International Journal of Allied Medical Sciences and Clinical Research (IJAMSCR)

ISSN:2347-6567

IJAMSCR |Volume 9 | Issue 2 | Apr - Jun - 2021
www.ijamscr.com

Research Study *Medical research*

Anti-Cyclic Citrullinated Peptide antibody and Rheumatoid factor and their association in Rheumatoid Arthritis in a tertiary care hospital

[1]Mamatha G. and *V G Thykadavil

*[1]*Department of Biochemistry, St John's Medical College and Hospital, Bengaluru-560034, Karnataka, India.*
***Corresponding Author**: **V G Thykadavil**
Email id: drvinodgeorge@gmail.com

ABSTRACT

Background: Rheumatoid arthritis (RA) is a systemic inflammatory disease of unknown etiology. Rheumatoid Factor (RF) is one of the criteria for diagnosis of RA, and there is fewer evidence for an early detection. In this study, we explored the levels of both Anti-Cyclic Citrullinated Peptide antibodies (Anti-CCP)and RF for an early diagnosis of RA before there is radiological structural changes in the joints.

Material and methods: In this prospective observational study conducted over a period of one-year, medical records of 355 subjects were evaluated. Data was compiled of Anti-CCP, RF levels, along with clinical diagnosis and other biochemical parameters. Based on clinical evaluation and serological evidence, 155 patients were diagnosed as positive cases of RA. Sensitivity and specificity were also calculated for Anti-CCP and RF.

Results: Compared to other age groups, female (72.7%) of 41-50 years ($p < 0.05$) were significantly affected as compared to male (42%) in similar age group. Our results showed sensitivity and specificity (80.5%, 100% respectively) for Anti-CCP and that for RF was (86.1%, 96.6 respectively).Association of Anti-CCP with RF was tested using Fisher's exact test, that showed a significant association ($p \leq 0.05$).

Conclusion: There is a significant association between Anti-CCP and RF in RA patient and Anti-CCP can be used as an early and specific marker for detection of RA.

Keywords: Rheumatoid factor, Rheumatoid arthritis, Anti-Cyclic Citrullinated Peptide antibodies, Rheumatology, Low back pain, Joint pain, Fatigue, Body Ache.

INTRODUCTION

Rheumatoid arthritis (RA) is a progressive, systemic inflammatory disease of unknown etiology which is diagnosed based on clinical features, biochemical parameters and radiological findings. Previous studies show Rheumatoid factor (RF) has a sensitivity of 31% to 54% and specificity of 91% to 93% for the eventual diagnosis of RA when the test was done at first presentation.[1]. Although RF was one of the criteria for the diagnosis of RA in previous studies, there are fewer evidence for an early detection. Anti - CCP has been proposed as a surrogate marker in the diagnosis of RA. In this study, we estimated Anti-CCP antibody and RF in RA, compared the data with RA negative subjects. The sensitivity and specificity of Anti-CCP and RF and the association between Anti-CCP and RF in RA positive and RA negative subjects was also done.

Rheumatoid arthritis (RA) is a chronic, inflammatory syndrome comprised of various disease phenotypes. RA is characterized by aggressive synovial hyperplasia causing destruction of articular joints. A combination of genetic, epigenetic, and environmental factors is responsible for the onset and development of RA. An array of susceptible genes (human leukocyte antigen (HLA)

class II and more than 100 susceptibility loci including PTPN22, PADI4, TRAF1, and CTLA4), nongenetic factors (sex hormones, smoking, periodontal infection, and microbiota), immune (macrophages, dendritic cells, mast cells, neutrophils, T cells, and B cells) and non immune (fibroblasts and chondrocytes) cells, and inflammatory mediators (autoantibodies, cytokines, chemokines, and proteases) are collectively involved in the inflammatory processes targeting the cartilage and bone effectuating functional loss of joints .

