

Original Article

A Comparative Assessment of the Diagnostic Value of Anti-cyclic Citrullinated Peptide Antibodies and Rheumatoid Factor in patients with Rheumatoid Arthritis in a Tertiary Care Hospital

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Introduction : Rheumatoid arthritis is a chronic, systemic inflammatory autoimmune disease that affects a variety of tissues and most commonly attacks the joints. Autoantibodies such as anti-cyclic citrullinated peptide antibodies and rheumatoid factor are useful diagnostic tools.

Objective: As modern therapy of Rheumatoid Arthritis (RA) focuses on aggressive aggressive initiation of the Disease-Modifying Antirheumatic Drugs (DMARD) and biologics to limit the joint destruction, diagnostic tests with high specificity are preferred. Using a synthetic peptide design, a new serologic test (anti-cyclic citrullinated peptide or anti- CCP antibody) measured by Enzyme-Linked Immunosorbent Assay (ELISA) was designed to detect the presence of antibodies directed against citrullinated peptides.

Methods : Among the 200 cases with a history of polyarthritis included in the study, 133 individuals are clinically diagnosed with rheumatoid arthritis. The control group consisted of 67 patients with recent onset undifferentiated polyarthritis. Individual sensitivity and specificity of the aforementioned tests, as well as the combined specificity of the two tests and the three tests, were computed and the test results were compared to see whether there was any association.

Results : Among the 133 patients with Rheumatoid Arthritis, Anti-cyclic Citrullinated Peptide antibodies were found in 94 patients (70.7%), while rheumatoid factor antibodies were found in 61 patients (66%). Anti-cyclic citrullinated Peptide antibodies have sensitivity, specificity, positive predictive value and negative predictive value of 70.76%, 85.07%, 90%, and 59% respectively, for diagnosing Rheumatoid Arthritis. In case of Rheumatoid Factor, the values were 66.26%, 90.29%, 90% and 45% correspondingly. Using both RA and non-RA sera, the Anti-CCP ELISA established as an exceptionally specific (98%) and sensitive (88%) tool. At ideal cut-off levels, the Anti-CCP ELISA demonstrated a considerably greater specificity than the IgM Rheumatoid Factor (IgM-RF) ELISA (96 % for CCP *versus* 91% for IgM-RF).

Conclusion: The anti-CCP ELISA could be highly effective for diagnosing and treating recent onset Rheumatoid Arthritis. When this test is used in conjunction with the RF IgM ELISA and the Latex test, the combined specificity can reach up to 99%.

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Key words : Anti-CCP Antibody, Rheumatoid Factor, Latex Agglutination Test, Enzyme Linked Immunosorbent Assay.

Rheumatoid Arthritis (RA) is a chronic inflammatory disease which is mainly associated with a symmetric pattern of polyarthritis mainly involving the small joints of the upper extremities¹. It is the

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Editor's Comment :

- Rheumatoid arthritis is the commonest form of chronic arthritis and needs early diagnosis to prevent complications.
- Anti-CCP antibody measurement, whenever combined with rheumatoid factor, enhances the diagnostic accuracy.
- Anti-CCP antibody much more specific than RF and higher value of this antibody indicates more severe disease.

commonest variety of chronic inflammatory arthritis and if not properly treated, may lead to deforming arthropathy. The estimated prevalence of RA is about 1% worldwide². The recent management of RA is mainly focused on the early and aggressive initiation of the Disease-Modifying Antirheumatic Drugs (DMARD) and biologics to limit the joint destruction and also to prevent the development of the extra-articular complications. As most of such drugs are associated with several adverse reactions, therefore, proper use of specific diagnostic tests for RA is of utmost importance³. The diagnosis of this auto-immune

disease is chiefly based on the inflammatory nature of the joint pain mainly involving the small joints of hands in a symmetric distribution with relative sparing of the distal inter-pharyngeal joints and elevation of the biochemical markers of inflammation combined with the presence of high titre of Rheumatoid Factor (RF).

Rheumatoid Factor is an Immunoglobulin-M (IgM) type of antibody directed against the Fc fraction of Immunoglobulin-G (IgG)⁴. Elevated levels of RF are seen in about 75-80% of the patients of RA, therefore, a negative result can not negate the possibility of RA. It is also found in 1-5% of the healthy population and in several other connective tissue diseases, such as Sjogren syndrome, systemic lupus erythematosus, mixed connective tissue disease, type-2 essential mixed cryoglobulinemia; and also in some chronic infective conditions like Hepatitis-B and C induced chronic liver disease and subacute bacterial endocarditis⁵. Furthermore, several studies are going on to differentiate "pathological" and "physiological" RF. This lack of sensitivity and specificity limits the usefulness of this serological marker of RA and necessitates the use of some other useful marker. Presence of serum anti-CCP antibodies has almost same or slightly higher sensitivity than RF, but the specificity is as high as 95%⁶. Therefore, its presence in early inflammatory arthritis helps to differentiate RA from the other chronic inflammatory conditions. According to recent data, Anti-CCP had an excellent specificity and a relatively high sensitivity for RA, especially for recent onset RA^{7,8}.

A hospital-based comparative study was conducted to determine the diagnostic value of anti-cyclic citrullinated peptide (anti-CCP) antibodies and rheumatoid factor in patients with Rheumatoid Arthritis and the study results were also compared with a group of patients of undifferentiated arthritis.

