

Original Research Article

Occurrence of Anti-Cyclic Citrullinated Antibodies in Rheumatoid Arthritis and Other Rheumatological Disorders

Dr. Saumya Agarwal¹, Dr. Nandini Duggal², Dr. Vivek Arya³¹Senior resident, Department of Microbiology, Dr. RML hospital & PGIMER, New Delhi-110001, India²Professor & head, Department of Microbiology, Dr. RML hospital & PGIMER, New Delhi-110001, India³Professor, Department of Medicine, Dr. RML hospital & PGIMER, New Delhi-110001, India

*Corresponding author

Dr. Saumya Agarwal

Email: drsaumya.agarwal@gmail.com

Abstract: Anti-cyclic citrullinated peptide antibodies (anti-CCP) have been shown to have high specificity in the early diagnosis of rheumatoid arthritis (RA). However, these antibodies are sometimes present in patients with other rheumatic diseases and rarely in healthy individuals. We evaluated the occurrence of these antibodies in patients with RA, other rheumatic diseases and in healthy controls. Fifty two patients with RA, 39 patients with other rheumatological disorders (like ankylosing spondylitis SLE, systemic sclerosis and psoriatic arthritis) and 30 controls were recruited. Tests for immunological indicators (rheumatic factor and anti-CCP) were performed for each subject. Anti-CCP antibodies were found in 34 out of 52 patients with RA, 3 out of 39 patients with other rheumatic diseases and in none of the 30 healthy controls. Sensitivity and specificity of anti-CCP for RA were 65.4% and 95.7%, respectively while for rheumatoid factor, sensitivity and specificity were 50% and 89.9%. Anti-CCP antibodies were detected in nearly 70% of patients with RA. Fewer than 10% of patients with other rheumatic diseases and no healthy controls had anti-CCP antibodies. It is important to correlate the result of anti-CCP testing with the clinical presentation in order to avoid misdiagnosis.

Keywords: Anti-CCP antibodies, rheumatoid arthritis, sensitivity, specificity.

INTRODUCTION

Rheumatoid arthritis (RA) is a systemic inflammatory disease, characterized by chronic and erosive polyarthritis with abnormal growth of synovial tissue or pannus that causes irreversible joint disability [1]. Early diagnosis and treatment are important to avoid unwanted complications of the disease.

However, patients with rheumatoid arthritis do not always show typical symptoms and signs early in their disease course which can make early diagnosis difficult.¹ Laboratory testing for rheumatic diseases helps in rapid diagnosis and appropriate management. Serological markers like rheumatoid factor (RF), anti-cyclic citrullinated peptide (CCP) antibodies and inflammatory indicators like C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) play an important role in the early diagnosis of rheumatoid arthritis [2].

Rheumatoid factor, a serological marker commonly used in patients suspected to have

rheumatoid arthritis, has a sensitivity of 60- 80% in RA. However, it lacks specificity since RF can also be found in many other rheumatic diseases like systemic lupus erythematosus (SLE), Sjogren's syndrome, scleroderma and non-rheumatic diseases like viral infections, tumours and even in normal healthy subjects, particularly in ageing individuals [1,3,4].

The presence of antibodies to cyclic citrullinated peptides (anti-CCP) has been shown to be more specific than RF for the diagnosis of RA [5-7]. Anti-CCP antibodies are autoantibodies that bind with antigenic determinant of unusual amino acid citrulline, formed by post translational modification of arginine residues [8]. The sensitivity of anti-CCP antibodies for a diagnosis of rheumatoid arthritis varies from 50 to 75%, while the specificity is relatively high, usually more than 90% [8,9]. Increased anti-CCP antibody levels are associated with disease progression, more severe disease and radiological damage [4,10-12].

Table- Classification Criteria for Rheumatoid Arthritis [13]

		Score
Joint involvement	1 large joint (shoulder, elbow, hip, knee, ankle)	0
	2–10 large joints	1
	1–3 small joints (MCP, PIP, Thumb IP, MTP, wrists)	2
	4–10 small joints	3
	>10 joints (at least 1 small joint)	5
Serology	Negative RF and negative ACPA	0
	Low-positive RF or low-positive anti-CCP antibodies (3 times ULN)	2
	High-positive RF or high-positive anti-CCP antibodies (>3 times ULN)	3
Acute-phase reactants	Normal CRP and normal ESR	0
	Abnormal CRP or abnormal ESR	1
Duration of symptoms	<6 weeks	0
	6 weeks	1

Owing to widespread use of testing for anti-CCP antibodies and their diagnostic value in RA, this serological parameter has been included in the new 2010 ACR/EULAR classification criteria for rheumatoid arthritis [13].

