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Original Article

Microbiology Section

Diagnostic utility of Anti-cyclic Citrullinated Peptide Antibodies for Very Early Rheumatoid Arthritis

SUCILATHANGAM G, ANNA T, VELVIZHI G

ABSTRACT

Background and Objectives: Rheumatoid arthritis (RA) is a systemic autoimmune disorder characterized by widespread joint inflammation with synovial hyperplasia. The most well known diagnostic marker is rheumatoid factor (RF).However; RF is not a good marker for RA, because it can be detected in autoimmune disorders other than RA as well as in 3-5% of healthy individuals. Recent studies indicated that Anti-CCP testing is particularly useful in the diagnosis of RA with high specificity present early in the disease process. Therefore, we aimed in our study to evaluate the diagnostic performances of anti-cyclic Citrullinated peptides antibody (anti-CCP) ELISA and Rheumatoid Factor in a group of patients with RA and non RA patients in Tirunelveli.

Materials and Methods: This prospective study, which was carried out for a period of six months in a tertiary care hospital. Blood was collected with informed consent in 65 RA patients and 30 non RA patients and serum was subjected to Rheumatoid Factor latex kit (AGAPPE Diagnostics, Kerala) and anti-CCP antibody detection by Enzyme Linked Immunosorbent assay (Euroimmun, Germany) with the cut-off at 8 IU/ml and 5 RU/ml respectively. The cut-off values recommended by the respective manufacturers were used to determine the sensitivity and specificity.

Results: This study shows that Sensitivity of anti-CCP was 58.3% with better specificity (100%) in RA patients whereas Sensitivity of anti-CCP was 50% with specificity (89.3%) in non RA patients. The combination of Rheumatoid factor (RF) and antibodies against cyclic Citrullinated provide better specificity to anti-CCP assay.

Conclusion: Anti-CCP assay could be a useful serological test for the diagnosis of patients with early stages of RA, because anti-CCP revealed higher diagnostic specificity than RF. The high specificity of anti-CCP antibodies is particularly useful in RF-negative RA patients. Anti-CCP antibodies are more specific than RF for diagnosing rheumatoid arthritis. In Sudanese patients with RA, anti-CCP antibodies exhibit a better diagnostic value than RF.

Keywords: Anti-cyclic Citrullinated peptides antibody ELISA, Rheumatoid arthritis, Rheumatoid factor latex kit, Sensitivity, Specificity

INTRODUCTION

Rheumatoid arthritis (RA) is a common lifelong rheumatic disease of uncertain aetiology with significant morbidity and mortality. It is one of the most common autoimmune diseases affecting 0.5–1% of the population. The disease is diagnosed on basis of a set of clinical, serological and radiological criteria. With significantly improved therapy options now available, early treatment could be shown to prevent irreversible joint damage reducing signs and symptoms of erosion and improving physical function. At the same time, an early and accurate diagnosis may protect other patients who do not have RA, from aggressive therapies with potential toxicity [1].

Historically, rheumatoid factor (RF) has long been the serological indicator for RA. Rheumatoid factor is an antibody directed against the Fc region of IgG that has been used as a diagnostic marker for RA. However, it is non-specific and may be present in healthy elderly persons or in patients with other autoimmune and infectious diseases [2]. In India, the routine serological marker for the diagnosis of RA is the RF, which may lead to missing of some individuals with early undifferentiated arthritis.

In 1998, Schllekens et al., showed that citrullin is an essential constituent of antigenic determinant, recognized by the above-mentioned RA specific auto-antibodies [3]. This discovery led to the development of a new serological test so called anti-cyclic Citrullinated peptide antibody (anti-CCP) (ELISA) test and proved to be more helpful to diagnose RA [4].

Anti-cyclic Citrullinated peptide (anti-CCP) antibody testing is particularly useful in the diagnosis of rheumatoid arthritis, with high specificity, presence early in the disease process, and ability to identify patients who are likely to have severe disease and irreversible damage.

Despite overwhelming evidence of its specificity, anti-CCP antibody detection for the diagnosis of RA is not requested on a regular basis in our country probably due to the lack of

comparative studies or the non-availability of this test in routine laboratories. This study aims to assess the diagnostic value and usefulness of anti-CCP antibodies assay in distinguishing RA from other rheumatic disorders in comparison with RF and thus find the sensitivity and specificity of anti-CCP antibodies in the diagnosis of early RA.

