

Diagnostic Value of Rheumatoid Factor and Anti-CCP in Seropositive and Seronegative Rheumatoid arthritis patients at a Tertiary Hospital in Tamil Nadu

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ABSTRACT

Rheumatoid Arthritis (RA) is not an uncommon disease and its rate of prevalence is estimated about 1- 1.5% across the globe. It is a non-organ specific autoimmune disease which is categorized by systemic, chronic and symmetric inflammation involving multiple joints, predominantly the small joints. The aim of this study is to evaluate the diagnostic value of Anti-CCP autoantibodies with the standard rheumatoid factor (RF) among seropositive and negative patients attending a tertiary care hospital. This is a cross sectional study was conducted in a tertiary care hospital in and around Coimbatore district. The total study population was categorized into two groups 80 RF positive and 80 RF negative for the performance of anti-CCP ELISA. The age of the RA positive patients ranges from 28 to 83 years and for negative patients about 18 to 75 years old. Among the RA-positive group, 19 patients (23.7%) were tested positive for anti-CCP antibodies, while the remaining 61 (76.3%) were negative. In the RA-negative group, 15 patients (18.7%) were anti-CCP positive, whereas 65 (81.3%) tested negative. Rheumatoid factor is the significant diagnostic marker for RA till the discovery of Anti –CCP autoantibodies. The study concludes that anticitrullinated protein antibodies are more sensitive than RF antibodies. Anti-CCP is considered to be early diagnostic marker for the diagnosis of RA than other serological tests.

Keywords: Rheumatoid Arthritis, anti-cyclic citrullinated peptide, Rheumatoid Factor, IgG ELISA, Autoimmune disease

1. INTRODUCTION

Rheumatoid arthritis (RA) is a non-organ specific autoimmune disease which is categorized by systemic, chronic and symmetric inflammation involving multiple joints, predominantly the small joints. T-lymphocytes play a significant role in ankylosis because of the proliferation of synovial cells, which leads to bone and cartilage destruction (1). It is often considered as a multifactorial origin which may result due to genetic and environmental factors. This may be considered as more substantial in pathogenesis (2). Though there are many theories that have been studied to the onset of this clinical autoimmune condition, and they lack on the etiological manifestation of the disease. This condition may produce auto antibodies causing destruction of joints and further leads to loss of movements, deformities and disabilities (3, 4). RA is not an uncommon disease and its rate of prevalence is estimated about 1- 1.5% across the globe. It is more common in women when compared to men (5). This disease can be identified serologically with the production of auto antibodies like rheumatoid factor (RF), anti-cyclic citrullinated peptide (CCP), anti-keratin antibodies (AKA), anti-perinuclear factor (APF), anti-RA33, anti-flaggerin antibodies etc (6). Though there are many auto antibodies produced in this condition, where RF is the more

specific and commonly used for the detection of RA serologically with the recent addition of anti-CCP (3). Rheumatoid factor (RF) is an auto antibody which is IgM in nature and it is directed against the Fc region of immunoglobulin G which the other autoantibody anti CCP is formed as a result of citrullination (3,7). The routine diagnostic test performed for RA patients is the detection of the rheumatoid factor status which has a low specificity but high sensitivity especially in the early course of the disease (8). According to the ACR- American College of Rheumatology and EULAR - European League against Rheumatism , the recent criteria is inclusive of anti –CCP in the identification of the RA condition of the patient (9). These autoantibodies are also considered to predict the severity of the patients' condition when combined with the RF factor detection , though some studies claim anti- CCP to have high specificity but moderate sensitivity when compared with the rheumatoid factor (10,11). Hence this study is conducted to diagnostic value of Anti-CCP autoantibodies with the standard rheumatoid factor (RF) among seropositive and negative patients attending a tertiary care hospital.

2. MATERIALS AND METHODS

Study Group:

This is a cross sectional study was conducted in a tertiary care hospital in and around Coimbatore district for a period of about four years, from January 2020 – April 2024. After getting approval from the Institutional Human Ethics Committee (EC/2020/0411/CR/85) After getting the consent about 2 ml of blood sample was collected from the patients and serum was separated by centrifugation and it was preserved at -20°C. The patients with joint pain especially small joints, symmetric pain were included while pediatric age group, ante- natal women, elderly beyond 80 years, pain only in large joints were excluded from the study. The total study population was categorized into two groups 80 RF positive and 80 RF negative. for the performance of anti-CCP ELISA. The basic information of the patients like age, ethnicity and gender were collected along with the details of the disease duration, onset of the disease, joints involved, other systemic infections (if any), medication for the condition etc.