MATERIALS AND METHODS

Institutional ethics committee approval was obtained for this observational study. All patients were selected consecutively from outpatients being treated at the Calcutta National Medical College and Hospital. Serum samples were obtained from 133 patients with RA and 67 patients of Undifferentiated Polyarthritis (UPA) (157 women and 43 men, mean age: 56.7 years, range: 24–83 years, mean duration of the disease \pm SD: 8.2 \pm 10.5 years).

Approximately, 4-5 ml of venous blood was collected by aseptical methods from the patients of diagnosed RA and also from the control group. Serum was separated by appropriate technique (centrifugation) and vials were also marked carefully. Those samples were being stored at -80°C until the assay. Presence of Anti-CCP antibodies was detected by Enzyme-Linked

Immunoassay (ELISA), by using a commercial kit. Detection of Rheumatoid Factor (RF) was performed by Latex Agglutination slide test. A comparison between the results of those laboratory tests between study group and control group was performed by Chi-square test or the Fisher's exact test wherever applicable. Categorical variables are expressed as number and percentage of patients; they are compared across the groups by using Pearson's Chi-Square test for determining independence of Attributes. Continuous variables have been expressed as Mean \pm Standard Deviation and they are compared across the 2 groups using the Mann-Whitney U test since the data does not follow normal distribution. Statistical software SPSS version 20 has been used for the complete analysis. An Alpha level of 5% has been taken, ie, if any p value is less than 0.05 it has been considered as significant.

RESULTS

Presence of Anti-CCP antibodies were detected in 94 out of 133 patients with RA (70.7%) and RF was detected in 61 out of 133 patients with RA (46%). Of the 133 RA patients, 48 were Anti-CCP positive/RF positive, 46 were Anti-CCP positive/RF negative, 13 were anti-CCP negative/RF positive and 26 were anti-CCP negative/RF negative. In the group of 67 patients, of undifferentiated Polyarthritis (uPA) both Anti-CCP and IgM-RF tested was performed 6 were Anti-CCP positive/RF positive, 8 were anti-CCP positive/RF negative, 22 were Anti-CCP negative/RF positive and 31 were Anti-CCP negative/RF negative (Table 1).

DISCUSSION

The clinical diagnosis of RA is largely based on the presence of chronic inflammatory Arthritis with suggestive laboratory and radiologic abnormalities. The 1987 classification criterion for RA proposed by the American College of Rheumatology (ACR) was revised in 2010 by a collaborative effort between the ACR and European League Against Rheumatism (EULAR)⁹. In this revised criteria proposed by the ACR-EULAR, there

Table 1 — Test results at optimal cut-off values for the Anti-CCP and the IgM-RF ELISAs in patients with and without RA*

	No (%) of RA patients (n=133)	No (%) of undifferentiated Polyarthritis (uPA) patients (n=67)
CCP positive and		
RF Positive	48(36.09)	6(8.95)
RF Negative	46(34.6)	8(11.94)
CCP Negative and		
RF Positive	13(9.8)	22(32.83)
RF Negative	26(19.54)	31(46.26)

*The optimal cut-off value for the Anti-cyclic citrullinated peptide (Anti-CCP) enzyme-linked immunosorbent assay (ELISA) was 52 units; that for the IgM rheumatoid factor (IgM-RF) ELISA was 10 units. RA = rheumatoid arthritis.

is a point for positive Anti-citrullinated Peptide (anti-CCP) antibodies.¹⁰ This diagnostic test is much more specific than the widely used RF. The use of both of these markers may add some incremental value in diagnosing RA, as some patients of RA may be positive for anti-CCP antibodies but negative for RF and vice versa. Therefore, Anti-CCP antibodies should not be an alternative to RF for the serological diagnosis. Moreover, Anti-CCP antibodies estimation is helpful for prognostication, as higher level of this antibody in patients with RA indicate worse outcome¹¹.

Our study results indicated that Anti-CCP positivity correlated with RF positivity. However, there are a significant proportion of patients in our study who were negative for RF but positive for Anti-CCP antibody and vice versa.

Saroux A *et al* conducted a study to compare the diagnostic values of serological markers such as Anti-keratin Antibody (AKA), Anti-perinuclear Factor (APF), and anti-CCP antibodies for determination of diagnostic value of anti-CCP when performed alone or combined with other markers; and to diagnose Rheumatoid Arthritis (RA) by using it¹². They found that Anti-CCP (with a cut off value of 53 UI was 93% specific and 47% sensitive. On the other hand, the sensitivity of RF by Latex test was only 45%.

Another study conducted by Binesh F *et al* found the sensitivity of RF Latex test of only 46%, whereas the specificity was more than 90%¹³. Similar results were seen in another study conducted by Aflaky E *et al*¹⁴. They found the sensitivity of Anti-CCP, Citrullinated Protein Antibodies (CPA), and RF of 82, 83 and 61%, respectively; whereas specificities of those markers were 91, 79 and 90%, respectively.

Swedler W *et al* found that, the sensitivity and specificity of RF by ELISA was 91 and 75% respectively¹⁵. Slightly decreased sensitivity and higher specificity of RF IgM ELISA was observed in another study conducted by Bas S *et al*¹⁶.

The sample size of both the disease and the control arm were small. Due to relatively smaller sample size, randomization could not be performed. Our study findings indicate that, combining RF with anti-CCP antibody may provide a much higher sensitivity without compromising specificity for the diagnosis of RA.

Conflict of interest : None of the researcher has any financial or other interest in the products that were used for this study.

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