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Journal homepage: [www.Sjournals.com](http://www.Sjournals.com)**Original article****Estimation of serological marker for the diagnosis of rheumatoid arthritis****S.S.Haque<sup>a,\*</sup>, S. Kumar<sup>b</sup>, M. A.Muzaffar<sup>c</sup> R. Kumari<sup>d</sup>, U. Kumar<sup>e</sup>, A. Saran<sup>f</sup>, M.D.Tanweeruddin<sup>g</sup>  
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## ABSTRACT

Rheumatoid arthritis (RA) is a chronic auto-immune disease that affects multiple joints. The most promising candidates are the autoantibodies to antigens containing one or more than one citrulline residues (cyclic citrulline peptides, CCP) - the anti-CCP antibodies apart from C-reactive protein. They play an important role in the diagnosis, prognosis and therapeutic approach to patients with RA. Their high specificity, the ability to diagnose RA early in its development and distinguish it from other nonerosive type of arthritis, make the anti-cyclic citrullinated peptide antibody (ACCP) a key serologic marker. The aim of this study was to investigate the role of Anti-CCP in addition to C-reactive protein (CRP) for the diagnosis and therapeutic management of RA. Anti-cyclic citrullinated peptide antibodies were determined quantitatively by Enzyme-Linked Immunosorbent assay (ELISA) and serum C-reactive protein detected using Avitex CRP kit, which is a rapid latex agglutination test. The results showed a statistically significant anti-CCP levels in serum of patients with rheumatoid arthritis ( $p < 0.001$ ). CRP test was found to be positive in 37/40 cases of RA and none of the controls. Conclusion, anti-CCP assay can be a reliable, sensitive and specific test, and CRP is an important inflammatory marker for rapid diagnosis of rheumatoid arthritis.

## 1. Introduction

Rheumatoid arthritis (RA) is one of debilitating inflammatory autoimmune disorder characterized by chronic proliferative synovitis that leads to ultimately bone destruction (Van Boekelet al.,2001) RA appears in Europe only in the early 17th century, when Sydenham published a case report in 1676, and was fully described by Garrod in 1859 and named 'rheumatoid' arthritis to distinguish it from rheumatic fever and gout, two recognised forms of arthritis (Firestein et al.,2003). The main cause of RA is unknown, however autoimmune mechanisms have been involved. The genetic factor predisposes to the disease, but also person-related and environmental factors, such as age, gender, infectious agents, smoking and dietary factors are thought to play a role in the disease pathogenesis. In clinical practice, RA is diagnosed respecting revised criteria of ACR (American College of Rheumatology) from 1987 and must last at least for 6 weeks (Popović et al., 2000). ACR criteria for RA are mostly used as a "golden standard" in RA diagnosing although they have certain limitation for early diagnosing of RA. At the same time, those are the reasons why it is necessary to find a new "golden standard" that would be less dependent on RA clinical symptoms (Popović et al., 2000). Anti-cyclic citrullinated peptide antibody (Anti-CCP) antibodies are often detectable in early RA. Anti-CCP levels are not useful in the longitudinal monitoring of disease activity. Anti-CCP antibodies are potentially important markers for diagnosis and prognosis in rheumatoid arthritis (RA), due to their sensitivity around 50% to 75% and more specificity usually over 90 % (Schellekens et al., 1998, Vincent et al., 2002) than, IgM rheumatoid factors (RF) in early and fully established disease and may be detected in healthy individuals years before onset of clinical RA.

Another potential marker for increased risk of RA may be C-reactive protein (CRP), since CRP is a sensitive marker of systemic inflammation and is elevated in patients with RA Tishler et al., 1985 and Otterness et al.,1994). My aim of the study is to investigate the role of Anti-CCP level along with CRP for the diagnosis of RA.

## 2. Subjects and Methods

The study involved 80 subjects who were divided into two groups. The control group consisted of 40 healthy subjects (28 women and 12 men) with an average age of 55.38 years, who were from 35 to 70 years old; they also did not have family history of rheumatoid arthritis and they were not medically treated. From the remaining 40 subjects were diagnosed with rheumatoid arthritis. All the subjects were diagnosed with RA by specialized rheumatologists. Criteria for involving the patients in the study and for their exclusion were the revised ACR criteria from 1987.

Blood samples were collected from eighty patients who were attending to Indira Gandhi Institute of Medical Sciences Patna teaching hospital from January 2013 to August 2013. Sera were separated and stored at -20 OC until use.

