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Original Research Article

Anti- Cyclic Citrullinated Peptide Antibodies: Clinical utility and their role as early prognostic markers in Erosive Rheumatoid Arthritis

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Abstract

Objectives: To assess the diagnostic utility of anti- cyclic citrullinated protein antibody (anti-CCP) in comparison to rheumatoid factor (RF) in diagnosis of rheumatoid arthritis (RA) and to determine its prognostic value in erosive rheumatoid arthritis.

Materials and Methods: The study included 60 patients with clinically suspected RA. Anti-CCP was measured by enzyme-linked immunosorbent assay (ELISA) and rheumatoid factor (RF) by nephalometry. The seropositivity with RF and ACCP antibodies were studied in comparison with radiographic changes and joint symptoms which included presence and duration of joint involvement- multiple joints, small joints, symmetric joints, stiffness or swelling of joints.

Results: Among the 60 patients, the mean age for RA was found to be 43 years. A higher seropositivity was found among the females. The sensitivity and specificity of anti-CCP reactivity for the diagnosis of RA were 38.3 and 100 respectively. Among the symptoms studied, small joint involvement and involvement of multiple joints showed a positive association with serologically positive individuals. Patients with high anti-CCP antibody titres showed higher RF titres and severe radiographic erosions.

Conclusion: Anti CCP antibodies are highly specific for rheumatoid arthritis. A combined diagnosis using both high titers of anti-CCP antibodies and a positive RF test markedly improves RA diagnostic specificity. ACCP antibody detection helps in early diagnosis as well as detection of progressive joint damage.

Keywords: Anti-cyclic citrullinated peptides, autoantibodies, citrullinated proteins/peptides, rheumatoid arthritis

Rheumatoid arthritis (RA) is the most common chronic autoimmune joint disease of unknown etiology [1]. The course of RA ranges from mild arthritis to progressive joint destruction [2, 3]. Genetic factors like presence of HLA -DRB1*04 alleles combined with smoking have shown to increase the risk of developing RA [2]. Various circulating auto antibodies are preset in the sera of RA patients. Among them, IgM autoantibody against IgG i.e. the Rheumatoid factor (RF) is most widely used in the diagnosis of RA. RF has an acceptable sensitivity but lacks specificity [4]. RF is also detectable in other connective tissue disorders like SLE and Sjogren's syndrome and in patients with chronic hepatitis, 3-5% of general population and around 30% of the elderly, thus making it a non specific marker for RA [5,6].

The focus of recent research is on the development of markers for early diagnosis of RA to prevent absolute crippling deformity of joints and extra articular complications [7]. Anti Cyclic Citrullinated Antibodies and antibodies to Citrullinated Vimentin have now been described as specific antigens of RA and antibodies to citrullinated proteins/peptides (ACPAs) are included in 2010 ACR/EULAR Rheumatoid Arthritis (RA) Classification Criteria[3,8]. ACPAs are a family of autoantibodies seen in RA patients and include antiperinuclear factor (APF), antikeratin antibodies (AKA), antifilaggrin antibodies, and anticyclic citrullinated peptide (anti-CCP) antibodies. Studies have shown that anti-CCP was the most valuable marker in the diagnosis of RA, among the 3 ACPAs[9].

Citrullination is a protein degradation mechanism in which a peptidylarginine is deimided to peptidylcitrulline by peptidylarginine deiminase enzyme (PADI), a process that takes place in both the healthy and the arthritic population. Citrullination of synovial antigens, especially fibrin, is an active process during synovial inflammation that probably allows the induction of anti-cyclic citrullinated peptide (anti-CCP) antibody in RA patients, through an antigen-conducted activation of B cells and multiple genetic and environmental influences leading to development of a rheumatoid phenotype [4]. A genetic polymorphism of the citrullinating enzyme, PADI has also been suggested which may lead to breakage of self tolerance and induction of autoimmunity against citrullinated proteins [10]. Studies have shown that anti-CCP antibody is highly specific for RA with similar sensitivity to RF, and is able to predict the progression of disease long before the onset of clinical RA [4].

This study was conducted to compare the utility of anti-CCP and RF in the diagnosis of RA in patients presenting with joint pain to Vydehi Institute of Medical Sciences and research Centre, a tertiary care centre in Bangalore, India.

2. Materials and methods

Sixty patients >18 years of age with complaints of joint pains were included in the study. Classification criteria for rheumatoid arthritis as per the 2010 ACR/EULAR [3] were considered while including the patient for study. The study was approved by the Institutional Ethics Committee. 5 ml of blood was acquired from all patients for anti-CCP and RF tests.

The detection of anti-CCP was performed by ELISA, (EUROIMMUN, Medizinische Labordiagnostika AG. D-23560 Lubeck) Standard protocol was followed according to manufacturer's instructions. Results were reported with reading of > 5 Relative Units/ml were considered as positive as per the manufacturers' instructions.

RF factor was analyzed by nephalometry (Beckman Coulter IMMAGE, Ireland Inc). RF concentrations of 20 IU/ml or more were considered as positive as per the manufacturers' instructions. Calibrators/positive and negative controls were included every time.

