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Original Article

Orthopaedic Section

Anti-Cyclic Citrullinated Peptide Antibody: An Early Diagnostic and Prognostic Biomarker of Rheumatoid Arthritis

D. MANIVELAVAN, VIJAYASAMUNDEESWARI C.K.

ABSTRACT

Objectives: To evaluate the role of Anti-Cyclic Citrullinated Peptide (anti-CCP) antibody and Rheumatoid Factor (RF) in Rheumatoid Arthritis (RA) patients.

Methods: The present study comprised of 60 clinically diagnosed rheumatoid arthritis patients and 30 apparently healthy subjects as controls. Among 60 RA patients, 30 were <2 years duration and 30 were 3 to15 years duration. Anti-CCP and RF levels were analyed by ELISA and immunoturbidimetric assay respectively. Disease activity was assessed by disease duration, duration of morning stiffness, hand deformity and radiological findings. Anti-CCP and rheumatoid factor were measured.

Result: A valid comparison showed that autoantibodies directed to citrullinated antigen–anti-CCP superior to RF for the detection of RA. Anti-CCP antibodies have an independent role in predicting radiological damage and progression in RA patients.

Conclusion: With their excellent specificity, anti-CCP antibodies used as serological marker in establishing the diagnosis of RA. Anti-CCP antibodies discriminated accurately between erosive and nonerosive RA making them a potentially good prognostic marker for the disease.

Key Words: Rheumatoid arthritis, Anti-cyclic citrullinated peptide antibodies, Rheumatoid factor

INTRODUCTION

Rheumatoid arthritis is a systemic inflammatory disease [1,2,3] affect nearly 0.75% of adult Indian population frequently characterized by circulating autoantibodies. It is characterized by multiple deformities and is associated with considerable morbidity and mortality [4]. Although the precise aetiology of RA remains unknown, there is a strong evidence for autoimmunity [5] since several autoantibodies are associated with disease.

RA is diagnosed primarily according to ACE [6] criteria which are based mainly on clinical manifestations and serological support. The only serological marker included in the criteria is RF. Rheumatoid factor, an antibody directed against the constant region of IgG is elevated in 75% of patients with RA [7,8,9,10]. However its diagnostic specificity for RA is poor, since RF is also found in many other rheumatic, non-rheumatic disease and in elderly healthy individuals [11,12]. In addition to RF, autoantibodies targeting cyclic citrullinated peptide are commonly observed in the serum of patients with RA. These antibodies are known as anticyclic citrullinated peptide antibodies. Because of their early presence and high specificity [13] Anti-CCP antibodies represent a superior marker for the diagnosis and prognosis of RA, in contrast to RF, which has only modest disease specificity.

A good marker should ideally not only indicate the development of the disease but also be able to predict its erosive or non-erosive progression. The serological parameter that meets these requirements is Anti-CCP antibody. These autoantibodies bind antigenic determinants that contain unusual amino acid citrulline. Citrulline is a nonstandard amino acid as it is not incorporated into proteins during protein synthesis [8]. It can, however, be generated via post translational modification [14]. Citrullination or deimination is an enzyme- catalysed process in which the positively charged NH2 – group of amino acid arginine is hydrolyed to a neutral oxygen group. It is this oxygen group of peptidylcitrulline that is specifically recognized by autoantibodies in RA. The citrulline residues are essential part of the antigenic determinants recognized by the RA antibodies. So Anti-CCP testing is particularly useful in the diagnosis of RA with high specificity present early in the disease process [1,11] and ability to identify patients who are likely to have severe disease and irreversible damage [4]. The high specificity of Anti-CCP can be valuable in distinguishing RA from other diseases which are clinically very similar to RA in its early stages and in which RF positivity is relatively frequently observed.

MATERIALS AND METHOD

This cross sectional, case control study was carried out in Rheumatology Clinic, Vinayaka Missions Kirupanandha Variyar Medical College Hospital, Salem, Tamilnadu, India from Sep 2011-April 2012.The study protocol was approved by the ethical committee of the institution. The patients gave written informed consent to participate in the study.

Group I: Comprises of 30 age matched apparently healthy subjects as controls.
Group II: Comprises of 30 RA patients <2 years duration.
Group III: Comprises of 30 RA patients of 3 to 15 years duration.

At inclusion, the patients fulfilled either 4 of 7 American criteria and also all the patients had first 4 criteria for at least 6 weeks

The American College of Rheumatology 1987-revised criteria for the classification of Rheumatoid arthritis [6]

1. Morning stiffness at least one hour before maximal improvement.
2. Arthritis of three or more joint areas.
3. Arthritis of hand joints.

4. Symmetric arthritis.
5. Rheumatoid nodules.
6. Rheumatoid Factor (RF) positivity.
7. Radiographic changes on hand and wrist radiographs (erosions or decalcification).

A patient will be said to have rheumatoid arthritis if he/she has satisfied at least four of these seven criteria. Criteria 1 to 4 must have been present for at least 6 weeks.

