Possible Role of Anti-Cyclic Citrullinated Peptide Antibody (Anti-CCP Ab) in Rheumatic Diseases

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Abstract

Background: Anti-cyclic citrullinated peptide (anti-CCP) antibodies are highly specific for RA, but are not detectable in all RA patients, in addition their usefulness to identify rheumatic arthritis (RA) from other rheumatic diseases presenting with joint pain is not well studied.
Objective: To establish whether the clinical phenotypes of anti-CCP positive and negative disease are distinct at the earliest clinically apparent phase of disease and to assess the diagnostic accuracy of the anti-CCP antibodies assay to separate RA patients from a group of patients with (SLE).
Patients and Methods: One hindered ten blood samples were collected from patients, 75 with RA (58 seropositive and 17 seronegative) and 35 patients with SLE attending the Rheumatology Clinic of the Al-Yarmook Teaching Hospital for period between March 2009 and November of 2010 were included in this study. Then sera were stored at -20° and tested for anti-CCP antibodies by a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Euroimmun, while CRP and Rheumatoid factor was test by latex agglutination (LA).
Results: Forty five of 58 seropositive RA patients (77.6%) were positive for anti-CCP, while 13 patients tested negative for anti-CCP antibodies. Among the seronegative group, antibodies to anti-CCP could be demonstrated in 22.4% of patients. Anti-CCP positive patients were rheumatoid factor positive (77.6% vs. 22.4%, $p < 0.005$). There was no significant difference in the pattern of joint involvement. In the SLE group, anti-CCP antibody was positive in only 8 of 35 (22.8 %) patients.
Conclusions: Patients with and without anti-CCP antibodies present in a similar way, and anti-CCP antibody have high sensitivity for diagnosis of RA.
Keywords: Anti-CCP antibodies, C-reactive protein, rheumatoid arthritis, rheumatoid factor, systemic lupus erythematosus.

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الخلاصة

الخلفية: ان الاضداد المضادة للبيبتيد الحلقي cyclic citrullinated peptide هي عالية التخصص كأجسام مضادة لالتهاب المفاصل الروماتويدي، ولكن لا يمكن كشفها في كل مرضى التهاب المفاصل الروماتويدي، بالإضافة الى ذلك فان فائدتها لتحديد التهاب المفاصل الروماتيزمية (RA) من الأمراض الروماتيزمية الأخرى المصحوبة بآلام في المفاصل لم تدرس جيدا.

الاهداف: لتحديد ما إذا كانت الظواهر السريرية للمرض الموجب او السالب لمضادات ال CCP هي مميزة في المرحلة السريرية المبكرة للمرض وتقييم الدقة التشخيصية لفحص الأجسام المضادة المقاومة لل CCP لفصل مرضى التهاب المفاصل الروماتويدي عن مجموعة من المرضى الذين يعانون من (SLE)

النتائج: اظهرت النتائج ان خمسة وأربعين من ٥٨ مريضا من المرضى الموجبين مصليا لمرضى التهاب المفاصل الروماتويدي (٧٧.٦٪) موجبين ايضا لاضداد الCCP ، في حين أن ١٣ مريضا ظهروا سالبين لاضداد الCCP .اما المرضى السالبين مصليا لمرض التهاب المفاصل فقد تبين ان ٢٢,٤% منهم هو موجب لاضداد الCCP . وظهر ايضا ان المرضى الموجبين لاضداد الCCP هم ايضا موجبين ل rheumatoid factor (p<٠,٠٠٥ ، ٢٢,٤% vs. ٧٧,٦%) لم تظهر اية فروق معنوية في نمط شمول المفاصل.وظهر في مجموعة الSLE ان ٨ مرضى من ٣٥ مريضا (٢٢,٨%) هم موجبين لاضداد ال CCP.

الاستنتاجات: المرضى مع وبدون اضداد ال CCP ظهروا بشكل متشابه وان اضداد ال تمتلك حساسة عالية لتشخيص الامراض الروماتيزمية.