The synovium is one of the major target tissues in RA [2]. During joint inflammation, macrophage-like synoviocytes (MLS) and fibroblast-like synoviocytes (FLS) proliferate to form the pannus, which invades and destroys the cartilage. These cells are the major sources of factors that can promote inflammation and joint destruction. Autoantibodies contribute to the inflammatory process by acting as the mediator of joint inflammation and bone erosion [3]. Rheumatoid arthritis is a chronic inflammatory disorder that can affect more than just your joints. In some people, the condition can damage a wide variety of body systems, including the skin, eyes, lungs, heart and blood vessels.

An autoimmune disorder, rheumatoid arthritis occurs when your immune system mistakenly attacks your own body's tissues. Unlike the wear-and-tear damage of osteoarthritis, rheumatoid arthritis affects the lining of your joints, causing a painful swelling that can eventually result in bone erosion and joint deformity.

The inflammation associated with rheumatoid arthritis is what can damage other parts of the body as well. While new types of medications have improved treatment options dramatically, severe rheumatoid arthritis can still cause physical disabilities.

Symptoms

Signs and symptoms of rheumatoid arthritis may include:

- Tender, warm, swollen joints
- Joint stiffness that is usually worse in the mornings and after inactivity
- Fatigue, fever and loss of appetite

Early rheumatoid arthritis tends to affect your smaller joints first — particularly the joints that attach your fingers to your hands and your toes to your feet.

As the disease progresses, symptoms often spread to the wrists, knees, ankles, elbows, hips and shoulders. In most cases, symptoms occur in the same joints on both sides of your body.

About 40 percent of the people who have rheumatoid arthritis also experience signs and symptoms that don't involve the joints. Rheumatoid arthritis can affect many nonjoint structures, including:

- Skin
- Eyes
- Lungs
- Heart
- Kidneys
- Salivary glands
- Nerve tissue
- Bone marrow
- Blood vessels

Rheumatoid arthritis signs and symptoms may vary in severity and may even come and go. Periods of increased disease activity, called flares, alternate with periods of relative remission — when the swelling and pain fade or disappear. Over time, rheumatoid arthritis can cause joints to deform and shift out of place.

Causes

Rheumatoid arthritis vs. osteoarthritis Open pop-up dialog box

Rheumatoid arthritis occurs when your immune system attacks the synovium — the lining of the membranes that surround your joints.

The resulting inflammation thickens the synovium, which can eventually destroy the cartilage and bone within the joint.

The tendons and ligaments that hold the joint together weaken and stretch. Gradually, the joint loses its shape and alignment.

Doctors don't know what starts this process, although a genetic component appears likely. While your genes don't actually cause rheumatoid arthritis, they can make you more susceptible to environmental factors — such as infection with certain viruses and bacteria — that may trigger the disease.

Risk factors

Factors that may increase your risk of rheumatoid arthritis include:

- **Your sex.** Women are more likely than men to develop rheumatoid arthritis.
- **Age.** Rheumatoid arthritis can occur at any age, but it most commonly begins in middle age.
- **Family history.** If a member of your family has rheumatoid arthritis, you may have an increased risk of the disease.
- **Smoking.** Cigarette smoking increases your risk of developing rheumatoid arthritis, particularly if you have a genetic predisposition for developing the disease. Smoking also appears to be associated with greater disease severity.
- **Environmental exposures.** Although poorly understood, some exposures such as asbestos or silica may increase the risk of developing

rheumatoid arthritis. Emergency workers exposed to dust from the collapse of the World Trade Center are at higher risk of autoimmune diseases such as rheumatoid arthritis.