Although several Indian studies have assessed the role of anti-CCP antibodies in the diagnosis of RA, there is paucity of data on the occurrence of these antibodies in patients with other rheumatic diseases. We studied the presence of these antibodies in patients with rheumatoid arthritis, those with other rheumatic diseases and in healthy controls.

METHODS

A cross-sectional, observational study was performed from November 2013 to March 2015. Study subjects included consecutive patients with rheumatoid arthritis, diagnosed as per ACR/EULAR 2010 classification criteria; patients with other rheumatological disorders like SLE, primary Sjogren’s syndrome, progressive systemic sclerosis, psoriatic arthritis, ankylosing spondylitis, reactive arthritis, inflammatory bowel disease (IBD) associated arthritis and palindromic rheumatism; and healthy age and sex matched controls.

A detailed history was obtained and a comprehensive clinical examination was performed in all patients. For each patient with rheumatoid arthritis,

the disease activity score on 28 joints (DAS28) was calculated using the following formula-

$$DAS28 = 0.56 \sqrt{\text{(tender joint count)}} + 0.28 \sqrt{\text{(swollen joint count)}} + 0.70 \ln(\text{ESR}) + 0.014 \text{ GH}$$

(GH= patient global assessment of health)

This score was used to categorize patients into low, moderate and high disease activity levels. On the basis of disease duration, patients were classified as having early RA (≤2 years) and late RA (>2 years).

Blood samples of all study participants were collected in plain vacutainer vial after obtaining written, informed consent. Anti-CCP antibodies (positive in a titre ≥15 U/ml) and RF were detected by ELISA.

RESULTS

A total of ninety one (91) patients and thirty (30) controls were included in the study. Out of these 91 patients, 52 had rheumatoid arthritis and 39 had other rheumatological disorders like ankylosing spondylitis (n=13), SLE (7), systemic sclerosis (5), undifferentiated spondyloarthropathy (5), reactive arthritis (4), psoriatic arthritis (3), polymyositis (1) and primary Sjogren’s syndrome (1).

The age of the entire study population ranged from 18 to 70 years with 94 women and 27 men. The age and gender distribution of patients in each group is shown in Table 1.

Table 1: Age and gender distribution in each study group

Group	Females	Age range (years)	Age (mean ± s.d.) (years)
Rheumatoid arthritis (n=52)	90.4%	18 -70	42 ± 14
Other rheumatological disorders (n=39)	51.3%	18 -60	32 ± 10
Healthy controls (n=30)	90%	23-59	39 ± 11

Of patients with rheumatoid arthritis (n=52), 24 had early RA (disease duration less than 2 years) and 28 had late RA. Disease activity was low in 6, moderate in 18 and high in 28 patients.

Rheumatoid factor was positive in 35 out of 52 patients with RA; in 7 out of 39 patients with other rheumatological disorders and in none of the healthy

controls. Anti-CCP antibodies were positive in 34 of 52 patients with RA; in 3 out of 36 patients with other rheumatological disorders and in none of the healthy controls.

A comparison between positivity for rheumatoid factor and anti-CCP antibodies is shown in Table 2.

Table-2: Comparison between positivity for rheumatoid factor and anti-CCP antibodies in patients with rheumatoid arthritis (n=52)

Anti-CCP antibodies	Rheumatoid factor		Total
	Negative	Positive	
Negative	9	9	18
Positive	8	26	34
Total (no.)	17	35	52

In 30 patients with RA, the results of the two tests were concordant. However, in the remaining 22 patients, one test was positive while the other test was negative. In 43 patients with RA, either RF or anti-CCP antibodies were positive. Only nine patients were truly seronegative with neither test being positive.

No significant correlation was found between anti-CCP positivity and disease duration or disease

activity according to DAS28 score in patients with rheumatoid arthritis.

The sensitivity, specificity, positive predictive values and negative predictive values of rheumatoid factor and anti-CCP antibodies in the diagnosis of RA are summarized in Table 3.

Table-3: Performance characteristics of rheumatoid factor and anti-CCP antibodies in patients with rheumatoid arthritis

Parameter	Rheumatoid factor (ELISA)	Anti-CCP antibodies
Sensitivity	67.3%	65.4%
Specificity	89.9%	95.7%
Negative predictive value	78.5%	78.6%
Positive predictive value	83.3%	91.9%

Taken together, a combination of both tests [either anti-CCP antibodies or RF being positive] increased the sensitivity to 82.7%.

