Anti-Cyclic Citrullinated Peptide Antibodies in Rheumatoid Arthritis and Their Relationship with Extra-Articular Manifestations

Romatoid Artritte Anti-Siklik Sitrulinli Peptid Antikorlar ve Bunların Ekstra-Artiküler Bulgular ile İlişkisi

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Objectives: In this study, we investigated the relationship between extra-articular manifestations (EAM) and anti-cyclic citrullinated peptide (anti-CCP) antibodies.

Patients and methods: Sixty-seven patients (53 females, 14 males; mean age 57.4±13.5 years; range 26 to 82 years) diagnosed with rheumatoid arthritis (RA) according to the ACR (American College of Rheumatology) criteria between January 2009 - December 2009 were included in the study. The age, gender, disease duration, and EAMs (rheumatoid nodules, pulmonary nodules, pulmonary fibrosis, keratoconjunctivitis sicca) of each patient were recorded, and the anti-CCP antibody and rheumatoid factor (RF) positivity and serum RF levels were evaluated.

Results: Extra-articular manifestations were present in 28 (41.8%) of the patients. Anti-CCP antibodies were present in 34 (50.7%) patients. Positivity of anti-CCP antibodies was found in 16 (57.1%) patients with EAM compared to 18 (46.2%) patients without EAM (p>0.05). Rheumatoid factor positivity was found in 23 (82.1%) patients with EAM compared with 30 (76.9%) patients without EAM (p>0.05). The serum levels of RF were 176.5±201.7 IU/ml in the patients with EAM, and 114.1±144.8 IU/ml in the patients without EAM (p>0.05). The mean serum titers of RF were significantly higher in the patients with pulmonary fibrosis than in those without pulmonary fibrosis (237.8±204.8 IU/ml and 118.9±158.6 IU/ml respectively; p=0.036).

Conclusion: It is known that EAMs increase morbidity and mortality in patients with rheumatoid arthritis. Thus, the markers that help identify patients with severe disease have an important role in making a decision for early aggressive treatment. Studies that investigate the relationship between anti-CCP antibodies and EAM have conflicting results. In this study, no relationship was found between the antibodies against citrullinated peptides and the presence of EAMs. We think that further studies with a larger sample size are necessary to evaluate the relationship between EAM and anti-CCP antibodies.

Key words: Anti-CCP antibodies; extra-articular involvement; rheumatoid arthritis; rheumatoid factor.

Amaç: Bu çalışmada anti-siklik sitrulinli peptid (anti-CCP) antikorlar ile ekstra-artiküler tutulum (EAT) arasındaki ilişki araştırıldı.

Hastalar ve yöntemler: Çalışmaya Ocak 2009 - Aralık 2009 tarihleri arasında ACR (American College of Rheumatology) tanı kriterlerine göre romatoid artrit (RA) tanısı konulan 67 hasta (53 kadın, 14 erkek; ort. yaş 57.4±13.5 yıl; dağılım 26-82 yıl) dahil edildi. Tüm hastaların yaşları, cinsiyetleri, hastalık süreleri ve EAT'leri (romatoid nodül, pulmoner nodül, pulmoner fibroz, keratokonjonktivitis sikka) kaydedildi ve romatoid faktör (RF) ve anti-CCP antikor pozitifliği ve serum RF seviyeleri değerlendirildi.

Bulgular: Hastaların 28'inde (%41.8) EAT saptandı. Otuz dört hastada (%50.7) anti CCP antikorlar mevcuttu. Anti-CCP antikor pozitifliği EAT'li 16 hastada (%57.1) saptanırken, EAT'si bulunmayan 18 hastada (%46.2) saptandı (p>0.05). Romatoid faktör pozitifliği EAT'li 23 (%82.1) hastada mevcut iken, EAT'si olmayan 30 hastada (%76.9) mevcuttu (p>0.05). Serum RF seviyeleri EAT'li hastalarda 176.5±201.7 IU/ml iken, EAT'siz hastalarda 114.1±144.8 IU/ml idi (p>0.05). Pulmoner fibrozisi olan hastaların ortalama serum RF titreleri pulmoner fibrozisi olmayanlara göre anlamlı şekilde daha yüksek (sırasıyla 237.8±204.8 IU/ml ve 118.9±158.6 IU/ml; p=0.036) idi.

