Original Article / *Özgün Araştırma*

Correlation of Pulmonary Involvement with Serum Anti-CCP Antibodies and Disease Activity in Patients with Rheumatoid Arthritis

Romatoid Artritli Hastalarda, Akciğer Tutulumunun Serum Anti-Ccp Düzeyleri ve Hastalık Aktivitesi İle Korelasyonu

Songül Çildağ, Taşkın Şentürk, Alparslan Ünsal*, Berna Gültekin**

Adnan Menderes Üniversitesi Tıp Fakültesi, İmmünoloji-Romatoloji, Aydın, Türkiye

**Adnan Menderes Üniversitesi Tıp Fakültesi, Radyoloji, Aydın, Türkiye*

***Adnan Menderes Üniversitesi Tıp Fakültesi, Mikrobiyoloji ve Klinik Mikrobiyoloji, Aydın, Türkiye*

Abstract

Aim: Lung involvement substantially increases the morbidity and mortality rates in patients with rheumatoid arthritis (RA), thus, the early detection of lung involvement is essential for proper management. Several recent reports revealed that anti-CCP is an important parameter in the early diagnosis of RA and is closely related with the extraarticular manifestations of the disease. The aim of this study was to determine the relationship between serum anti-CCP antibodies and disease activity score-28 (DAS28), using the Stanford Health Assessment Questionnaire (HAQ) in patients with RA, whose pulmonary involvement was detected by computed tomography (CT).
Methods: According to the high-resolution CT findings, the patients were divided into two groups - with pulmonary involvement (24 patients), and without a pulmonary disease (25 patients).
Results: Statistically significant association was not found between pulmonary involvement and anti-CCP antibodies. There was no significant correlation between pulmonary involvement and disease activity evaluated by the HAQ.
Conclusion: The disease activity determined by the DAS28 was significantly negatively correlated with pulmonary involvement *(The Medical Bulletin of Haseki 2010; 48: 142-5)*
Key Words: Rheumatoid arthritis, Pulmonary Involvement, Anti-CCP, Stanford Health Assessment Questionnaire, DAS28

Özet

Amaç: Romatoid artritli hastalarda akciğer tutulumu morbidite ve mortalite oranlarını önemli derecede etkiler. Bu nedenle, akciğer tutulumunun erken dönemde saptanması ve tedavi modalitesinin düzenlenmesi gerekir. Son zamanlarda Anti-CCP'nin, erken romatoid artrit tanısındaki önemi ve romatoid artritin ekstraartiküler bulgularıyla ilişkisi gösterilmiştir. Bu çalışmanın amacı romatoid artritli hastalarda bilgisayarlı tomografi ile belirlenen akciğer tutulumunun, serum anti-CCP düzeyleri ve DAS 28, Stanford Sağlık Değerlendirme Anketi ile belirlenen hastalık aktivitesi ile korelasyonunu belirlemektir.
Yöntemler: Hastalar yüksek çözünürlüklü bilgisayarlı tomografi sonuçlarına göre akciğer tutulumu olan (24 hasta) ve akciğer tutulumu olmayan (25 hasta) şeklinde iki gruba ayrıldı.
Bulgular: Sonuç olarak, akciğer tutulumu ile serum anti-CCP düzeyleri arasında istatistiksel olarak anlamlı bir ilişki saptanmadı. Akciğer tutulumu ile Stanford Sağlık Değerlendirme Anketi ile belirlenen hastalık aktivitesi arasında anlamlı bir korelasyon saptanmadı.
Sonuç: Akciğer tutulumu ile DAS 28 ile belirlenen hastalık aktivitesi arasında anlamlı negatif bir korelasyon saptandı. *(Haseki Tıp Bülteni 2010; 48: 142-5)*
Anahtar Kelimeler: Romatoid artrit, akciğer tutulumu, Anti-CCP, Stanford Sağlık Değerlendirme Anketi, DAS 28

Introduction

Rheumatoid arthritis (RA) is a systemic inflammatory auto-immune disease characterized by chronic polyarthritis of unknown etiologies (1). Although it is manifested principally by involvement of joints, extra-articular tissues, such as the skin, heart, lungs, eyes, and the nervous system may also be affected. The number and severity of extra-articular symptoms may vary depending on the duration and severity of the disease (2,3). Pulmonary involvement is a well-known extra-articular involvement form of RA and it is the most common second factor causing death, following infection (4).