Patients' medical records were looked through for demographic data as well as clinical signs and symptoms. The presence and duration of, joint involvement- multiple joints, small joints, symmetric joints, stiffness or swelling of joints were the main symptoms considered. X-ray findings were studied for the joint damage.

2.1 Statistical analysis

The sensitivity, specificity, positive and negative predictive value were calculated based on the standard table for sensitivity and specificity. Odd's ratio and 95% confidence intervals were considered to study the association of clinical and laboratory parameters with the seropositivity. The p value of <0.05 was considered as significant.

3. Results

The study included 60 patients who fulfilled ACR 2010 criteria for RA. The ages ranged from 20 years to 63 years mean age being 43 years. It consisted of 43 females (71.6%) and 17 males (28.3%). Majority of the patients belonged to areas in and around Bangalore.

Table I: Demographic data and duration in ACCP seropositives and seronegatives

	ACCP Seropositives	ACCP Seronegatives
Age(Years)		
Range	29-61	20-63
Mean	43	40
Gender		
Male	**07**	**10**
Female	**16**	**27**
Duration of symptoms		
0-6 months	**02**	**02**
6 months-1yr	**07**	**11**
1-2yrs	**08**	**14**
>2yrs	**06**	**10**

Most of the patients came with complaints of joint pains for a period ranging from 6 months to 10 years. Symptoms of joint involvement included pain in multiple joints, small joints, symmetric joints, stiffness or swelling of joints.

In this study anti CCP antibodies and RF were detected in 23/60 and 17/60 patients respectively. Sixteen of the 17 RF positive patients exhibited ACCP antibodies. One patient who was positive for RF and negative for ACCP antibodies had cervical spondylosis on X-ray and was diagnosed to have Sjogren's syndrome (mixed connective tissue disorder). Five patients who were negative for RF and positive for ACCP antibodies characteristically showed low titres of ACCP antibodies and joint pains of small joints of less than 1 year duration. Among the 16 RF and ACCP positive patients', high concentrations of RF very well correlated with higher ACCP titres and erosive joint involvement.

The sensitivity and specificity of anti-CCP were 38.3% and 100% respectively. The sensitivity and specificity of RF on the other hand were 69.5% and 97.3% respectively. The positive predictive value of ACCP was 100% and the negative predictive value was found to be 50%. The association of symptoms and clinical findings in ACCP seropositives and seronegatives are presented in Table II.

Table II: Association of presenting symptoms and clinical findings in ACCP seropositives and seronegatives.

Symptom	Seropositives (N=23)	Seronegatives (N=37)	Odd's ratio (95% CI*)	P- value
Multiple joints	13 (56.52%)	15(40.54)	4.76(1.30-17.46)	0.018(<0.05)
Small joints	10(43.7%)	07(18.91)	6.12(1.72-21.77)	0.005(<0.05)
Symmetric joints	06 (26.08%)	07(18.91)	2.33(0.64-8.49)	0.197(>0.05)
Stiffness or swelling of joints	08(34.78)	10(27.02)	2.40(0.72-7.94)	0.158(>0.05
Clinical and lab Findings				
RF	16(69.56%)	01(2.70)	576.0(33.8-97)	<0.0001(<0.05)
X-ray showing joint Damage	12(52.17%)	02(5.40)	5.36(3.76-62.66)	0.0001 (<0.05)

*- 95% Confidence Interval.

4. Discussion

In this study we found that among 60 patients who came with symptoms of RA, 24 had a serological evidence of RA. Twenty three patients were positive for ACCP antibodies and 1 patient had only RF. Patients' age ranged from 20-63 years. The mean age of ACCP antibody seropositives did not differ significantly (43 yrs 40years) from the seronegatives. Females were more predominant. Several studies have shown that middle aged adult females are commonly afflicted by RA [5, 7]

Many studies have found a similar sensitivity and specificity pattern as ours. In most of the studies specificity was more than 90% and sensitivity varied from 33% to 87%. This may be possibly related to diverse genetic backgrounds, differences in antigens and techniques. An ACCP antibodies detection is reported to be more specific than the anti vimentin antibodies [8]

Among the symptoms studied, involvement of small joints and involvement of multiple joints had a positive association with occurrence of higher levels of ACCP antibodies. Also the ACCP levels positively co-related with the radiological evidence of joint damage. A positive association for occurrence was found with symptoms of small and multiple joint involvements with significant p-values. Likewise among the clinical laboratory findings, both the presence of RF and erosions were found to be associated with RA. Similar findings have been cited in the literature that higher ACCP levels are associated with progressive and erosive joint damage [11-15].

Apart from their role in early diagnosis of RA, and progression to erosive joint changes, ACCP antibodies have also played an important role in therapeutic intervention. Infliximab used to treat RA significantly reduces RF titres but no change in the ACCP titres and the condition remains refractory. Treatment with TNF-alpha has shown reduction in titres of both RF as well as ACCP antibodies along with clinical improvement, thus indicating the role of ACCP antibodies in prognosis of RA [16]. Anti CCP antibodies have also been shown in relatives of patients with RA and interestingly in relatives who are younger than the patients[17]. This finding may be further studied using ACCP antibody detection as a diagnostic tool in undiagnosed RA and in turn help in early detection and prevention of crippling complications.