- **Obesity.** People — especially women age 55 and younger — who are overweight or obese appear to be at a somewhat higher risk of developing rheumatoid arthritis.
- Complications
- Rheumatoid arthritis increases your risk of developing:
- **Osteoporosis.** Rheumatoid arthritis itself, along with some medications used for treating rheumatoid arthritis, can increase your risk of osteoporosis — a condition that weakens your bones and makes them more prone to fracture.
- **Rheumatoid nodules.** These firm bumps of tissue most commonly form around pressure points, such as the elbows. However, these nodules can form anywhere in the body, including the lungs.
- **Dry eyes and mouth.** People who have rheumatoid arthritis are much more likely to experience Sjogren's syndrome, a disorder that decreases the amount of moisture in your eyes and mouth.
- **Infections.** The disease itself and many of the medications used to combat rheumatoid arthritis can impair the immune system, leading to increased infections.
- **Abnormal body composition.** The proportion of fat to lean mass is often higher in people who have rheumatoid arthritis, even in people who have a normal body mass index (BMI).
- **Carpal tunnel syndrome.** If rheumatoid arthritis affects your wrists, the inflammation can compress the nerve that serves most of your hand and fingers.
- **Heart problems.** Rheumatoid arthritis can increase your risk of hardened and blocked arteries, as well as inflammation of the sac that encloses your heart.
- **Lung disease.** People with rheumatoid arthritis have an increased risk of inflammation and scarring of the lung tissues, which can lead to progressive shortness of breath.
- **Lymphoma.** Rheumatoid arthritis increases the risk of lymphoma, a group of blood cancers that develop in the lymph system.

MATERIALS AND METHODS

This prospective observational study was performed from September 2018 to 2019, at the Department of Biochemistry, St John's Medical College, Bengaluru in India. All data of samples received for the analysis of RF and Anti-CCP in the biochemistry laboratory and serology of St. John's Medical College Hospital were included in the study after ethical clearance from the institutional ethic review board (IERB). Neonatal or children samples, hemolyzed, lipemic and icteric samples were excluded as these interfere with the assay. Data for analysis from LIS and medical records was obtained after necessary clearance from IERB.Of the 355 subject's medical data evaluated, 155 positive subjects were identified for RA and were further evaluated for other biochemical parameters levels (CRP, AST, ALT, Hb and ESR). We analyzed data of 155 patients with RA at first presentation, who met the ACR 2010 classification criteria. 200 control subjects included were,patients with low back pain, osteoarthritis, and individuals with non-RA rheumatic diseases.The Anti-CCP test was done by Electro chemiluminescence Immunoassay using e6000analyzer (Roche Diagnostics). Concentrations of >7 U/ml were considered positive for Anti-CCP antibodies. The RF test was done by nephelometry using (MISPA-i_2) analyzer. Concentration of >10 IU/ml were considered positive for RF. The positivity levels were according to manufacturer's recommendations. CRP, AST, ALT among other biochemical parameters were alsoanalyzed using ABBOTT Architect analyzer. Quality control material from Bio-rad and Roche were used to study pression and accuracyof the assays. Hb and ESR were done in Sysmex Autoanalyzer. The data obtained were entered along with patients details in excel sheet along with other biochemical parameters. Further the data were analyzed using SPSS version 18.The quantitative variables were analyzed with Fisher's exact test. ROC curve was used for determining the sensitivity and specificity of the laboratory markers. The area under the curve of each test was calculated and compared with each other.

RESULTS

In the 155 patients' group with RA, 112 were females (72.70%) and 42 males (27.30%)(Table:1)

Table-1: Gender distribution among cases and controls

Gender	CASES		CONTROLS	
	NO	(%)	NO	(%)
MALE	42	27.30%	58	29%
FEMALE	113	72.70%	142	71%
TOTAL	155	100	200	100

The mean age of the RA positive group was 45±13years. The control group consisted of 58 males (29%) and 142 females (71%) with the mean age of 48±13years. (Table:2)

Table-2: Comparison of cases and control according to Age groups

Age group	Cases		Controls	
	No.	(%)	No.	(%)
20-30	16	10.32	31	15.5
31-40	35	22.58	49	24.5
41-50	40	25.80	56	28
51-60	36	23.22	44	22
>60	28	18.06	20	10
TOTAL	155	100	200	100
MEAN± SD	**45±13**		**48±13**	

In the RA patients' group, Anti-CCP was positive in 134 cases (86%) and RF in 121 cases (80%).