Sonuç: Romatoid artritli hastalarda EAT'lerin mortaliteyi ve morbiditeyi artırdığı bilinmektedir. Bu nedenle şiddetli hastalığı olan hastaların saptanmasına yardımcı olan belirleyiciler erken agresif tedaviye karar verilmesinde önemli rol oynamaktadır. Anti-CCP antikorları ile EAT arasındaki ilişkiyi araştıran çalışmalarda çelişkili sonuçlar bulunmuştur. Bu çalışmada CCP pozitifliği ile EAT varlığı arasında herhangi bir ilişki saptanmadı. Anti-CCP antikorları ile EAT arasındaki ilişkiyi değerlendirmek için daha fazla sayıda hasta içeren çalışmalara gereksinim olduğu düşüncesindeyiz.

Anahtar sözcükler: Anti-CCP antikorları; ekstra-artiküler tutulum; romatoid artrit; romatoid faktör.

Rheumatoid arthritis (RA) is a chronic inflammatory disease in which the peripheral joints are the primary sites of inflammation. It often leads to destruction in the joints involved. Extra-articular manifestations (EAM) occur, especially in patients with long-standing RA.[1] The exact etiology of the disease has not been clearly defined to date. However, strong evidence suggests autoimmunity since several autoantibodies are associated with RA. Rheumatoid factor (RF), which is currently used in the diagnosis of RA, represents one of the classification criteria proposed by the American College of Rheumatology (ACR).[2] In recent years, the assay for anti-cyclic citrullinated peptide (anti-CCP) antibodies, another diagnostic test for RA, has been developed. These serological markers are considered to be important in determining the prognosis of the disease.[3-9] The presence of anti-CCP antibodies and IgM RF are associated with a higher probability of radiological signs of joint damage.[10] In a former study which investigated the relationship between anti-CCP antibodies and radiologic damage in Turkish patients with RA, it was found that there were no significant relations between anti-CCP antibodies and radiologic parameters. They also stated that further studies with larger groups were indicated to investigate this association.[11] In studies on patients with active, severe extra-articular disease, high levels of RF were determined, suggesting that RF contributes to the pathogenesis of EAM.[12] Studies investigating the association between CCP and EAM have had contradictory results. The aim of this study was to analyze the association of EAM (rheumatoid nodules, pulmonary nodules, pulmonary fibrosis, keratoconjunctivitis sicca) with anti-CCP antibodies.

PATIENTS AND METHODS

Sixty-seven patients (53 females, 14 males; mean age 57.4±13.5 years; range 26 to 82 years) attending the Physical Medicine and Rehabilitation Clinic of Zonguldak Karaelmas University Hospital and fulfilling the American College of Rheumatology (ACR) criteria for RA[2] were included in the study. The local ethics committee of Zonguldak Karaelmas University approved the study.

Blood samples were obtained from all the patients, and the age, gender, and disease duration along with the presence of EAM (rheumatoid nodules, pulmonary nodules, pulmonary fibrosis, keratoconjunctivitis sicca) were recorded.

Serum IgM RF concentrations were measured by automated immunonephelometry on the BN ProSpec System (Dade Behring Inc., Newark, USA) by using a commercially available N Latex RF kit. (Dade Behring Inc., Newark, USA). The cut-off value was 15 IU/ml. Second generation anti-CCP was analyzed by using a microplate ELISA kit (Euroimmun Medizinische Labordiagnostika AG, Luebeck, Germany) according to the manufacturer's instructions.

Statistical analysis

The statistical analyses were performed using SPSS (SPSS Inc., Chicago, Illinois, USA) for Windows 11.0 version software. Differences in means between the groups were analyzed with the Mann-Whitney U-test. Proportions were compared using the chi-square test. A binary multiple logistic regression model was created to evaluate the effects of RF and anti-CCP antibody positivity along with duration of disease for the development of EAM. While creating the model, duration of disease was divided into two categories through ROC analysis. The cut-off values of this group were found to be 5.5 years (sensitivity: 60.7%, specificity: 66.6%, likelihood ratio: 1.8). P values less than 0.05 were considered statistically significant.