All patients underwent clinical evaluation by the physician and the following data were recorded; age, gender, disease duration, duration of morning stiffness, and presence of hand deformity. Anti-CCP, RF and ESR were estimated. Antero Posterior radiographs of hand (including wrist) and feet were taken to assess the erosive changes. Radiological progression was evaluated by the modified Larsen score.

In this study, we have evaluated the sensitivity and specificity of Anti-CCP and RF in RA patients with <2 years duration and RA patients with 3 to 15 years disease duration. We have also determined the role of Anti-CCP, RF in RA and their correlation with radiological findings.

Serology: Anti-CCP antibodies were analyzed by 3rd generation ELISA (CCP3, INOVA DIAGNOSTICS, and UNITED STATES OF AMERICA) according to the manufacturer's instruction. An ELISA with third generation CCP3 shows 5% greater sensitivity at detecting RA patients than the second generation CCP2, while maintaining a very high specificity [15]. Sera were stored at –20ºC until use. 100µl of Anti-CCP calibrators (250, 125, 62.5, 31.2, 15.62 units), controls and patient samples were distributed into appropriate wells. The microtitre plates were coated with highly purified synthetic cyclic citrullinated peptide. After incubation for 30 minutes at room temperature, the wells were washed three times with 300µl of wash buffer. The microplates were then incubated for 30 minutes at room temperature with HRP CCP immunoglobulin conjugate and washed again three times. A chromogenic substrate (TMB) was added to each well. After 30 minutes of incubation, the reaction was stopped using HRP stop solution. Bichromatic measurements were done at 450nm and 620nm on Bio-Rad ELISA reader. Serum Anti-CCP concentrations were calculated according to the standard curve and results were expressed as units RF was analyzed by immunoturbidimetric assay. Erosive changes are scored by modified Larsen score. Larsen has introduced the following guidelines for scoring.

Scores for the thumbs and 1st MTP were deleted, the wrist was divided into four quadrants (therefore, the joints considered are PIP 2 to 5 and MCP 2 to 5 in each hand, 4 quadrants in the wrist, and MTP 2 to 5 in each foot), soft tissue swelling and osteoporosis were omitted and distinction was made between erosions of different sizes. The grading scale range from 0 to 5. 0 = intact bony outlines and normal joint space, 1 = erosion less than 1 mm in diameter or JSN, 2 = one or several small erosions (diameter more than 1 mm), 3 = marked erosions, 4 = severe erosions (usually no joint space left and the original bony outlines are only partly preserved), and 5 = mutilating changes (the original bony outlines have been destroyed). The score range from 0 to 160 [16].

STATISTICAL ANALYSIS

Results are expressed as mean ±SD. statistical comparison between the two groups carried out using Student's t test. P value less than 0.05 was considered significant. All statistical tests were analyzed using SPSS software.

RESULTS

Total of 60 patients with RA and 30 apparently healthy subjects as controls (group I) were included in this study. Among 60 RA patients, 30 patients were <2years duration form group II and 30 patients were 3 to 15 years duration form group III.

[Table/Fig-1,2 & 3] shows the percentage distribution of Anti-CCP and RF in group I, group II and group III respectively.

[Table/Fig-4] shows the sensitivity and specificity of Anti-CCP and RF tests in group II vs. group I. In group II patient's 83% sensitivity and 96% specificity were observed for Anti-CCP test and 70% sensitivity and 86% specificity were observed for RF test.

[Table/Fig-5] shows sensitivity and specificity of Anti-CCP and RF tests in group III vs group I. In group III patients 93% sensitivity and 96% specificity were observed for Anti-CCP test. 76.6% sensitivity and 86% specificity were observed for RF test.

Sensitivity for Anti-CCP test was significantly higher than that RF test in group II and also in group III but the sensitivity for Anti-CCP were higher in group III compared to group II. Data here confirm that the presence of Anti-CCP is a specific marker for diagnosing RA.This observation correlates well with Suzuki et al. [2].

	Anti-CCP+ve 1 (3.33)	Anti-CCP-ve 29 (96.67)
RF+ve 4(13.33)	0 (0.00)	4 (13.33)
RF-ve 26(86.67)	1 (3.33)	25 (83.33)

[Table/Fig-1]: Cross Tabulation of Anti CCP and Rheumatoid Factor in group I
Results are shown as No (%)

	Anti-CCP+ve 25 (83.33)	Anti-CCP-ve 5 (16.67)
RF+ve 21(70.00)	18 (60.00)	3 (10.00)
RF-ve 9(30.00)	7 (23.33)	2 (6.67)

[Table/Fig-2]: Cross Tabulation of Anti-CCP and Rheumatoid Factor in group II
Results are shown as No (%)

	Anti-CCP+ve 28 (93.33)	Anti-CCP-ve 2 (6.67)
RF+ve 23(76.67)	23(76.67)	0(0.00)
RF-ve 7(23.33)	5(16.67)	2(6.67)