DISCUSSION

There is growing evidence that therapeutic intervention early in the course of RA can lead to earlier disease control, less joint damage and a better prognosis. However, the diagnosis of RA is often difficult as patients do not always present with typical clinical features. Moreover, many other rheumatic or immune diseases can mimic the clinical presentation of RA. The use of diagnostic serological markers can help to make an early diagnosis, to predict the course of the disease and to guide treatment.

In this study, we recruited 52 patients with rheumatoid arthritis who had moderate to high disease activity and compared their RF and anti-CCP antibody status with patients who had other rheumatological diseases and with healthy controls.

Rheumatoid factor was present in 67.3% of RA patients, in 17.9% of patients with other rheumatological disorders and in none of the healthy controls. Positivity of RF in RA patients in our study is comparable to other studies by Bizzaro *et al.* [5], Ioannis *et al.* [7], Quinn *et al.* [14], Mehandiratta *et al.* [15] which have reported a positivity of 62.2%, 59.1%, 63% and 68%, respectively. In patients with other rheumatological disorders and in healthy controls, the positivity of RF was similar to that reported in previous studies by Ioannis *et al.* [7], Lin *et al.* [16] and Mehandiratta *et al.* [14].

Anti-CCP antibodies were positive (with a cut-off 15 U/ml) in 65.4% of patients with rheumatoid arthritis, a finding similar to that reported in by Ioannis *et al.* [7], Sakineh *et al.* [17], Bizzaro *et al.* [5] and Mehandiratta *et al.* [14].

Gupta *et al.* [18], Sghiri *et al.* [19] and Bombardieri *et al.* [20] have reported a higher positivity rate of anti-CCP antibodies in patients with RA. A

possible explanation for this finding is the use of a lower cut-off value (5 U/ml) of anti-CCP antibodies by these authors compared to the cut-off value (15 U/ml) used in our study.

Though, anti-CCP positivity was seen more in patients with disease duration > 2 years, no statistically significant correlation was found between anti-CCP positivity and duration of RA. Similar finding have been reported by Sghiri *et al.* in 2007 [19].

Anti-CCP antibodies were found to be positive in 7.7% (3/39) of patients with other rheumatological disorders and in none of the healthy controls. The three patients with other rheumatological disorders, who were anti-CCP antibodies positive, had ankylosing spondylitis, polymyositis and reactive arthritis. Previous studies have shown that anti-CCP antibodies can also be detected in a small percentage of patients with immune diseases other than RA like SLE, psoriatic arthritis, ankylosing spondylitis, systemic sclerosis and Sjogren's syndrome [21-23].

Ioannis *et al.* recruited 115 patients with RA and 100 healthy controls and tested RF and anti-CCP antibodies by nephelometry and ELISA, respectively. They found anti-CCP antibodies in 63.2% of patients with RA and in none of the healthy controls while RF was found to be positive in 59.1% of patients with RA and in 3% of healthy controls [7].

Lin *et al.* conducted a study on 145 patients with RA and 75 patients with other rheumatic diseases. They found anti-CCP antibodies in 119/145 patients (82.1%) with RA and 9/75 patients (12.0%) with non-RA rheumatic diseases while RF was positive in 116/145 RA patients and 27/75 non RA rheumatic diseases patients [16].

Bizzaro *et al.* studied 98 patients with RA, 174 with non RA rheumatological diseases and 58 controls. They found that anti CCP antibodies were present in 40 patients with RA (40.8%) and in 5 patients with other rheumatological diseases (3.0%) and in none of the controls. RF was positive in 61 patients with RA (62.2%) and in 36 patients with other rheumatic diseases (16%) [5].

However, the low prevalence of anti-CCP antibodies in these diseases as compared to RA suggests that anti-CCP antibodies are a reliable marker for diagnosing RA and for distinguishing RA from other rheumatological disorders.

Since the clinical presentation of ankylosing spondylitis, reactive arthritis and polymyositis is very different from that of rheumatoid arthritis, the finding of anti-CCP antibodies in patients clinically suspected

to have one of these disorders is unlikely to lead to a mistaken diagnosis of rheumatoid arthritis. At the same time, the fact that anti-CCP antibodies may be detectable in patients who do not have rheumatoid arthritis cautions against the use of this test as a diagnostic "gold standard" for rheumatoid arthritis. It would be erroneous to conclude that the mere presence of these antibodies establishes the diagnosis of RA.

To conclude, our study showed that anti-CCP antibodies are a more specific marker than rheumatoid factor for the diagnosis of RA. Anti-CCP antibodies also help in differentiating RA from other rheumatological disorders. Testing for both anti-CCP antibodies and RF increases the sensitivity for diagnosing RA thus helping in timely diagnosis and early institution of therapy to prevent complications and functional limitation due to the disease.

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