Introduction

Rheumatoid arthritis (RA) is a chronic, inflammatory condition typically manifesting clinically as a symmetrical polyarthritis. Rheumatoid synovitis is characterised by complex leukocyte and cytokine networks. The persistence of inflammation is mediated, in part, by the stromal micro-environment, but the underlying causes remain unclear [1,2]. Over the last decade there has been particular interest in antibodies to citrullinated peptides and proteins as important aetiological and predictive factors in early RA [3,4,5].

These autoantibodies(anti-cyclic-citrullinated peptide [anti-CCP] antibodies) are highly specific serological markers for rheumatoid arthritis (RA) that are thought to be directly involved in the disease pathogenesis [6] . Citrullinated proteins are not exclusively located in synovial tissue of RA patients, but can also be found in synovium samples of patients with other inflammatory joint diseases [7] – suggesting that the specificity of anti-CCP antibodies for RA is not due to the expression of citrullinated proteins, but might be the result of an abnormal humoral response.

Intriguingly, this antibody response may occur years before any clinical symptoms, as shown by the presence of anti-CCP antibodies several years before the clinical onset of arthritis [8,9] . Furthermore, a proportion of RA patients do not harbour anti-CCP antibodies, suggesting that the presence of anti-CCP antibodies is not obligatory for the development of arthritis or that the pathogenic mechanisms underlying anti-CCP-positive RA and anti-CCP-negative RA are different [9].

These observations inspired subsequent research addressing the question of whether RA patients with anti-CCP antibodies are different from those who are anti-CCP-negative.

Although a number of studies have assessed the role of anti-CCP antibodies assay in the diagnosis of RA, the ability of this assay to differentiate an RA patient from another rheumatic disease patient has not been adequately addressed [10,11,12]Because the usefulness of this assay largely rests in distinguishing RA from other rheumatic disorders, we aimed to assess the diagnostic accuracy of the anti-CCP antibodies assay to separate RA patients from a group of patients

with SLE, and thus find the sensitivity and specificity of anti-CCP antibodies in RA.

Patients and Methods

A total of 110 patients were obtained from the Rheumatology Consultation Clinic of Al-Yarmook Teaching Hospital from 2009 to end of 2010. These patients had joint pain as one of the clinical presentation. There were divided into two groups:

Group (1): included 75 patients with RA as defined by the American collage of Rheumatology 1987, there were 54 female (75 %) and 21 male (28 %) , female to male ratio is 2.5:1 with range between 17-69 years with mean (41.9± 12.5).

Group (2): included 35 patients with SLE considered as control group, diagnosed by applying the respective American College of Rheumatology criteria 1997. There were 27 female (77%) and 8 male (23%) male to female ratio is 3:1, with range between 18-68 years with mean age (52.4±19.6).

Methodology

Sera were stored at -20° and tested for anti-CCP antibodies by a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Euroimmun, Germany), according to manufacture instructions a reading >5 units was considered positive. Rheumatoid factor (for the IgM isotype) and CRP were test by latex agglutination (Euroimmun, Germany). RA patients positive or negative for RF are termed as seropositive and seronegative, respectively. Radiological assessment was done in radiographs of both hands and feet.

Statistical analysis

Statistical analysis was performed using SPSS statistical software version 11.5. The anti-CCP positive and negative groups were compared, with differences in means assessed using a two-tailed unpaired student t-test. Proportions were compared using a chi-squared test. P values < 0.05 were considered significant.

Results

Fifty eight of the 75 RA patients (77.3%) were rheumatoid factor positive by latex agglutination assay and 17 patients (22.7 %) were seronegative.

Forty five out of 58 seropositive RA patients (77.6%) were positive for anti-CCP, while 13 (22.4%) were negative for anti-CCP antibodies, as shown in table (1).

Table (1): The study groups with and without anti-CCP.