Table 3 depicts sensitivity, specificity, positive and negative likelihood ratio of Anti-CCP, RF. In RA patients' group, both Anti-CCP and RF were negative in 1 case, both were positive in 130 cases, positive Anti-CCP and negative RF in 15 cases and negative Anti-CCP and positive RF in 20 cases.

Table-3: Association between RF and Anti-CCP

Association between RF and Anti-CCP				
		ACCP		
		Negative	Positive	Total
RF	Negative	1	20	21
	Positive	29	101	130
	Total	30	125	155

Association with RF is tested using Fisher's exact test with test statistics 3.5 andp = 0.05

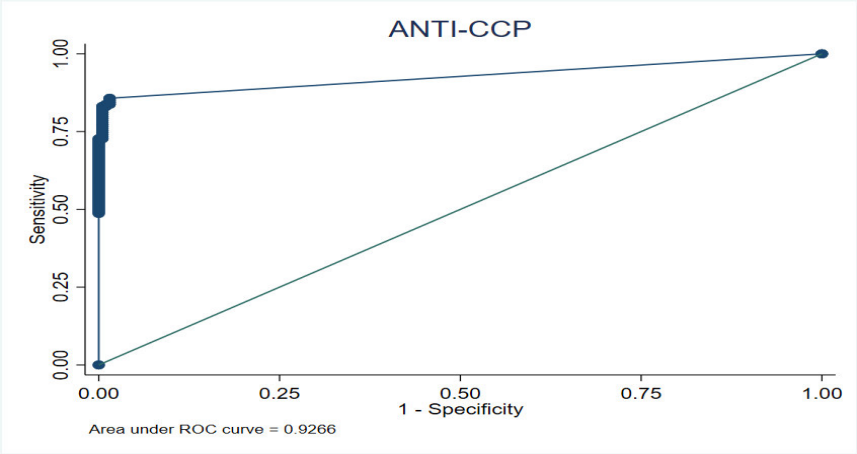
Sensitivity, specificity, predictive values and diagnostic accuracy of RF for the diagnosis of Rheumatoid arthritis, and anti-CCP as the gold standard were calculated along with the clinical findings. The Table-4 showed that sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), true positives (TP), true negatives (TN), false positives (FP), false negatives (FN) and diagnostic accuracy.

Table-4: Sensitivity, specificity, predictive values and diagnostic accuracy for RA

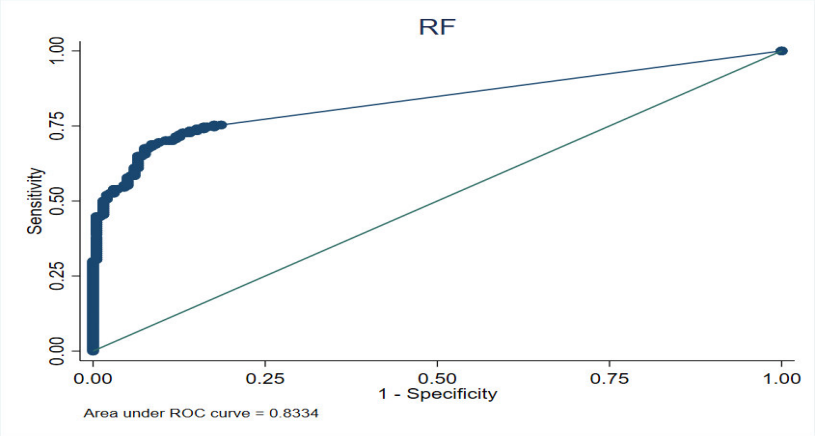
	Sensitivity	Specificity	PPV	NPV	TP	TN	FP	FN	Accuracy
Anti-CCP	0.805	1.00	1.00	0.909	124	300	0	30	0.934
RF	0.861	0.966	0.928	0.931	130	283	14	21	0.863

As per Receiver Operator Characteristic (ROC) curve analysis, the area under the curve (AUC) for anti-CCP is 0.92 and for RF, it is 0.85 (Graph 1 & 2).