MATERIALS AND METHODS

Study design and settings

This prospective study was carried out in Orthopaedics Department, Tirunelveli Medical College hospital, TamilNadu, India from April 2013 to September 2013.The study protocol was approved by the ethical committee of the institution. The patients gave written informed consent to participate in the study.

Study population

Patients and controls

The study population comprised of adult RA patients who fulfilled the American College of Rheumatology (ACR) criteria as cases and patients with symptoms of arthritis or arthralgia as controls.

Inclusion criteria

1. Patients who fulfilled the ACR criteria
2. Age of 18 years old or above

Exclusion criteria

1. Patients who were already been diagnosed or treated for Sarcoidosis, Systemic Lupus Erythematosus and Sjogrens syndrome.
2. Pregnant women

Sample collection

After obtaining approval from the ethical committee, the samples were collected from 95 patients, of which 31 are male patients and 64 of them are female patients. Most of them have early disease (less than a year). Sera from patients with RA and controls were stored at - 20°C until the assay.

Laboratory procedures

Test Procedure for RF Latex Agglutination Test: RF is a rapid agglutination procedure for the detection and semi-quantitation of Rheumatoid Factor (RF). The antigen, a latex particles suspension coated with human gamma globulin, agglutinates in presence of rheumatoid factors in the patient serum. RF was determined qualitatively and Semi quantitatively in all samples using latex kits (AGAPPE Diagnostics, Kerala). RF (IgG) was measured in IU/ml according to the manufacturer's instructions. Results were considered to be positive at a cut-off value of 8 IU/ml.

Anti - CCP ELISA test

Determination of the concentration of anti-CCP antibodies was performed using anti- CCP IgG ELISA kit (EUROIMMUN, Germany) in which synthetic cyclic Citrullinated peptides (CCP) containing modified arginine residues are used as the antigen for the detection of anticitrullinated protein antibodies. The assay was performed according to the manufacturer's instructions. Anti-CCP antibodies were measured in RU/ml and were considered to be positive at a cut-off value of 5RU/ml.

RESULTS

We analyzed data from 95 patients for whom RF was tested, of which 31 are male patients and 64 of them are female patients. RA patients were recruited according to ACR criteria. Among the 65 RA patients, 16 are males and 49 are females whereas 15 were males and 15 were females among 30 non-RA patients.

In this study, the number of RF positive cases is 10, of which 4 were males and 6 were females. Number of Anti-CCP positive cases is 14 of which 4 were males and 10 were females. Number of cases both positive for rheumatoid factor and Anti-CCP is 7 of which one was male and six were females. Females have a greater predisposition for Rheumatoid arthritis than males. Positivity for RF and Anti-CCP test was greater for women than for men.

Total of 95 patients have been recruited in this study, among them there are 65 RA patients who were < 2years duration and 30 non RA patients. Below [Table/Fig-1,2] shows the percentage distribution of Anti-CCP and Rheumatoid factor in the 65 Rheumatoid arthritis patients and the 30 non Rheumatoid arthritis patients. Sensitivity and specificity of Anti-CCP in comparison with RF is 58.3% and 100% in RA group whereas Sensitivity and specificity of Anti-CCP in comparison with RF is 50% and 86.3% in Non RA Group.

In the RA group, 12 sera were positive for anti-CCP at ≥5 units. In the non-RA group, only 2 sera were positive. The sensitivity and specificity were 18.54% and 93.3% respectively. In the RA group, 7 sera were positive for RF at ≥8 IU/ml.In the non-RA group,3 sera were positive for RF at ≥8 IU/ml. The sensitivity and specificity were 10.77%. and 90% respectively. [Table/Fig-3,4].

DISCUSSION

Rheumatoid arthritis is a systemic autoimmune disease characterized by chronic inflammation of synovial joints, which leads to destruction of cartilage and bone and eventually to disability of the patient [5]. Though not directly life threatening, RA severely affects the quality of life of a patient and also has major economic consequences for society. Therefore, every attempt should be made to prevent the erosive processes to occur.

Currently, the classification of RA relies mainly on the criteria described by the American College of Rheumatology (ACR) [6]. These criteria, originally formulated 50 years ago and last adjusted in 1987, are based mainly on clinical parameters. Since these parameters are often only sufficiently fulfilled when the damaging effects of the inflammatory process are already in progress, this set of criteria is not very suitable for the early diagnosis of RA [7].