Address for Correspondence/Yazışma Adresi: Dr. Songül Çildağ,
Adnan Menderes Üniversitesi Tıp Fakültesi, İmmünoloji-Romatoloji, Aydın, Türkiye
Phone: +90 372 261 23 83 Fax: +90 256 213 60 64 E-mail: songulcildag@yahoo.com
Received/Geliş Tarihi: 20 Eylül 2010 **Accepted/Kabul Tarihi:** 21 Ekim 2010
Sunulduğu Kongre: 10. Ulusal Romatoloji Kongresi 2009

Incidence rate of pulmonary involvement in RA is known to range between 16 and 81 percent (5). Recently, specific antibodies against epidermal filagrin (filament-aggregating protein) were determined in 40-60% of patients with RA. Citrulline is a rare amino acid found in filagrin molecule. In recent studies, it was shown that citrulline found in filagrin is a structural component of antigenic epitope. Antibodies against cyclic peptide containing citrulline (anti-CCP; anti-cyclic citrullinated peptide) are reported as a new parameter, which is far more specific than rheumatoid factor (RF). Anti-CCP antibodies are mostly in IgG class, and their specificity rate for RA is 97 percent. Those antibodies can be easily detected using ELISA (Enzyme-Linked Immunosorbent Assay) due to development of synthetic peptides containing citrulline (6,7).

The aim of this studywas to determine the relationship between serum anti-CCP antibodiesand disease activity score-28 (DAS28), using the Stanford HealthAssessment Questionnaire (HAQ) in patients with RA, whose pulmonary involvement was detected by computed tomography (CT).

Methods

Totally 49 patients with RA diagnosis fulfilling 1987 the American Rheumatism Association (ARA) classification criteria, who had referred to outpatient clinic of Immunology-Rheumatology Division, Internal Medicine Department, were enrolled into the present study. Considering inclusion of patients, no age-and sex-related limitations were applied. Patients were divided into two groups: patients with and without pulmonary involvement according to the results of high-resolution computed tomography examinations (HRCT). The HRCT images were acquired using single-detector CT in the supine position and at maximum inspiration. The scans covered the whole lung with a slice thickness of 1 mm and interslice gaps of 10 mm. Images were reconstructed using an edge-enhancing algorithm and a matrix of 512-512 pixels. One and the same HRCT scanner was used in all patients. One observer (radiologist) documented the following features on HRCT images.

Disease Activity was quantified using the DAS28 and the Stanford Health Assessment Questionnaire (HAQ). According to the results of disease activity assessment performed using DAS28, two shoulders, 2 elbows, 2 wrists, 10 metacarpaphalangeal joints, 10 proximal interphalangeal joints, 2 knee joints were examined for tenderness and swelling. Tender joint count (TJC) and swollen joint count (SJC) were recorded. Erythrocyte sedimentation rate (ESR) and general health status (GHS) were also required for calculation of the DAS28 using the formula given below:

DAS 28= (0,56x√TJC) + (0,28x√SJC) + (0,70 log ESR) + (0,014xGHS)

It is considered that a DAS-score below 2.6 indicates remission, a score between 2.6 and 3.2 indicates low clinical activity, a score between 3.2 and 5.1 indicates intermediate clinical activity, and a score over 5.1 indicates high clinical activity. For assessment of the GHS, the patients were asked to rate their GHS on a self-administered scale ranging between 0 and 100.. 0= no pain, 100= intolerable pain. The point marked by the patient was used as GHS.

Using the HAQ, the patients were questioned about dressing, raising, eating, walking, hygiene, reaching, griping and common daily activities over the past week. Scores included 0=without any difficulty, 1=with some difficulty, 2= with much difficulty, 3=unable to do, and total of scores were recorded. They were also asked about special instruments used for performing those activities and need for another person.