RESULTS

A total of 67 patients were involved in the study with 53 (79.1%) being female and 14 (20.9%) being male. They had a mean age of 57.4±13.5 (26-82) years old, and the mean duration of disease was 10.1±11.7 (1-46) years. Extra-articular manifestations were present in 28 (41.8%) of the patients [Keratoconjunctivitis sicca: three (4.5%), subcutaneous nodules: 10 (14.9%), pulmonary nodules: nine (13.4%), pulmonary fibrosis: 12 (17.9%)].

Anti-CCP antibodies were present in 34 (50.7%) patients. Positivity of anti-CCP antibodies was found in 16 (57.1%) patients with EAM compared to 18 (46.2%) patients without EAM (p>0.05). Fifty-three (79.1%) patients were positive for RF. Rheumatoid factor positivity was found in 23 (82.1%) patients with EAM compared to 30 (76.9%) patients without EAM (p>0.05). Fifteen patients with EAM (65.2%) were both anti-CCP positive and RF positive compared to 18 (60%) patients without EAM (p>0.05). Serum levels of RF were 176.5±201.7 IU/ml (mean±SD) in the patients with EAM and 114.1±144.8 IU/ml in the patients without EAM (p>0.05). Disease duration was significantly longer in the patients with EAM (11.5±10.7 years) than in the patients without EAM (9.2±12.4 years; p=0.046; table 1). The mean serum titers of RF were significantly higher in the patients

Table 1. Clinical and laboratory findings of the patients

	n	%	Mean±SD	Median	(min.-max.)	n	%	Mean±SD	Median	(min.-max.)	p
			Patients with EAM					Patients without EAM			
Mean age (years)			58.1±12.5	56.5	(33-82)			56.9±14.4	60	(26-82)	>0.05
Disease duration (years)			11.5±10.7	8	(1-42)			9.2±12.4	3	(1-46)	0.046
Positivity of anti-CCP antibodies	16	57.1				18	46.2				>0.05
RF positivity	23	82.1				30	76.9				>0.05
RF levels (IU/ml)			176.5±201.7	72	(0-604)			114.1±144.8	53	(0-604)	>0.05

EAM: Extra-articular manifestations; SD: Standard deviation; min.: Minimum; max.: Maximum; CCP: Cyclic citrullinated peptide; RF: Rheumatoid factor.

with pulmonary fibrosis than in the patients without pulmonary fibrosis (237.8±204.8 IU/ml vs. 118.9±158.6 IU/ml p=0.036).

Positivity of anti-CCP antibodies was present in seven (70%) patients with subcutaneous nodules compared to 27 (47.3%) patients without subcutaneous nodules (p>0.05); in one (33.3%) patient with keratoconjunctivitis sicca compared to 33 (51.6%) patients without keratoconjunctivitis sicca (p>0.05); in five (55.5%) patients with pulmonary nodules compared to 29 (50%) patients without pulmonary nodules (p>0.05); and in seven (58.3%) patients with pulmonary fibrosis compared to 27 (49.1%) patients without pulmonary fibrosis (p>0.05).

A logistic regression model was created to evaluate the effects of RF in conjunction with anti-CCP antibody positivity and duration of disease for the development of EAM. A binary multiple logistic regression analysis with duration of disease and positivity of RF and anti-CCP antibodies revealed that duration of disease longer than 5.5 years caused a 2.96-fold increase in the risk for EAM. Positivity of RF and anti-CCP antibodies were not associated with the development of EAM (table 2).

DISCUSSION

Rheumatoid arthritis is an inflammatory and autoimmune disease that is responsible for joint destruction. Extra-articular manifestations may occur depending on the severity. Inadequate treatment of RA results in a significantly reduced quality of life, increased morbidity, and mortality in the long-term.[13] Thus, early diagnosis and proper treatment is highly crucial for the prognosis of RA. A decision can be made for early aggressive treatment using the markers that help determine patients with severe disease. Clinical factors such as age of onset, number of joints involved, persistently elevated C-reactive protein, and presence of RF are well-recognized predictors of severe disease.[14,15] Moreover, anti-CCP antibodies, which predict the development of RA in early arthritis, have been associated with severe disease.[16]

