Serum anti-cyclic citrullinated peptide antibodies before and after treatment by disease-modifying anti-rheumatic drugs

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Original Article

Abstract

BACKGROUND: Rheumatoid arthritis (RA) is the most common chronic disease involving joints. Anti-cyclic citrullinated peptide (anti-CCP) as a specific antibody is a reliable index to early diagnosis of RA. Disease-modifying anti-rheumatic drugs (DMARDs) can reduce progression of RA joint destruction. The present study aimed to investigate the effects of DMARDs in reducing serum anti-CCP.

METHODS: A cross-sectional study was performed on 30 patients including 22 females and 8 males RA patients according to the American College of Rheumatology (ACR) classification criteria, who referred to the Rheumatology Clinic. Treatment with DMARD group started at the beginning of the study (May 2009). At 1st and 6th month of the study, clinical findings and disease activities were recorded and anti-CCP was measured.

RESULTS: At the beginning and the end of the study, morning stiffness for more than 1 h and involvement of three areas were, 28 (93%) and 12 (40%), respectively. Indicators of disease severity in patients, the mean ± SD serum levels of erythrocyte sedimentation rate at the beginning and end, were 40.7 (30-59) mm/1 h and 13.4 (9-86) respectively. Anti-CCP at the beginning and end of the study was 141.83 (65.8-101.09) U/ml and 65.8 (62-92) U/ml respectively (P < 0.05). Disease Activity Score in 28 joints and rheumatoid factor positive and C-reactive protein positive were significantly different at the onset and at the end of the study (P < 0.05).

CONCLUSION: Measurement of serum anti-CCP is a helpful index of treatment response and monitoring of treatment efficacy in patients with RA.

KEYWORDS: Rheumatoid Arthritis, Anti-Cyclic Citrullinated Peptide Antibodies, Disease-Modifying Anti-Rheumatic Drug Group

Introduction

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Rheumatoid arthritis (RA) is a chronic inflammatory disease in which the Synovium tissue can be attacked more than any other organs. Despite of increased medical costs, the

quality of life is impaired in many patients clearly. The American College of Rheumatology (ACR) criteria usually is used for classification of RA,[1] which does help neither early diagnosis nor determination the severity of disease. Laboratory tests can lead to fast diagnosis. Measurement of anti-cyclic citrullinated peptide (anti-CCP) antibodies besides other antibodies such as prinuclease antibodies, anti-cratin antibody, anti-filagrin antibody can confirm the diagnosis.[2,3]

The current blood marker is rheumatoid factor (RF), anti-Fc fragment of IgG, which is neither specific nor sensitive enough for diagnosing RA. RF titer in the early stages of the disease may not be sufficient enough for the diagnosis and disease prognosis. Therefore, treatments based on this finding may not be enough. Finding a marker that indicate rheumatoid arthritis diagnosis at early stages, could be helpful for prognosis and monitoring of responses to the treatment.[4] Citrulline, as an unusual amino acid, is produced by an enzymatic reaction of arginine. It is as a part of the protein building such as filagrin and profilagrin. During cell differentiation process, this protein will be produced. In RA patients, anti-CCP antibodies are synthesized against this atypical protein. Anti-CCP measurement is a method for determining the prognosis of arthritis because it accompanies with more destructive forms of RA. Anti-CCP test has a high sensitivity and specificity and it is checked via enzyme-linked immunosorbent assay (ELISA) and using patients, serum.[5]

Several studies have pointed out the positive predictive value of anti-CCP for prognosis of RA. However, it has also been found in approximately 1.5% of healthy people and in the people with other joint diseases.[6] The disease-modifying anti-rheumatic drugs (DMARDs) group is one of the five group therapies that have been used in the treatment of rheumatoid arthritis. DMARDs including a group of drugs that are structurally uniform. Their common characteristic is slowing down the progression of the disease. They have been accepted as the basis of the treatment of RA.[7]

Anti-CCP levels are increasing in early RA disease leading to joint destruction.[8] There are several evidences showing that increased levels of anti-CCP are correlated with progression of radiological joint damage.[9,10]

Several studies have mentioned the relation between serum anti-CCP and DMARD therapy, but they have found some conflicting results.[11,12] disease activity score in 28 joints (DAS28) is a qualitative scale for evaluation of the disease activity and contains four parameters [frequency of swollen joints and joints with tenderness, erythrocyte sedimentation rate (ESR), and visual analog scale (VAS)] and calculated mathematically. Values less than 2.6 were considered as a response to treatment. Anti-CCP in severe forms of RA which can clearly be identified. DMARDs delay progression of the disease.[13]

The present study aimed to investigate the effects of DMARDs in reducing serum anti-CCP. In fact it was designed and performed to address the possibility of if DMARDs interfere in RA pathology by reducing serum anti-CCP as well as application of anti-CCP for monitoring of the responses to DMARD therapy.

Materials and Methods

A cross-sectional study was performed on 30 RA patients who referred to the Clinic of Rheumatology in Towhid Hospital, Sanandaj, Iran. The serum anti-CCP was measured before and after treatment with DMARD group in RA patients.