Anti-CCP antibodies were studied by ELISA IgG kit following the below-mentioned steps. Serum samples were diluted 1:101 with sample buffer. Calibrators, control and diluted serum samples, all in volume of 100 µl, were placed in ELIZA wells and were incubated at room temperature for 60 minutes. After rinsing the wells three times using rinsing solution, 100 µl of the enzyme conjugate (peroxidase-labeled anti-human IgG) was added and the mixture was incubated at room temperature for 30 minutes. 100 µl of stop solution was added to each well and the reading was performed using a spectrophotometer at 450 nm. Samples with value >5 RU/ml were considered positive.

Statistics

All analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 13. All data were expressed as mean±standard deviation (SD) or as a percentage, as appropriate. Qualitative parameters were analyzed with the Chi-Square test and Fisher's exact test. Nonparametric variables were analyzed using the Mann-Whitney U test.

Results

A total of 49 patients with the diagnosis of RA-42 females (85.7%) and 7 males (14,3%) aged between 23 and 96 years (mean: 55.86±13.31 years) were examined. The duration of the disease was 0-2 years in 17 patients (34.7%) and over 2 years in 32 patients (65.3%) (Table 1).

The mean value of DAS 28 in all cases was 3.52±1.55, but it was 2.99±1.41 in 24 patients with pulmonary involvement and 4.03±1.52 in 25 patients without pulmonary involvement. When the difference in the values of DAS 28 between the groups with and without pulmonary involvement was regarded, it was found that the mean DAS 28 was significantly higher in the group with pulmonary involvement (p= 0.017).

The mean score of the HAQ in all cases was 10.10±12.59, but it was 6.75±9.41 in 24 patients with pulmonary involvement and 13.32±14.50 in 25 patients without

Table 1. Demographic characteristics of patients

	Patients number (n= 49)
Female / Male	42/7
Mean age, years	55.86±13.31
Disease year (0-2 year)	17
Disease year (over 2 years)	32

pulmonary involvement. No significant difference was found between the mean HAQ scores of groups with and without pulmonary involvement (p= 0.067) (Table 2).

When the disease activity was examined using DAS 28 and the HAQ, although DAS28 activity was found higher in the patients with positive anti-CCP, no statistically significant difference could be observed (p=0.119; 0.787) (Table 3).

19 patients were anti-CCP-positive (38.8%) and 30 were negative (61.2%). Anti-CCP was positive in 10 out of 24 patients with pulmonary involvement (41.6%) and negative in the remaining 14 patients (58.4%), whereas it was positive in 9 out of 25 patients without pulmonary involvement (36%) and negative in the remaining 16 patients (64%). No significant difference could be found between pulmonary involvement and positivity and negativity of anti-CCP test (p=0.773) (Table 4).

HRCT findings in RA patients with pulmonary involvement included pulmonary nodule in 17 cases (70.8%), bronchiectasia in 14 (58.3%), interlobular septal thickening in 13 (54.2%), peribronchial thickening in 7 (29.2%), ground glass opacity in 5 (20.8%), reticular infiltration in 5 (20.8 %), pleural thickening in 4 (16.7%), emphysema in 4 (16.7%), atelectasia in 3 (12.5%), air trapping in 3 (12.5%), fibrous band in 2 (8.3%), pleural nodule in 2 (8.3%), honeycomb formation in 2 (8.3%) and bullae in 2 cases (8.3%) (Table 5).

Table 2. Correlation between pulmonary involvement with disease activity

	Pulmonary involvement (-)	Pulmonary involvement (+)	p
Mean of DAS 28	4.03±1.52	2.99±1.41	0.017
Mean of Stanford	13.32±14.50	6.75±9.41	0.067

Table 3. Correlation between serum Anti-CCP antibodies with disease activity

	Anti CCP (+)	Anti CCP (-)	p
Mean of DAS 28	3.96±1.50	3.24±1.530,	119
Mean of Stanford	10.57±13.09	9.8±12.47	0.787

Table 4. Correlation between pulmonary involvement with serum Anti-CCP antibodies

	Pulmonary involvement (-)	Pulmonary involvement (+)	p
Anti-CCP (+)	9 (%36)	10 (%41.6)	0.773
Anti-CCP (-)	16 (%64)	14 (%58.4)	
Totally	25 (%100)	24 (%100)	

Table 5. High resolution computerized tomography findings in rheumatoid arthritis