Graph-1



Graph-2



An association study was done for parameters like Uric acid, CRP, ESR, AST, ALT, and glucose. Only AST was found to have significant association with RF (Table 5)

Table-5: Association of RF with AST

Association between AST and RF				
		AST		Total
		Negative	Positive	
RF	Negative	11	1	12
	Positive	63	5	68
	Total	**74**	**6**	**80**

Association with AST is studied using Fisher's exact test with test statistics 0.014 and p = 1.00.

DISCUSSION

The purpose of this study was to evaluate the sensitivity and specificity of anti-CCP antibodies in diagnosing rheumatoid arthritis. In our study, we found a significant association between Anti-CCP and RF. Anti-CCP antibodies had a high specificity (100%) and low sensitivity (80%) in comparison with RF which had high sensitivity (86%) and low specificity (96 %).

A study done by Khan et al showed the sensitivity, specificity, PPV, NPV of Anti-CCP to be 54.7%, 95.5%, 93.5% and 64.1% respectively. Another study by Dogan et al shows the sensitivity, specificity, PPV and NPV as 69%, 95%, 97% and 59% respectively [2].

Shafia et al demonstrated the sensitivity, specificity, PPV and NPV as 78.7%, 100%, 100% and 48.4% respectively. All these results were similar to our study which showed sensitivity, specificity, PPV and NPV of anti-CCP as 69.2%, 98.5%, 97.8% and 77.0% respectively. There is an association between the presence of anti-CCP and structural damage in early RA [3].

Similar studies were conducted in Malaysia where the sensitivity and specificity of Anti-CCP was found to be 35% and 100% and for RF, 43% and 85% respectively. On combining both the assays, the sensitivity and specificity was 50% and 85% respectively. Their study also found an association of anti-CCP antibody with involvement of multiple joints, pain in the joints of the hand, symmetrical joint involvement, rise in CRP and RF [4].

The concentration of anti-CCP antibodies increases as the disease becomes more severe, it predicts the course of the disease, and patients with positive anti-CCP antibodies show more joint destruction than RA patients without anti-CCP antibody positivity[5].

Earlier studies have used the 1987 American College of Rheumatology (ACR) criteria for the Diagnosis of RA. These criteria have been criticized for their lack of sensitivity and the newer2010 ACR/EULAR criteria has now been incorporated Anti-CCP in the diagnostic criteria. These Criteria use the signs and symptoms in the early stages of the disease rather than the late-stage Features for diagnosing RA. High serum values of Anti-CCP were present in more severe Disease as seen by the scoring of the symptoms in the ACR/EULAR criteria [6].

Anti-CCP assays are useful in the early diagnosis of RA, in cases where there is RF-negative RA and in cases of hepatitis C related joint involvement in which RF may be positive. In Undifferentiated arthritis, anti-CCP positivity seems to have value in the diagnosis, prognosis and prediction of the disease course [7].

Diagnostic performance was compared using ROC curves, sensitivity, specificity, Likelihood ratios, and predictive values. Logistic regressions were used to investigate whether using both tests (anti-CCP2 and anti-CCP3) gives a better prediction of rheumatoid arthritis. The manufacturer's cut-offs sensitivity and specificity were 79.4 and 61.0% for CCP3 and 80.9 and 69.5% for CCP2. No significant differences could be observed regarding the areas under the curve (AUC) of both ROC-curves. 80.0%) and 5.6 U/ml for CCP3 (sensitivity of 86.9% and specificity of 61.0%). Binary logistic regressions indicated that the likelihood of having rheumatoid arthritis (RA) is significantly higher when testing positive on both CCP2 and CCP3 compared to CCP2 or CCP3 alone.In their study comparable performance was found between the two CCP assays. Positivity for both CCP2 and CCP3 resulted in the most specific identification of RA patients [8].