Studied group		**Anti-CCP positive**	**Anti-CCP negative**
Patients with RA	Sero positive (58)	45 (77.6%)	13 (22.4%)
	Sero negative (17)	7 (41.17%)	10 (58.82%)
Total number	75 (100%)		

The patient with anti-CCP positive and anti-CCP negative were comparable in many terms and show no significant differences in gender, age and durations of symptoms. The exception, however, was that anti-CCP positive patients were significantly more likely to be seropositive for rheumatoid factor (77.5% vs. 28.5%, p = 0.005), as demonstrated in table (2).

Table (2): characteristic of patients with and without anti-CCP antibody.

	Anti-CCP antibody positive(n=45)	Anti-CCP antibody negative (n=13)	P-value
Female (%) **Male (%)**	73.3% 26.7%	69.2% 30.8%	0.3
Age in years (mean±SD)	50.2±18.4)	54.8 ±15.2)	0.6
Disease duration in month (median)	34 (3-220)	33(2-216)	0.7
RF test positivity %	77.5%	28.5%	0.005
CRP test positivity %	65.7%	66.8%	0.4
ESR mm/1h (mean±SD)	45 ± 10.0	42± 12.0	0.7

The present study showed that there were no significant differences between anti-CCp positive and negative with different clinical features like joint swelling, joint stiffness, ocular disturbances, joint effusion, rheumatoid and joint deformity based on t-test of analysis as shown in table (3).

Table (3): Association of anti-CCP Ab with clinical features of RA.

Clinical features	Anti-CCP Ab +ve % (N=45)	Anti-CCP Ab -ve % (N=13)	P-value
Joint swelling	81.9	80.7	0.5
Joint stiffness	84.2	85.1	0.8
Occular disturbances	47.5	22.6	0.4
Joint effusion	8.8	7.9	0.2
rheumatoid nodules	13.8	15.1	<0.7
joint deformity	45.6	44.8	0.2

In the SLE group, the result of ELISA technique have demonstrated that 8 out 35 (22.8%) were positive for anti-CCP while 27 out 35 (77.2 %) were negative for anti-CCP as shown in table (4).

Table (4): The result of ELISA in SLE group.

Studied group		
Patients with SLE	Anti-CCP positive	Anti-CCP negative
	8 (22.8%)	27(77.2%)
Total number	35(100%)	

During the samples collection dimorphic detail were recorded, which include radiographs of hands and feet that showed erosions in 50.8% of the patients, other clinical presentation were also recorded.

Discussion

The above results denoted a high prevalence of RA among women rather than men, which may be due to the hormonal differences between them and in turn, theirs effects on the immune responses. Those make women normally tend to mount more robust IRs and these responses tend to be more TH1 - like responses rather than TH2 response, which are pro-inflammatory, hence may enhance the development of autoimmunity [13].

The female to male ratio was 2.5:1 which is nearly comparable to that reported by Ubaid, (2.7:1) [14], and Al-Haidary, (2.9:1) [15]. Meanwhile were this result compatible to abroad that showed the ratio of 3:1, 2.7:1,

and 2.4:1, which had been reported by many researchers (16,17,18) respectively.

The mean age of the disease was (41.9 ±12.5), however Al-Haidary study in 2003 showed that the average of age is 42.1, which was almost comparable to this result [15]. While it was to some extent lower than that of Anaya, et. al. [19] and Pascual et.al. [20] who observed that the mean age was 47±12.7 years for the Colombian RA woman and 49 ± 2.5 among Spanish patients respectively. The lower mean of the age probably is due to the fact that the life spans of Iraqi are lowers than that for European.The positivity of RF was observed 77.3% in the sera of RA patients. While other studies showed 88% in Nebraska, 66% in Germany, 70.2% in Colombia and 72.2% in North America and 80% in Southern Spain of the RA cases as mentioned by [19,21,22,23] respectively. The high RF positivity in this study was probably related to use ELISA technique, which is highly sensitive one than Latex agglutination test that has been used by many other investigators.

