCP levels correlated significantly (p=0.000) with the rheumatoid factor titers.

Similarly, a study by Alexiou et al[16] (2007) on Greek patients did not find correlation between anti-CCP antibodies and DAS28 score. But, anti-CCP positive RA patients had increased swollen joint count and serum CRP concentration compared to anti-CCP-negative RA patients (p=0.01, and p<0.001). A prospective study by Papadopoulos, et al[17] on Greek patients found no association of serum levels of anti-CCP with disease activity and severity. Similarly, Serdaroglu et al[18] found no significant correlation between anti-CCP antibody and serological markers of disease activity (ESR, CRP, VAS, DAS28) or radiological assessment but significant correlation was found between RF and anti-CCP antibody (p=0.02, r=0.35).

Sockalingam, et al[19] did not find a correlation of anti-CCP levels with the number of tender joints (p=0.478), swollen joints (p = 0.417), patient's global assessment of disease activity (p=0.562) or physician assessment of RA activity (p= 0.282). No correlation was observed between anti-CCP antibody levels and either ESR or CRP (p- > 0.05). Anti-CCP levels correlated significantly with rheumatoid factor. Onder, et al[20] reported that anti-CCP positivity was associated with higher scores of DAS-28, longer duration of morning stiffness, serum RF positivity, while it was not associated with disease duration, VAS, HAQ, ESR, CRP and hemoglobin. Vanichapuntu, et al[21] found that anti-CCP

significantly correlated with parameters of inflammation, but not with DAS 28.

## CONCLUSION

No correlation exists between serum anti-CCP antibody with DAS-28 and other disease activity parameters besides RF titers, so the treatment decisions cannot be based on the serum anti-CCP testing alone.

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## REFERENCES

1. Lee DM, Weinblatt ME. Rheumatoid Arthritis. Lancet 2001; 358: 903-11.
2. Combe B. Progression in early rheumatoid arthritis. Best Pract Res Clin Rheumatol 2009; 23(1): 59-69.
3. Saraux A, Berthelot JM, Chales G, Le Henaff C, Thorel JB, Hoang S, et al. Ability of the American College of Rheumatology 1987 criteria to predict rheumatoid arthritis in patients with early arthritis and classification of these patients two years later. Arthritis Rheum 2001; 44: 2485-91.
4. Cush JJ. Early Rheumatoid arthritis- is there a window of opportunity? J Rheumatol. 2007; 80: 1-7.
5. Marianna MN. Rheumatoid factors: What do they tell us? J Rheumatol. 2002; 29: 2034-40.
6. Saraux A, Berthelot JM, Charles G, et al. Value of laboratory tests in early prediction of rheumatoid arthritis. Arthritis Rheum 2002; 47: 155-65.
7. Zendman AJ, van Venrooij MJ, Pruijn GJ. Use and significance of anti-CCP autoantibodies in rheumatoid arthritis. Rheumatology. 2006; 45(1): 20-5.
8. Schellekens GA, de Jong BA, van den Hoogen FH, van de Putte LB, van Venrooij WJ. Citrulline is an essential constituent of antigenic determinants recognized by rheumatoid arthritis- specific autoantibodies. J. Clin Invest 1998; 101: 273-81.
9. Vossenaar ER, Zendman AJ, van Venrooij WJ, Pruijn GJ. PAD, a growing family of citrullinating enzymes: genes, features and involvement in disease. Bioessays 2003; 25: 1106-18.
10. Bang H, Egerer K, Gauliard A, Berg W, Fredenhagen G, Feist E, et al. Mutation and citrullination modifies vimentin to a novel autoantigen for rheumatoid arthritis. Arthritis & Rheumatism. 2007; 56(8): 2503-11.
11. Bizzaro N, Mazzanti G, Tonutti E, Villalta D, Tozzoli, R.Diagnostic accuracy of the anti-citrulline antibody assay for rheumatoid arthritis. Clin Chem. 2001;47(6): 1089-93.
12. Nielen MJ, van Schaardenburg D, Reesink HW, van de Stadt RJ, de Koning MHT, Habibuw MR, et al. Specific aotuantibodies precede the symptoms of rheumatoid arthritis-a study of serial measurements in blood donors. Arthritis & Rheumatism. 2004; 50(2): 380-6.
13. Bruce B and Fries JF. The Health Assessment Questionnaire (HAQ). Clin Exp Rheumatol., 2005; 23: 14-18.
14. Prevoo ML, van't Hof MA, Kuper HH, van Leeuwen M.A, van de Putte LB and van Riel PL. Modified disease activity scores that include twenty-eight-joint counts. Development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. Arthritis Rheum.1995;38:44–8.
15. Shovman O, Gilburd B, Zandman-Goddard G, Sherer Y, Orbach H, Gerli R, et al. The diagnostic utility of anti-cyclic citrullinated peptide antibodies, matrix metallo-proteinase-3, rheumatoid factor, erythrocyte sedimentation rate, and C-reactive protein in patients with erosive and non-erosive rheumatoid arthritis. *ClinDevImmunol.*2005,12**:** 197-202.
16. Alexiou I, Germenis A, Ziogas A, Theodoridou K and Sakkas LI. Diagnostic value of anti-cyclic citrullinated peptide antibodies in Greek patients with rheumatoid arthritis. BMC Musculoskeletal Disorders. 2007;8: 37.
17. Papadopoulas NG, Tsiaousis GZ, Pavlitou-Tsionti A, Giannakou A. and Galanopoulou VK. Does the presence of anti-CCP antibodies and their serum levels influence the severity and activity in rheumatoid arthritis patients? Clinic Rev AllergImmunol 2008; 34: 11-5.
18. Serdaroglu M, Cakirbay H, Deger O, Cengiz S and Kul S. The association of anti-CCP antibodies with disease activity in rheumatoid arthritis. Rheumatol Int. 2008, 28: 965-70.
19. Sockalingam S, Khuan CS and Sthaneshwar P. Prevalence of anticycliccitrullinated peptide antibodies in Malaysian rheumatoid arthritis patients and its correlation with disease activity. Int. J. Rheum. 2009, 12: 211-5.
20. Onder B, Kurtaran A, Kimyon S, Selcuk B and Akyuz, M. Association of anti-CCP positivity with serum ferritin and DAS-28. Rheumatol Int.2009;30: 223-7.
21. Vanichapuntu M, Phuekfon P, Suwannalai P, Verasertniyom O, Nantiruj K. and Janwityanujit, S. Are anti-citrulline autoantibodies better serum markers for rheumatoid arthritis than rheumatoid factor in Thai population? Rheumatol Int.2010;30: 755-9.