### 2.1. Methods

Antibodies to cyclic citrulline peptide (CCP) were measured by Enzyme-Linked Immunosorbent assay (ELISA).

C - Reactive protein Detection, For the detection of CRP in serum, Avitex -CRP kit was used which is a rapid latex agglutination test. The test is based on the principle that Avitex- CRP latex particles are coated with antibodies to human CRP, i.e. when the latex suspension is mixed with serum containing elevated CRP levels on a slide; clear agglutination is seen within 2 minutes. Avitex -CRP has detection limit of 6 mg/ litre of CRP in the patient's serum. The test is considered as positive when the CRP serum concentration is above 6mg/litre and negative when it is at 6 mg / litre and below.

Statistical Analysis, The data of the study subjected to statistical analysis is expressed as mean  $\pm$  SD. Statistical comparisons were performed by Student 't' test.

## 3. Results

Form 40 patients, 28 (70%) of them were women while only 12 (30%) of who were men. The mean age of the patients was  $53.84 \pm 9.05$ .

A mean Anti-CCP value in serums of the control group was  $X = 17.57 \pm 2.07$  U/L. As shown in table 1 results indicated that there is a significant differences between RA patients ( $12.38 \pm 32.60^*$ ) and control group ( $0.035 \pm 0.019$ ).

CRP levels estimated in the RA patients and controls are presented in table 3. In the present study 37/40 cases of RA were found to be positive to CRP while all of controls was negative for the test. Serum dilutions were performed to detect the titer of CRP in all positive cases.

**Table 1**

Sex and mean age of rheumatoid patients

Groups	Number of subjects(N)	Mean $\pm$ SD
Age		
1. Male	12	53.84 $\pm$ 7.10
2. Female	28	54.36 $\pm$ 7.53

**Table 2**

Serum Anti-CCP in patients with Rheumatoid Arthritis and Controls.

Anti-CCP	Number of subjects(N)	Positive Cases
RA patients	40	12.38 $\pm$ 32.60*
Controls	40	0.035 $\pm$ 0.019

Significant at \* $p < 0.001$ .

**Table 3**

Serum C-reactive protein in patients with rheumatoid arthritis and controls

Groups	No. of cases	No of cases positive for CRP
Rheumatoid arthritis Patients	40	37
Controls	40	Nil

In this study, significant difference was found between the mean value of serum Anti-CCP among RA patients when compared with the control group (table 2) and this may be due to increase activity of Anti-CCP, which may be detected in healthy individuals years before onset of clinical RA.

#### 4. Discussion

In RA, immunological studies play an important role, such as testing for the presence of C-reactive protein and anti-cyclic citrullinated peptide (Anti-CCP). Anti-CCP was the first autoantibody detected in patients with RA. It was discovered in the early twentieth century and became the primary serological test used in the diagnosis of RA (Renaudineau et al., 2005). In RA patients autoantibodies identified which are specific for epitopes containing the unusual amino acid citrulline that is generated by post-translational deimination of arginyl residues by the enzyme peptidyl arginine deiminase. Autoantibodies recognizing citrullinated epitopes are now generally named anti-citrullinated protein antibodies (Anti-CCP). Citrullinated epitopes were first identified in filaggrin, a protein expressed exclusively in squamous epithelial cells. Interestingly, filaggrin forms the target of antikeratin antibodies that had been described previously to be highly specific for RA (Youinou and Serre, 1995). Since filaggrin is not expressed in the joint, it presumably represents a cross-reacting antigen rather than the primary target structure of Anti-CCP.

One attractive candidate antigen is fibrin and its precursor fibrinogen, since citrullinated forms of these proteins have been demonstrated to be present in the synovial tissue of patients with RA and other arthritides.

Citrullinated fibrinogen appears to be a dominant B cell antigen that is targeted by the majority of RA patients with established disease (Vincent et al., 2005).

C-reactive protein (CRP) is acute-phase marker of inflammation produced by liver. It helps in the disease progression. In addition, CRP determination is easy to perform and of low cost, making it the preferred biomarker of disease activity and play a pivotal role in pathogenesis of rheumatoid arthritis. In the present study the levels of C-reactive protein were significantly high in patients compared to controls and high values of CRP indicates of active inflammation in RA patients.

## 5. Conclusions

For estimation anti-citrullinated protein antibodies and C-reactive protein in patients suspected with rheumatoid arthritis are recommended because anti-citrullinated protein antibodies (ACPA) have been established as the most specific serological marker antibodies for RA, since a positive result for either test increase diagnostic sensitivity and high specificity.

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