This study shows that the phenotype of RA patients with or without anti-CCP antibodies does not differ at clinical presentation. This is perhaps surprising given the emerging consensus that anti-CCP positive and negative states represent distinct clinical entities. There is mounting evidence suggesting not only separate molecular mechanisms underlying these two patient groups, but also different genetic and environmental predispositions as well as a different clinical progression. In a large, prospective, early arthritis cohort , the scientists observed neither a significant difference in the reported first symptoms nor in the signs found in the physical examination at initial presentation between anti-CCP-positive patients and anti-CCP-negative patients. During follow-up, however, anti-CCP-positive RA patients have more swollen joints and show more radiological destruction than anti-CCP-negative RA patients [13].

However, our data provide evidence that, despite these pathological differences, there is a shared clinical phenotype for RA presentation regardless of anti-CCP status. Not only do patients with anti-CCP positive and negative disease present with similar distributions of joint disease, but they also have comparable levels of inflammation. However, Lee AN and co-workers reported that, anti-CCP positive patients do fulfill significantly more ACR criteria for RA than anti-CCP negative patients. This is predominantly due to the fact that anti-CCP positive patients more often express rheumatoid factor, a well described phenomenon [24].

Seven of the 17 seronegative patients were also positive for anti-CCP antibodies as shown in table (1). Therefore, a positive anti-CCP antibody supports the diagnosis of RA when RF is negative in the appropriate clinical setting. Thus, anti-CCP antibody serves as a better diagnostic marker in the diagnosis of RA, especially to detect the seronegative group.

Anti-CCP antibodies are important for diagnosis in RA because they are as sensitive as and more specific than the IgM RFs in early and fully established diseases. In addition, they may predict the eventual development into RA when found in undifferentiated arthritis and they may be detected in healthy individuals' long before onset of clinical RA [17,18,19]. Anti-CCP antibodies and RF are superior to several genetic markers in predicting the diagnosis of RA from undifferentiated arthritis in early arthritis patients [20]. In addition, the combination of anti-CCP antibodies and IgM-RF has been found to have a high positive predictive value for RA [21]. Most of these studies had a normal group as the control population and a few other studies used patients with other rheumatic diseases

as the control group [8,13].In this study, we compared the diagnostic specificity of anti-CCP antibodies in RA with respect to patients who have rheumatic diseases other than RA. Bizzaro *et al.* (2001) found anti-CCP antibodies to be 41% sensitive and 97% specific in diagnosing RA compared to patients with other rheumatic diseases [25]. The low frequency of anti-CCP antibodies in the SLE group, leads us to conclude that a patient with joint pain with anti-CCP antibodies positivity is most likely to have RA rather than a different rheumatic disease.

In addition, the determination of anti-CCP antibodies is important for prognosis. Kroot *et al*.(2000) reported anti-CCP positivity in 70% of 273 RA patients and after 6 years of follow-up, patients who were anti-CCP positive at entry had more radiological damage [26]. Similarly, Schellekens *et al*.(1998) found that both anti-CCP antibodies and IgM RF positivity at first visit predicted erosive changes after 2 years of follow-up [3]. Some studies have shown that anti-CCP antibodies were not associated with severe disease (erosions on radiographs). In this work, we found that anti-CCP antibody positive patients did not have any significant erosion on plain radiograph when compared with the anti-CCP antibodies seronegative group. This is probably because the sample size is small.

In conclusion, the present study shows that, although separate risk factors for anti-CCP-positive RA and anti-CCP-negative RA have been recently described, the clinical presentation of RA patients with or without anti-CCP antibodies is not different. The use of anti-CCP antibodies may allow the clinical rheumatologist to better predict the diagnosis and prognosis of individual patients with RA.

In addition, a positive anti-CCP antibody results in seronegative RA patients, strongly supports the diagnosis of RA serologically.

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