Anti-CCP antibodies are known to be an important serological marker in the diagnosis of RA. As a result, since 1998 several tests have been developed for a more accurate identification of RA patients. Most recently, third generation anti-CCP tests have been developed to increase the sensitivity for the detection of patients with RA. However, the academic literature comparing anti-CCP2 and anti-CCP3 assays present conflicting evidence [9].

A study done by Dos Anjos LM et al aimed to advance current knowledge by investigating the diagnostic performance of anti-CCP2 and anti-CCP3 assays separately as well as in combination, in a setting of a routine patient population. Analysis reveals no significant differences between the AUC of both ROC-curves, indicating there is no advantage of using one over the other test [10].

This could be due to the method of selection of suspected cases. In present study, specificity of RF was found to be less (82.4%) as compared to that of anti-CCP antibody (98.4%). Our study goes parallel with the studies conducted by Ayesha et al., (86%, 98.6%), Sibel et al., (86.4%, 98.6%) and Lee et al., (80.3%, 90%) of RF and anti-CCP antibodies (Ayesha, 2017; Sibel et al., 2004; Lee and Weisman, 2006) [11].

It was also interesting to evaluate anti-CCP and RFbehavior in RA patients in relation to the duration ofdisease. In patients with early arthritis, the correlation with anti-CCP was highly significant, thus indicating that this assay maybe used evenin the early phases of disease. [12]

For serological tests, both RF and anti-CCP antibody were positive in more than half of the patients with prevalence of 52.2%, consistent with previous studies[13]. The study demonstrated the RF was significantly associated with anti-CCP antibody positivity and this finding was supported by Chou et al [14]. Where RF was found to be significantly associated with anti-CCP antibody in cohort of 155 Chinese RA patients at early stages of diseases, RA is often difficult to differentiate from other inflammatory arthritis condition and RF alone has low sensitivity in diagnosing early RA. Combination of both RF and anti- CCP antibody has been shown to improve the sensitivity to improve the sensitivity of early diagnosis [15].

Smoking has also been found to have a strong relationship to RA especially in HLA-DRB1 positive patients and substances in tobacco also induce citrullination [16].

The role of smoking in RA has not been addressed in this study. Further work needs to be done in this regard. There is an association between the presence of anti-CCP and structural damage in early RA. In case of established RA, this

association is not so conclusive. Anti-CCP and insulin resistance has been shown to be helpful in the early detection of sub-clinical atherosclerosis in RA patients [17].

CONCLUSION

Our study suggests that Anti CCP and RF provides valuable information to diagnose RA. The specificity of Anti CCP were higher when compared to RF in this study.Initial observations shows that Anti-CCP antibodies have the ability to distinguish between erosive and non-erosive disease, making it a good prognostic marker. Our study also found that there was a significant association between age and RF using Fisher's exact test with p value <0.05 (0.048) which signifies RF positivity with increasing age. Majority of patients were diagnosed in the age group 40 to 50 years. Females were more affected than males. It was also shown that the RA patients had extraarticular manifestations when the Anti CCP levels were higher. There was no follow up done for the cases, as some of the patients didnot come for follow up for further evaluation. In this study, continuous monitoring of anti-CCP and RF positive cases, were not carried out. Further studies are required to assess if anti-CCP could be used as a predictor or a exclusive marker for RA.

REFERENCES

[1]. Niewold TB, Harrison MJ, Paget S. Anti-CCP antibody testing as a diagnostic and prognostic tool in rheumatoid arthritis. QJM, 2007; 100: 193-201.

[2]. Khan AH, Jafri L, Husain MA, Ishaq S. Diagnostic utility of anti-citrullinated protein antibody and its comparison with rheumatoid factor in rheumatoid arthritis. J Coll Physicians Surg Pak 2012;22(11):711–5.

[3]. Shafiaa S, Shaha ZA, Sofib FA, Rasoola R, Gulla A. Anti-CCP is associated with greater disease burden in Kashmiri population with rheumatoid arthritis. Rheumatology (Sunnyvale) 2016; 6(1):2161-1149.