HRCT	Patients number (n)	%
Pulmonary nodule	17	70.8
Bronchiectasis	14	58.3
Interlobular septal thickening	13	54.2
Peribronchial thickening	7	29.2
Ground glass opacity	5	20.8
Reticular infiltration	5	20.8
Pleural thickening	4	16.7
Emphysema	4	16.7
Atelectasis	3	12.5
Air trapping	3	12.5
Fibrous band	2	8.3
Pleural nodule	2	8.3
Honeycombing	2	8.3
Bullae	2	8.3

Discussion

RA is a disease that particularly affects the joints, however, it may involve all the organ systems (3). In approximately 50 percent of RA cases, occurrence of extra-articular manifestations including serositis, pneumonitis, myocarditis, renal involvement, myositis, arteritis, peripheral neuritis, involvement of the central nervous system and hematological changes have been demonstrated. Extra-articular involvement may occur at any age following onset of the diseases. Although the true prevalence of pulmonary involvement is unknown, it has been reported to be 1-40 % of all RA patients (8,9). Middle age, male sex, serious destructive arthritis, high RF titrations, presence of subcutaneous nodule and other extra-articular involvement findings are known predisposing factors for pulmonary involvement (10). Respiratory involvement (9.9%) is the third most common cause of death in patients with RA, following infections (23.5%) and cardiovascular diseases (17,3 percent) (11). Particularly, development of pulmonary fibrosis may adversely affect the clinical course. Following diagnosis of pulmonary fibrosis, five-year survival was reported to be 39 percent (12). Since pulmonary involvement increases mortality and morbidity, the diagnosis should be made and treatment should be started at an early stage.

While anti-CCP antibodies are highly specific to RA, the relationship of these antibodies with clinical activity of the disease is not clear. It was shown that patients with anti-CCP positive RA have relatively higher disease activity and DAS than patients with anti-CCP negative RA (13). In the present study, disease activity determined using DAS 28 was found higher in anti-CCP(+) group, although it was not significant. No association could be found between disease activity determined using the HAQ and anti-CCP positivity-negativity.

It has been claimed that anti-CCP antibodies have prognostic value and are dominantly present in individuals with erosive disease (1,14). Erre et el. stated in their study that anti-CCP antibody, among all anti-filagrin antibodies, is the most definitive antibody for diagnosis of RA and, moreover, it has determinant role in the early phase of arthritis in terms of persistency of the disease and development of

radiologically evidenced damage (15). Meyer et al. had prospectively observed 191 patients with recently diagnosed RA for 5 years and found using the Sharp-van der Heijde method that radiological damage, erosions and narrowing in joint space had been three times more prevalent in anti-CCP positive patients (16). Similarly, this relationship had also been demonstrated in studies conducted by Vencovsky et al. and Lee et al. Nevertheless, in the study conducted by Dündar et al., no significant difference could be found between modified Larsen scores of right and left hand in anti-CCP positive and negative patients (17). In the present study, using the Sharp-van der Heijde method, no significant difference could be found in terms of joint erosion and narrowing on hand images of anti-CCP positive and negative patients using Sharp-van der Heijde method.

In pulmonary involvement, one of extra-articular findings in patients with RA, known predisposing factors include middle age, male sex, serious destructive arthritis, high RF titrations and presence of subcutaneous nodules (18). However, we could not find any study in the literature examining the relationship between anti-CCP antibodies and pulmonary involvement. In the present study, we could find no significant relations between pulmonary involvement and anti-CCP positivity-negativity.

In conclusion, as pulmonary involvement significantly influences morbidity and mortality in patients with RA, it is important to determine pulmonary involvement at an early phase and to plan treatment modality. Recently, significance of anti-CCP antibodies in early diagnosis of RA and their relationship with extra-articular symptoms of RA has been demonstrated. In the present study, it was observed that pulmonary involvement in RA is not related with levels of anti-CCP antibodies, which have high specificity for early diagnosis of RA and that disease activity is decreased as patients with pulmonary involvement are more aggressively treated. However, we decided that future studies in larger patient series are required.