5. Conclusion

Anti CCP antibodies are a highly specific and moderately sensitive sero marker in the diagnosis of RA than rheumatoid factor. ACCP antibody detection helps in early diagnosis as well as detection of progressive joint damage thus adjuncting in early therapeutic intervention to prevent crippling complications.

References

[1] Shovman O, Gilburd B, Zandman- Goddard G, Sherer Y, Orbach H, Gerli R et al. The diagnostic utility of anti-cyclic citrullinated peptide antibodies, matrix metalloproteinase-3, rheumatoid factor, erythrocyte sedimentation rate, and C-reactive protein in patients with erosive and non-erosive rheumatoid arthritis. *Clin & Develop Immunol* 2005; 12(3): 197–202.

[2] FathiN A, Ezz-Eldin AM, Mosad E, Bakry RM, Hamed HB, Ahmed S et al. Diagnostic performance and predictive value of rheumatoid factor, anti-cyclic-citrullinated peptide antibodies and HLA-DRB1 locus genes in rheumatoid arthritis. *Int Arch Med* 2008; 1:1-7.

[3] Puszczewicz M, Iwaszkiewicz C. Role of anti-citrullinated protein antibodies in diagnosis and prognosis of rheumatoid arthritis. *Arch Med Sci* 2011; 7, 2: 189-194.

[4] Del Val Del Amo N, Ibanez Bosch R, Fito Manteca C, Gutierrez Polo R, Loza Cortina E. Anti-cyclic citrullinated peptide antibody in rheumatoid arthritis: Relation with disease aggressiveness**.** *Clin Exp Rheumatol* 2006; 24: 281-6.

[5] Wahab AA, Mohammad M, Rahman MM, Said MSM. Anti-cyclic citrullinated peptide antibody is a good indicator for the diagnosis of

rheumatoid arthritis. *Pak J Med Sci* 2013; 29(3):773-7.

[6] Miriovsky B J, Michaud K, Thiele G M, O'Dell J R, Cannon G W, Kerr G et al. Anti- CCP antibody and rheumatoid factor concentrations predict greater disease burden in U.S. veterans with rheumatoid arthritis**.** *Ann Rheum Dis*. 2010; 69(7): 1292–7.

[7] Khan AH, Jafri L, Hussain MA, Ishaq S. Diagnostic Utility of Anti- Citrullinated Protein Antibody and its Comparision with Rheumatoid Factor in Rheumatoid Arthritis. *J Coll Physicians Surg Pak* 2012, 22 (11): 711-5.

[8] Dejaco C, Kotz W, Larcher H, Duftner C, Schirmer M, Herold N. Diagnostic value of antibodies against a modified citrullinated vimentin in rheumatoid arthritis. *Arthritis Res Ther* 2006; 8:119-24.

[9] Zhao J, Liu X, Wang Z, Liu R, Li Z. Is it necessary to combine detection of anticitrullinated protein antibodies in the diagnosis of rheumatoid arthritis?. *J Rheumatol* 2010; 37(12):2462-5.

[10] Mimori T. Clinical Significance of Anti –CCP Antibodies in Rheumatoid Arthritis. *Internal Med* 2005: 44: 1122-6.

[11] Manivelavan D, CK Vijayasamundeeswari. Anti-Cyclic Citrullinated Peptide Antibody: An Early Diagnostic and Prognostic Biomarker of Rheumatoid Arthritis. *J Clin Diag Res* 2012; 6(8): 1393-6.

[12] Hamad MB, Marzouk S, Kaddour N, Masmoudi H, Fakhfakh F, Rebai A. Anticyclic citrullinated peptide antibody and rheumatoid factor in south Tunisian patients with rheumatoid arthritis: association with disease activity and severity. *J Clin Lab Anal* 2014; 28(1):21-6.

[13] H Li, W Song, Y Li, Y Liu, J Bai, X Li et al. Diagnostic value of anti-cyclic citrullinated peptide antibodies in northern Chinese Han patients with rheumatoid arthritis and its correlation with disease activity. *Clin Rheumatol* 2010; 29(4):413-7.

[14] Khosla p, Shankar S, Duggal L. Anti CCP Antibodies in Rheumatoid Arthritis. *J Ind Rheumatol Assoc* 2004: 12: 143 -6.

[15] D Predeteanu, L Varzaru, A Balanescu, V Bojinca , D Opris , V Vlad et al. Anti-cyclic citrullinated peptide antibodies--activity markers in rheumatoid arthritis. *J Med Life* 2009; 2(1): 36-41.

[16] Singh K, Mahajan P. Anti CCP Antibodies in the Diagnosis and Prognosis of Rheumatoid Arthritis. *JK Sci* 2011; 13(1): 1-5.

[17] Goeldner I, Skare TL, de Messias Reason LT, Nisihara RM, Silva MB, da Rosa Utiyama SR. Anti-cyclic citrullinated peptide antibodies and Rheumatoid factor in rheumatoid arthritis patients and relatives from Brazil. *Rheumatol* 2010; 49: 1590-93.