Antibody Detection:

Anti-CCP antibodies (IgG) ELISA, (Human Gessellschaft fur Biochemica und Diagnostica mbH, Germany) were performed according to the manufacturer instructions. Briefly, patient serum samples were diluted in 1:50 with diluent and 100µl of diluted sample, Calibration, positive and negative control was added into the appropriate wells. Diluent was used as a blank and the plate was covered with the adhesive tape. The plate was incubated for one hour at room temperature. After incubation, the solution was discarded from the plate and three times of washing has been done using 300µl of washing buffer. After washing, the plate was tapped on the absorbent paper or cloth for the removal of residues from the wells. About 100µl of conjugate was added to the respective wells and covered with adhesive strip. The plate was incubated for 30 mins at room temperature. Washing step was repeated as above mentioned. After washing and tapping, 100µl of substrate was added into each well and incubated for 30 min at room temperature. Finally, 100µl of stop solution was added to the each well and the absorbance values were calculated at 450nm with reference range from 600 nm to 690nm using multimode ELISA Reader. Each patient's sample was run in duplicates. As per the kit instructions, the results above 20U/ml (Cut-off value) are considered as positive for anti-CCP. As per the results, the cut-off value 20 U/ml with the OD absorbance value >0.812 is considered as positive

3. RESULT

In the present study overall 160 patients were collected and subjected to RA factor About 80 RA positive patients with 25 male and 45 female Mean $\pm$ SD age of the patients = 51.7 ± 13.4 with 95% Confidence Interval (51.14 ± 3.15). A 80 RA negative patients with 40 male and 40 female with Mean $\pm$ SD age of the patients 41.5 ± 15.6 with 95% Confidence Interval (41.5696 ± 3.445). The age of the RA positive patients ranges from 28 to 83 years and for negative patients about 18 to 75 years old. Joint Pain + Swelling was statistically significant in RA positive patients ($P=0.0019$), and anemia in RA negative patients ($P=0.0207$) were showed in Table - 1. Other symptoms like Joint Pain + Fever + Stiffness, Fever, Fever + Malaise, and Morning Stiffness showed no statistically significant differences ($P > 0.05$). The data suggest that joint pain with swelling is strongly associated with RA positivity, while anemia appears more prevalent among RA negative patients. A total of 80 each RA-positive and negative patients were evaluated for the presence of anti-cyclic citrullinated peptide (anti-CCP) antibodies. Among the RA-positive group, 19 patients (23.7%) were tested positive for anti-CCP antibodies, while the remaining 61 (76.3%) were negative. In the RA-negative group, 15 patients (18.7%) were anti-CCP positive, whereas 65 (81.3%) tested negative. The difference in anti-CCP antibody positivity between the two groups was not statistically significant ($P > 0.05$).

Table – 1: Clinical conditions of RA positive (n=80) and negative patients (n=80)

Clinical Symptoms	RA positive patients (n=80)	RA negative Patients (n=80)	P value*
Joint Pain +Swelling	26	12	0.0019
Joint Pain + Fever + Stiffness	05	12	0.1961
Fever	13	20	0.3662
Fever + malaise	09	16	0.2744
Anemia	12	27	0.0207
Stiffness of joints in the morning	05	03	0.4738

4. DISCUSSION

Rheumatoid arthritis is more common systemic autoimmune diseases and it was shown with classical symptoms of chronic joint pain and later may develop into destruction of joints (12). RA is also observed in oral manifestations especially the patients with long term arthritis (5). Serum antibodies to oral bacteria have been found at increased levels in RA patients. In addition, extraction of nucleic acid from these bacteria has been identified from samples of RA patients. Several antibiotics are effective on RA. Finally, oral bacteria are responsible for the etiology of RA. Rheumatoid factor is the significant diagnostic marker for RA till the discovery of Anti –CCP autoantibodies. First of all the study was focused on the screening of RF and later with anti-CCP ELISA for symptomatic patients. We identified 15 RA seronegative patients who were positive for Anti RA33, which symbolizes the positive predictive value in the diagnosis of Rheumatoid arthritis. Most of the RF negative patients are seen to be positive for Anti – CCP antibodies, indicating that the production of autoantibodies even before the production of RF (13, 14). These antibodies are said to be associated with the erosive form of the diseases as well as they serve as an early marker in rheumatoid arthritis. Many studies have recorded that radiographical findings in both extremities with the support of serological studies were revealed to have indication of anti – CCP antibodies (15). Previous studies have reported that up to 40% of sero-negative RA patients tested positive for anti-CCP antibodies, were highlighting the diagnostic value of anti-CCP testing in sero-negative cases (16). Anti- CCP is useful for differentiating the diagnosis of RA from SLE patients, as they too present with the erosive form of the disease and also presents symptoms which are similar to RA (17). Despite their high specificity and low sensitivity, with the rheumatoid factor, they have been found on progression of the disease when compared to RF testing alone (18). In some observational studies, found that there is a significant association between periodontitis and rheumatoid arthritis (RA). There are some intervention studies that have been suggested that periodontal treatment can help to reduce the serum inflammatory biomarkers such as C-reactive protein or erythrocyte sedimentation rate etc. The study is highly limited to the serological detection of anti-CCP antibodies among clinically diagnosed RA patients.

5. CONCLUSION

The study concludes that anticitrullinated protein antibodies are more sensitive than RF antibodies. Anti-CCP is considered to be early diagnostic marker for the diagnosis of RA than other serological tests.

Conflict of interest: None to declare by the authors.

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