References

1. Kroot EJJA, de Jong BAW, Van Leeuwen MA et al. The prognostic value of anti-cyclic citrullinated peptide antibody in patients with recent-onset rheumatoid arthritis. Arthritis Rheum 2000;43:1831-5. [Abstract] / [PDF]
2. Lutra HS. Extraarticular rheumatoid arthritis. In: Koopman WI (ed). Arthritis and Allied Conditions 14th ed. Lippincott Williams and Wilkins 2001;1187-201.
3. Harris ED. Clinical features of rheumatoid arhritis. In: Ruddy S, Harris ED, Sledge CB (eds). Texbook of Rheumatology, 6th ed. Philadelphia: WB Saunders 2001;967-1000.
4. Anaya JM, Diethelm L, Ortiz LA et al. Pulmonary involvement in rehumatoid arthritis. Semin Arthritis Rheum 1995;24:242-54. [Abstract] / [PDF]
5. Schwarz MI. Pulmonary manifestations of the collogen vascular diseases. In: Fishman AP, Elias JA, Fishman JA, Grippi MA (eds). Fishman's pulmonary diseases and disorders Vol 1, 13 th ed. New York: The Mc Grow Hill Co 1998;1122-26.
6. Van Boekel MA, Vossenaar ER, Van De Hoogen FH, Van Venroo WJ. Autoantibody systems in rheumatoid arthritis: specificity, sensitivitiy and diagnostic value. Arthritis Res 2002;4:87-93.[Abstract] / [Full Text] / [PDF]
7. Schellkens GA, Visser H, De Jong BAW et al. The diagnostic properties of rheumatoid arthritis antibodies recognizing anti-cyclic citrullinated peptide. Arthritis Rheum 2000;43:155-63. [Abstract] / [PDF]
8. Turesson C, Jacobsson L, Bergstrom U. Extra-articular rheumatoid arthritis: prevalance and mortality. Rheumatology Oxford 1999;38:668-74. [Abstract] / [PDF]
9. Horton MR. Rheumatoid arthritis associated interstitiallung disease. Crit Rev Comput Tomogr 2004;45:429-40.
10. Chanin K, Vallefo-Manzur F, Sternbach GL, Fromm R, Varon J. Pulmonary manifestations of rheumatoid artritis. Hospital Physician 2001;23-28.
11. Turesson C, Jacobsson L, Bergstrom U. Extra-articularrheumatoid arthritis: prevalance and mortality. Rheumatology Oxford 1999;38:668-74. [Abstract] / [PDF]
12. Hakala M. Poor prognosis in patients with rheumatoid arthritis hospitalized for interstitial lung fibrosis. Chest 1988;93:114-18. [Abstract] / [PDF]
13. Bas S, Perneger TV, Seitz M, Tiercy J-M, Roux-Lombard P, Guerne PA. Diagnostic tests for rheumatoid arthritis: comparison of anti-cyclic citrullinated peptide antibodies, anti-keratin antibodies and IgM rheumatoid factors. Rheumatology 2002;41:809-14. [Abstract] / [Full Text] / [PDF]
14. Schellkens GA, Visser H, De Jong BAW et al. The diagnostic properties of rheumatoid arthritis antibodies recognizing anti-cyclic citrullinated peptide. Arthritis Rheum 2000;43:155-63. [Abstract] / [PDF]
15. Erre GL, Tocco A, Faedda R, Cossu A, Carcassi A. Diagnostic and prognostic value of antibodies to cyclic citrullinated peptide (Anti-CCP) in rheumatoid arthritis. Reumatismo 2004;56:118-23. [PDF]
16. Meyer O, Laberre C, Dougados M et al. Anticitrullinated protein/peptide antibody assays in early rheumatoid arthritis for predicting five year radiographic damage. Ann Rheum Dis 2003;62:120-6. [Abstract] / [Full Text] / [PDF]
17. Dündar Ü, Evcik D, Çakır T et al. Romatoid artritli hastalarda Anti-CCP antikorlarının hastalık aktivitesi ve radyolojik hasar ile ilişkisi. Romatizma 2005;20:9-14. [Abstract] / [PDF]
18. Chanin K, Vallefo-Manzur F, Sternbach GL et al. Pulmonary manifestations of rheumatoid artritis. Hospital Physician 2001;23-8. [PDF]