	Anti-CCP + ve 12 (18.46%)	Anti-CCP –ve 53 (81.54%)
RF + ve 7(10.77%)	7 (10.77%)	0 (0%)
RF –ve 58 (89.23%)	5 (7.69%)	53 (81.54%)

[Table/Fig-1]: Cross Tabulation of Anti CCP and Rheumatoid Factor in RA Group

	Anti-CCP + ve 2 (6.66%)	Anti-CCP –ve 28 (93.34%)
RF + ve 3 (10%)	0(0%)	3(10%)
RF –ve 27(90%)	2 (6.66%)	25(93.34%)

[Table/Fig-2]: Cross Tabulation of Anti CCP and Rheumatoid Factor in non RA Group

	RA patients	Non RA patients	P value
Anti-CCP Positive Negative	12 (18.46%) 53 (81.54%)	2 (6.66%) 28 (93.34%)	<0.05
RF Positive Negative	7(10.77%) 58 (89.23%)	3 (10%) 27(90%)	>0.05

[Table/Fig-3]: Comparison of Anti-CCP and RF reactivities in RA and non RA Groups

	Sensitivity	Specificity	PPV	NPV
Anti-CCP	18.54%	93.3%	85.7%	34.6%
RF	10.77%	90%	70%	31.8%

[Table/Fig-4]: Sensitivity and Specificity of Anti-CCP and RF.

Because RA patients at their first visit to the clinician often do not fulfil the criteria for the diagnosis/classification of RA, an early detectable, highly predictive marker would greatly help the clinician in reaching a diagnosis. Obviously, the sensitivity and specificity of such a marker should be as high as possible.

In recent years it has become clear that early aggressive treatment in rheumatoid arthritis (RA) reduces joint damage and improves function [5]. To use a potentially toxic therapy as early as possible we require an accurate diagnosis of RA and also information about prognosis in an individual patient. Apart from clinical features, auto antibodies progressive joint damage reflected in radiographs by bony erosion and as joint space narrowing. Since structural joint damage is irreversible, early recognition and treatment are currently being emphasized, with the goal of halting progression of the disease.

Anti-Citrullinated peptide antibody (ACPA) assays, developed and commercialized in the past decade, are now being employed clinically. Since ACPA are present before the onset of RA symptoms and are predictive of RA development, they are a valuable diagnostic test early in the course of the disease [8].

In the present study, the diagnostic performance of anti-CCP sera obtained from patients with RA and non RA group showed the anti-CCP sensitivity (58.3% vs. 50%) and specificity (86.3% vs. 100%), respectively. This result was similar with Sthaneshwar et al., reported the diagnostic sensitivity and specificity of anti-CCP (65%, 96%) [9], Manole [10], et al., showed that the diagnostic sensitivity and specificity of anti-CCP (69%, 99%) [10]. Serdaroflu et al., find that diagnostic sensitivity and specificity of anti-CCP (65%, 98%) [11]. The positivity rate of anti-CCP was greater for women than for men in contrast to Choi et al., study reported no significant difference between males and females [12].

In this study, (7.69%) RF negative RA patients were anti-CCP positive these result was lower than Sthaneshwar et al., (28%) [9] and Mobini M et al., (33%) [13]. This suggests that in anti-CCP could be an additional diagnostic marker for RA, especially in RF negative patient.

In a meta-analysis published in 2007 it was found that anti-CCP antibody displays sensitivities comparable to that of RF (approximately 80%) but with superior specificity (98%) [6]. In the current study, the sensitivity of anti-CCP antibody (18.54%) was comparable with that of RF (10.77%) but the specificity was much better 93.3% in contrast to RF (90%). The PPV and NPV was 85.7% and 34.6% for anti-CCP in contrast to RF which was 70% and 31.8% respectively. In this study, the sensitivity (probability that a test result will be positive when the disease is present (true positive rate)) is low compared to other studies since most patients are in a very early stage (<2 years), so even though the disease is present the test may be negative since they may become positive only at a little later. So, Anti-CCP provides a path for early detection of Rheumatoid arthritis cases and is also more specific, as it does not show positive results for non-RA, RF positive patients.