Many autoantibodies against various citrullinated peptides occur in RA. These antibodies can be detected by serological tests using synthetic citrullinated substrates.[17] First generation ELISAs (anti-CCP1) gave a specificity for RA of about 85% with a sensitivity of 65% to 70%.[13] A second generation anti-CCP ELISA (anti-CCP2), with higher sensitivity and specificity due to the optimalization of the substrate, was developed to improve on anti-CCP1.[18] Use of these cyclic citrullinated peptides has improved the specificity to between 96% and 98% without changing the sensitivity.[19] At present, the anti CCP2 ELISA is the most widely used test in international studies.[20] A third generation test (anti-CCP3) is commercially available. Citrullinated vimentin has been described

Table 2. Results of evaluation of risk factors for extra-articular manifestations with logistic regression analysis

	Odds ratio	95% CI for Odds ratio		
		Lower	Upper	p
Disease duration >5.5 years	2.960	1.067	8.212	0.037
Positivity of anti-CCP antibodies	0.678	0.226	2.035	0.488
Rheumatoid factor positivity	0.886	0.223	3.514	0.863

CI: Confidence interval; CCP: Cyclic citrullinated peptide.

as a relevant autoantigen expressed in synovial tissue. An ELISA based on mutated citrullinated vimentin (MCV) has been available for the diagnosis of RA.[13] The anti CCP3 test seems to be as sensitive as anti-CCP2 and more sensitive than MCV.[20]

Studies that investigate the association between anti-CCP antibodies and EAM have reported contradictory results. De Rycke et al.[1] found no relationship between anti-CCP antibodies and EAM. On the other hand, in a study by Kim et al.[21] from Korea, longer disease duration, smoking history, and positive anti-CCP antibodies were reported to have a significant role in the development of EAM. Similarly, in a case-controlled study in Sweden, a significantly larger proportion of patients with severe EAM had anti-CCP antibodies (p=0.03) than the patients without EAM. However, the titers of the two groups did not differ significantly.[12] In a study from Greece, it was suggested that RA patients with high anti-CCP2 levels (>100 IU/ml) were more likely to have EAM (serositis and pulmonary fibrosis) than RA patients without high serum CCP2 levels.[22] In a previous study on Turkish patients with RA, anti-CCP antibodies did not show any association with EAM.[23] Similarly, in our study, no relationships were found between antibodies against citrullinated peptides and the presence of EAM. Vencovský et al.[24] demonstrated that the combined use of RF and anti-CCP antibodies is highly predictive for more progressive disease. We found no difference between the positivity of anti-CCP antibodies and RF in patients either with or without EAM.

In this study, the relationship between RF positivity and EAM was also evaluated. In contrast to other studies,[1,25] we could not find any relationship between RF positivity and EAM. Nevertheless, RF levels were significantly higher in the patients with lung fibrosis. We concluded that our failure to find a significant relationship between RF positivity and EAM might be associated with the small sample size.

The prevalence of EAM in RA varies significantly depending on the ethnic populations or geographic areas. Another factor is the lack of definite inclusion criteria for EAM used in each study. For example, in the Caucasian population in the Great Britain and North America,[26,27] EAM was significantly prevalent compared with East Asian and African populations.[28-29] In this study, the rate of EAM was 41.8%. A previous study investigating EAM in the Turkish population reported a rate of 38.4% for EAM

presence.[30] The prevalence of EAM in Turkey seems to be higher than in East Asia and Africa but lower than in the Great Britain and North America.

In this study, disease duration was significantly longer in the patients with EAM than in the patients without EAM. Turesson et al.[31] have reported severe EAM in both patients recently diagnosed with RA and those with long-standing disease. Kim et al.[21] concluded that disease duration was closely associated with the development of EAM. According to Korkmaz et al.[23] EAM was 2.6 times more common in long-standing RA compared to an early RA group.

In conclusion, as EAM increases morbidity and mortality in patients with RA, markers that can help identify patients with severe disease may be used in the decision-making process for early institution of aggressive treatment. Studies investigating the association between anti-CCP antibodies and EAM have reported conflicting results. In this study, we could not find any association between antibodies against citrullinated peptides and the presence of EAM. Further studies with a larger sample size are necessary to evaluate the association of EAM and anti-CCP antibodies.

Declaration of conflicting interests

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