[Table/Fig-3]: Cross Tabulation of Anti-CCP and Rheumatoid Factor in group III
Results are shown as No (%)

	RA Patient (Group II) (N=30)	Controls (Group I) (N=30)	P Value
Anti-CCP			
Positive	25(83.33)	1(3.33)	
Negative	5(16.66)	29(96.66)	<0.0001
RF			
Positive	21(70)	4(13.33)	
Negative	9(30)	26(86.66)	<0.0001

[Table/Fig-4]: Comparison of Anti-CCP and RF reactivities in the study groups II and I

	Patient (Group III) (N=30)	Controls (Group I) (N=30)	P Value
Anti-CCP			
Positive	28(83.33)	1(3.33)	
Negative	2(6.66)	29(96.66)	<0.0001
RF			
Positive	23(76.66)	4(13.33)	
Negative	7(23.33)	26(86.66)	<0.0001

[Table/Fig-5]: Comparison of Anti-CCP and RF reactivities in the study groups III and I

The results were found to be statistically significant (p<0.0001) when Anti-CCP in group II and group III were compared with group I and statistical significance (p<0.0001) were also observed when RF in group II and group III were compared with group I.

Modified Larsen scores were calculated to assess joint destruction. For disease duration <2yrs (group II) the average Larsen score was 71 for Anti-CCP+ve,RF+ve RA patients, 69 for Anti-CCP+ve, RF-ve and 11 for Anti-CCP-ve, RF+ve. For disease duration 3-15 yrs (group III) the average Larsen score was 106 for Anti-CCP+ve, RF+ve RA patients, 91 for Anti-CCP+ve, RF-ve and 17 for Anti-CCP-ve, RF+ve.

Erosive changes quantitated by Larsen score. Anti-CCP+ve and RF+ve shows more erosive changes whereas Anti-CCP-ve and RF-ve shows no erosive changes. Erosive changes were also more in Anti-CCP+ve and RF-ve group II and group III RA patients. Minimal erosive changes were seen in Anti-CCP-ve and RF+ve of group II and group III. Larsen score in group III were high compared to group II suggesting that erosive changes more in RA patients as the duration of the disease increases which correlates well with the findings of S. Bas et al. [17].

DISCUSSION

Rheumatoid arthritis is a systemic autoimmune disease characterized by progressive joint damage reflected in radiographs by bony erosion and as joint space narrowing [4]. Since structural joint damage is irreversible, early recognition and treatment are currently being emphasized, with the goal of halting progression of the disease [18].

Recent studies have indicated that post-translational modification of proteins could be important in the initiation of autoimmune disease such as rheumatoid arthritis. Citrullination–conversion of peptidyl arginine to peptidyl citrulline by peptidyl arginine deaminase [12,19], induces the appearance of recently discovered autoantibody system, which can be measured efficiently by using cyclic citrullinated peptides as antigens. The high specificity of Anti-CCP antibodies for RA supports the importance of a modification process such as citrullination early in the development of the disease [14].

In the present study in group II and III, the sensitivity of Anti-CCP test were significantly higher compared to the sensitivity of RF tests. Previous multicentre studies showed that Anti-CCP antibodies present in about 80% of established RA patients and in 1% of healthy controls [9]. Anti-CCP antibody negativity does not exclude the diagnosis of RA. These findings correlates well with our findings.

Anti-CCP has high sensitivity for the diagnosis of rheumatoid arthritis and a very high specificity making this antibody more useful than RF.

The studies by Vencovsky et al., [20] Van jaarsveld et al., Kroot et al., and Visser et al., [11] support the idea that RA patients positive for Anti-CCP develop significantly more severe radiological damage than Anti-CCP negative patients [21], which correlate well with our findings.

CONCLUSION

Anti-CCP antibody is more sensitive and specific marker in the diagnosis of RA than RF. Our study provides good evidence of association of Anti-CCP with radiological joint damage and progression. The use of Anti-CCP in clinical practice contribute to enhance the ability of rheumatologists to make judicious treatment decision.

LIMITATION

This study was performed in a small group of patients with rheumatoid arthritis. These markers deserve to be evaluated further in a larger population of patients in order to determine their usefulness as diagnostic and prognostic marker.

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AUTHOR(S):
1. Dr. D. Manivelavan
2. Dr. Vijayasamundeeswari C.K.

PARTICULARS OF CONTRIBUTORS:
1. Assistant Professor of Orthopaedics, Vinayaka Mission's Kirupananda Variyar Medical College and Hospital, India.
2. Assistant Professor of Biochemistry, Vinayaka Mission's Kirupananda Variyar Medical College and Hospital, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Vijayasamundeeswari C.K.
D5, 259,Thoppukadu, Alagapuram Puthur,
Salem - 636016, Tamiladu, India.
Phone: 09894894445
E-mail: drvijimani@gmail.com

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