Measuring both RF and anti-CCP is clinically useful because there is an increase in sensitivity when either is positive and an increase in specificity when both are positive, compared to either marker alone. If a new set of criteria is made to classify early RA, anti-CCP should be included because it would increase the diagnostic power of serologic tests compared to RF alone.

CONCLUSION

Anti-CCP antibody is a more specific marker in the diagnosis of Rheumatoid arthritis than RF. Anti-CCP antibody is also an early marker of rheumatoid arthritis and may help early diagnosis before permanent joint damage sets in. The use of Anti-CCP in clinical practice contributes to enhance the ability of rheumatologists to make judicious treatment decision.

REFERENCES

[1] O'Dell JR.Rheumatoid arthritis: the clinical picture. In: Koopman WJ, Moreland LW, eds. Arthritis and Allied Conditions. A Textbook of Rheumatology. 2005. pp. 1165–94. *Lippincott Williams & Wilkins, Baltimore, MA*.

[2] Dörner T, Egerer K, Feist E, Burmester GR. Rheumatoid factor revisited. *Curr Opin Rheumatol.* 2004; 16:246-53.

[3] Schellekens GA, de jong BA, van den Hoogen FH, et al. Citrullin is an essential constituent of antigenic determinants recognized by RA, specific autoantibodies.J *Clin Invest.1*998; 101:273–78.

[4] Schellekens GA, Visser H, de jong BA, et al. The diagnostic properties of RA antibodies to cyclic citrullinated peptide. *Arthritis Rheum* 2000; 43:155–63.

[5] Feldmann M, Brennan FM, Maini RN. Rheumatoid arthritis. *Cel*l 1996; 85:307–10.

[6] Arnett FC, Edworthy SM, Bloch DA, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988; 31:315–24.

[7] Visser H, le Cessie S, Vos K, et al. How to diagnose rheumatoid arthritis early: a prediction model for persistent (erosive) arthritis. *Arthritis Rheum* 2002; 46:357–65.

[8] Rantapaa-Dahlqvist S. Diagnostic and prognostic significance of auto antibodies in early rheumatoid arthritis. *Scand J Rheumatol.* 2005; 34(2):83–96.

[9] Sthaneshwar P., Sokkalingam S., Zain A and Sook-Khuan C.Analytical and diagnostic performance of an automated anti-CCP assay: *Malaysian J Pathol.* 2011;33(2):101-06.

[10] Manole1C., Inimioara M., Lucia M., Isabela S.,Minerva1 G., Study of Anti-cyclic Citrullinated Peptides anti-bodies in early rheumatoid arthritis patient., *Conexium Medicale.* 2011; 6(1):61-66.

[11] Serdaroflu M., ÇakÂrbay H., Defer O., Cengiz S.,Kul S.,The association of anti-CCP antibodies with disease activity in rheumatoid arthritis: *Pub: Rheumatol Int. 2008;* 10:965-70.

[12] Choi SW, Lim MK., Shin DH. Park J., Shim SC.Diagnostic Performances of Anti-Cyclic Citrullinated Peptides Antibody and Antifilaggrin Antibody in Korean Patients with Rheumatoid Arthritis: *J Korean Med Sci*. 2005; 20(3):473-78.

[13] Mobini M., Kashi Z., Mahdavi MR., The role of Rheumatoid Factor and Anti-Cyclic Citrullinated Peptide Antibody in Diagnosis of Rheumatoid Arthritis: Pub: *Iranian Red Crescent Medical Journal Africa*n. 2010; 12(2):100-03.

AUTHOR(S):

1. Dr. Sucilathangam G
2. Dr. Anna T
3. Dr. Velvizhi G

PARTICULARS OF CONTRIBUTORS:

1. Senior Assistant Professor, Department of Microbiology, Tirunelveli Medical College, Tirunelveli, Tamil Nadu, India.
2. Professor & HOD, Department of Parasitology, Veterinary College, Tirunelveli, Tamil Nadu, India.
3. Senior Assistant Professor, Department of Microbiology, Tirunelveli Medical College, Tirunelveli, 627 011, Tamil Nadu, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:

Dr. G. Sucila Thangam,
Senior Assistant Professor, Department of Microbiology,
Tirunelveli Medical College, Palayamkottai-627011,
Tirunelveli Dt., Tamil Nadu, India.
Phone : 9442063819
E-mail : drgsucila@rediffmail.com

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