[4]. Abdul Wahab A, Mohammad M, Rahman MM, Mohamed Said MS. Anti-cyclic citrullinated peptide antibody is a good indicator for the diagnosis of rheumatoid arthritis. Pak J Med Sci 2013;29(3):773–7.

[5]. Sarah K, Nadeem A. Anti-CCP Antibodies: an emerging marker for the early diagnosis of rheumatoid arthritis. Int Med J Malaysia 2010;9(1):63–5.

[6]. Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT Bingham CO 3rd, et al. 2010 Rheumatoid arthritis classificationcriteria: an American College of Rheumatology/EuropeanLeague Against Rheumatism collaborative initiative. ArthritisRheum. 2010;62(9):2569–81.

[7]. Bizzaro N, Bartoloni E, Morozzi G, Manganelli S, Riccieri V, Sabatini P, et al. Anti-cyclic citrullinated peptide antibody titer predicts time to rheumatoid arthritis onset in patients with undifferentiated arthritis: results from a 2-year prospective study. Arthritis Res Ther 2013;15(1):R16.

[8]. Anti-citrullinated protein antibodies in the diagnosis of rheumatoid arthritis (RA): diagnostic performance of automated anti-CCP-2 and anti-CCP-3 antibodies assays Clin Rheumatol 2017 36:1487–1492.

[9]. Schellekens GA, de Jong BA, van den Hoogen FH, van de Putte LB, van VenrooijWJ Citrulline is an essential constituent of antigenic determinants recognized by rheumatoid arthritis-specific autoantibodies. J Clin Invest 1998 101:273–281.

[10]. Dos Anjos LM, Pereira IA, Orsi E, Seaman AP, Burlingame RW, Morato EF A comparative study of IgG second- and third generation anti-cyclic citrullinated peptide (CCP) ELISAs and their combination with IgA third-generation CCP ELISA for the diagnosis of rheumatoid arthritis. Clin Rheumato 2009; 28:153–158.

[11]. Sibel A., Reyhan C et al., a new marker in Rheumatoid Arthritis- autoimmunity relation: anticyclic citrullinated peptide antibody. Turk j immunol; 2004; 9: 21-265.

[12]. Lipsky PE, van der Heijde DMFM, St. Clair EW, Furst DE, BreedveldFC, Kalden JR, et al. Infliximab and methotrexate in the treatment of rheumatoid arthritis. N Engl J Med 2011;343:1594–602.

[13]. Abdul Wahab A, Mohammad M, Rahman MM, et al. Anti-cycliccitrullinated peptide antibody is a good indicator for the diagnosis ofrheumatoid arthritis. Pak J Med Sci 2013;29:773–7.

[14]. Chou C, Liao H, Chen C, et al. The clinical application of anti-CCP in rheumatoid arthritis and other rheumatic diseases. Biomark Insights2007;2:165–71.

[15]. Vencovsky J, Machacek S, Sedova L, et al. Autoantibodies can be prognostic markers of an erosive disease in early rheumatoid arthritis.Ann Rheum Dis 2003;62:427–30.

[16]. Porto LS, Tavares WC Junior, Costa DA, Lanna CC, Kakehasi AM. Anti-CCP antibodies are not a marker of severity in established rheumatoid arthritis: a magnetic resonance imaging study. Rev Bras Reumatol 2017;57(1):15–22.

[17]. Wahab MAKA, Laban AE, Hasan AA, Darweesh AF. The clinical value of anti-cyclic citrullinated peptide (anti-ccp) antibodies and insulin resistance (IR) in detection of early and subclinical atherosclerosis in rheumatoid arthritis (RA). Egypt Heart J 2016; 68(2):109–16.

How to cite this article: Mamatha G. and V G Thykadavil. Anti-Cyclic Citrullinated Peptide antibody and Rheumatoid factor and their association in Rheumatoid Arthritis in a tertiary care hospital. Int J of Allied Med Sci and Clin Res 2021; 9(2): 288-295.

Source of Support: Nil. **Conflict of Interest:** None declared.