Duration of symptoms, morning stiffness, number of joints and hand joints involvement, symmetric joint involvement, radiological changes due to RA, rheumatoid nodules, serum RF, ESR, VAS, and anti-CCP and index disease severity scale (DAS28) were also measured and recorded at the beginning of study. From May 2009 to February 2010 (8 months), 51 patients with a primary diagnosis of RA were referred to the rheumatology outpatient clinic. Patients who did not have an acceptable standard or whom refused of performing the experiments or those with medical

complications were excluded from the study.

Based on the American College of Rheumatology (ACR) criteria classification and clinical picture, 30 patients (22 women and 8 men) with diagnosis of RA were recruited. Treatment with DMARD group started at the beginning of the study. At the 1st and 6th month of the study, clinical findings, and disease activities were recorded and anti-CCP was measured.

After giving a simple explanation to patients, a written consent was obtained. Patient characteristics including the initial signs and symptoms of the disease were recorded. Hand radiographies (by radiologists and rheumatologists) was undertaken and recorded according to SHARP score.

Serum titration of anti-CCP and other tests were ordered and performed for free. To avoid errors and reducing the potential difference between the results of tests conducted in the laboratory, a standard kit was used. At the start of study DMARD, 7.5 mg prednisone daily, hydroxychloroquine 200 mg daily and oral methotrexate (10 mg/week) were prescribed.

By using a checklist, the side effects and clinical examination were investigated and recorded. In 2 of 30 patients, methylprednisolone 80 mg was injected intra-articular and in one patient methotrexate was increased to 15 mg weekly and hydroxychloroquine to 400 mg daily due to severe knee arthritis.

Six months after taking DMARDs, serum anti-CCP, ESR, C-reactive protein (CRP), RF, VAS, and hand radiographs were measured once again. Disease severity index DAS28 that shows the severity of pain and inflammation of the patients were calculated at the beginning and the end of the study. The DAS28 includes joint tenderness (0-28), number of swollen joints (0-28), and ESR. The VAS method was calculated by Excel software. This method is used for pain quality determination and is stated as a horizontal line from 0 to 10 cm long (0 means no pain and 10 means the most intense pain felt by the patient).

Of all 30 patients with RA, 10 cc of blood was taken and serum was separated for testing.

Collected samples were transported as frozen to the Unity Center of Immunology Laboratory in hospital. RF was measured via latex agglutination method by using commercial kits Genesis® Company. An anti-CCP antibody was measured by using commercial kits Genesis® that measures anti-CCP antibody of IgG class. In this method, the ELISA microplates have been covered with recombinant citrullinated filagrin.

Control samples with known concentration were also investigated to control the accuracy and reliability of standardized tests in parallel and the results were recorded.

McNemar and Bhapkar's test and Spearman Rank correlation test and Mann–Whitney test were used for data analysis. A probability value of 5% was statistically considered significant.

Results

Eligible patients for the present study were 30 individuals including 22 females (74%) and 8 males (26%). The mean age ± SD of subjects was 48.9 ± 9.72 years. The morning stiffness for more than 1 h and involvement of three areas were, 28 (93%) and 12 (40%), respectively.

Involvement of hand joints at the beginning and end of the study was observed in 30 cases (100%). Involving counterparts joints at the beginning and end of the study were 28 (93%) and 3 (10%), respectively. The corresponding rates was 3 (10%) and 1 (3%) for nodule and 25 (83%) and 9 (30%) for RF titer 1+ (P < 0.05).

Mean and standard deviation of ESR's serum levels at the beginning of study were 40.7 and 30.59 and 10.50 ± 9.86 at the end of study. Median VAS score ± interquartile range (IQR) at the beginning and end of the study were 58.17 ± 20.06 and 16.5 ± 8.92 respectively. Moreover, median DAS ± IQR at the beginning and end of the study, were 6.18 ± 0.94 and 3.17 ± 1.42, respectively.

CRP was positive in 21 patients (70%) at the beginning and only in 12 patients (40%) at the end of the study (P < 0.007). A total of 30 (100%) patients had theirserum anti-CCP positive (> 18 U/ml). The median level of anti-CCP at the

Table 1. Comparison of American College of Rheumatology criteria in rheumatoid arthritis patients before and after treatment with disease-modifying anti-rheumatic drugs

Variable	At the beginning, number (%)	At the end of 6th month, number (%)	P
Morning stiffness > 1 h	28 (93)	12 (40)	< 0.001
Involvement > 3 area	28 (93)	13 (43)	< 0.001
Involvement of hand joints	4 (100)	4 (13)	< 0.001
Involving of counterparts joints	28 (93)	3 (10)	< 0.001
Nodules	3 (10)	1 (3)	0.470
RF$^+$	25 (83)	9 (30)	< 0.001
Radiologic changes	29 (96)	8 (26)	< 0.001

Statistical test: McNemar; RF: Rheumatoid factor

beginning and end of the study was 127 and 25 U/ml, respectively (P < 0.001). The mean ± SD of duration of symptoms until diagnosis was 15.73 ± 13.41 weeks. The history and physical exams and supplementary laboratory tests were negative for all 30 patients. DAS28 as a measure of disease activity in 26 cases (86%) was more than 5.1 and in 4 cases (13%) was higher than 3.1. These finding represent the severe and moderate rates of disease activity. At the end of study, 9 of 30 patients treated with the index DAS28 lower than 2.6.

At the end of 6th month, a significant association was observed between low serum anti-CCP and low ESR, low CRP, lower VAS, and a decrease in DAS28 (P = 0.001). Majority of patients (97%) had an abnormal serum anti-CCP level while this proportion was 85% in RA (Tables 1-4).

Table 2. Changes in Disease Activity Score in 28 joints in rheumatoid arthritis patients before and after treatment with disease-modifying anti-rheumatic drugs

At the beginning	After 6 month				
	Rem	LDA	MDA	HDA	Total
Rem	0	0	0	0	0
LDA	0	0	0	0	0
MDA	1	1	1	1	4
HDA	8	11	5	2	26
Total	9	12	6	3	30

P < 0.001; Rem: Remission; LDA: Low dose activity; MDA: Moderate dose activity; HDA: High dose activity; Statistical test: Bhapkar's test

Table 3. Association between Disease Activity Score in 28 joints and other parameters of disease activity in rheumatoid arthritis patients after 6th month

Parameter	Rho	P
RF	0.43	0.016
ESR	0.30	0.081
CRP	0.27	0.205
VAS	0.002	0.521
Radiologic changes	0.22	0.239

Statistical test: Spearman Rank correlation test; RF: Rheumatoid factor; ESR: Erythrocyte sedimentation rate; CRP: C-reactive protein; Anti-CCP: Anti-citrullinated cyclic protein, VAS: Visual analogue acales

Table 4. Association between anti-cyclic citrullinated peptide levels and positive rheumatoid factor before and after of treatment in rheumatoid arthritis patients

Parameter	RF$^-$	RF$^+$	Anti-CCP$^+$	Anti-CCP$^-$	Total
RF$^-$	5	0	-	-	5
RF$^+$	16	9	-	-	25
Anti-CCP$^+$	-	-	19	11	30
Anti-CCP$^-$	-	-	0	0	0

P = 0.0002; Statistical test: McNemar; RF: Rheumatoid factor; P = 0.0025; Statistical test: McNemar; Anti-CCP normal: < 18 U/ml; Anti-CCP Abnormal: > 18 U/ml; Anti-CCP: Anti-cyclic citrullinated peptide

Discussion

In the present study, a clear reduction in the symptoms was observed and most patients showed a good response to treatment over 6 months. These findings are consistent with other studies.[12-14]

For all patients, the early indicators of disease activity were significantly decreased by treatment with a DMARD group. Previous studies have

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reported similar findings. However, decreasing the DAS28 has not been mentioned in these studies.[12-14]

For all 30 patients included in the present study, the anti-CCP had decreased significantly. In the Ronnelid et al. study a reduction in the serum anti-CCP on sulfasalazine was found at the 1st year only.[13] In the Mikuls et al. study, the RF level, but not the anti-CCP level was decreased significantly ($P \leq 0.010$).[14] Through, in the other study, serum IgM, but not anti-CCP was reduced significantly.[15,16]

According to the entering criteria for the study, all patients had unusual serum anti-CCP at the beginning of the study. At the end of study, due to drug intake 10 patients became RF negative. But anti-CCP was detectable which reflects the greater specificity of the anti-CCP. During the course of treatment, the RF in 11 patients became negative, but serum anti-CCP level was still higher than the normal values.

At the beginning of study, none of patients were RF positive and anti-CCP was lower than normal, but in all 30 RA patients who had ACR87 criteria, the anti-CCP levels were higher than normal. At the end of the study, there was only one positive RF with anti-CCP levels lower than normal. But at the same time in eight patients with positive RF, there was positive serum anti-CCP. These findings indicate a similar specificity (68%) and more sensitivity (89%) of RF compared with anti-CCP ($P < 0.050$), as shown in other reports.[15-18] This might be due to existing antibodies against heterogeneous citrulline or different epitopes of citrulline molecules. Every patient can produce antibodies of different affinity, while kits are used for identification anti-CCP in the laboratory, consisting limited antigens.

The main limitation of this study was the presence of patients who were taking other drugs, and referred to different clinics. However, they were excluded from the study. Another limitation of the study was the small sample size due to time constraints.

Conclusion

Measurement of serum anti-CCP is a helpful index of treatment response and monitoring of treatment efficacy in patients with rheumatoid arthritis. Early indicators of disease activity could be significantly decreased by treatment with DMARD group. For patients with a suspected RA, along with clinical examination, measurement of anti-CCP levels and the positive rate is recommended. Anti-CCP measurement along with the physical exam and index DAS28 can be useful as indicator in monitoring patients treated for rheumatoid arthritis.

Conflict of Interests

Authors have no conflict of interests.

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