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Anti-cyclic citrullinated peptide antibodies in patients with sarcoidosis

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Abstract. *Introduction:* Anti-cyclic citrullinated peptide (anti-CCP) antibodies have a high predictive value in rheumatoid arthritis (RA) patients and are associated with disease severity. Sarcoidosis is a chronic inflammatory disease characterized by non-calcified granuloma formations. *Aim:* To determining the prevalence of anti-CCP antibodies in patients with sarcoidosis, and identifying a possible correlation with clinical and laboratory findings. *Materials and Methods:* Forty-two patients presenting to the rheumatology polyclinic and diagnosed with sarcoidosis as a result of the examinations made, 45 RA patients and 45 healthy subjects were included in the study. Demographic, clinical, serological and radiological data of all patients were recorded. Anti-CCP antibodies were evaluated by using a second-generation ELISA method. Rheumatoid factor (RF) IgM was determined with the nephelometry method. *Results:* Forty-two patients (10 males) were included in the study. Mean patient age was 45.2 years (20-70 years) and mean duration of disease was 3.5 years. Two sarcoidosis patients (4.7%) and 38(84.4%) RA patients were found to be positive for anti-CCP antibodies while the antibody wasn't detected in any healthy subject. The two sarcoidosis patients found positive for anti-CCP were also diagnosed with rheumatoid arthritis. RF positivity was detected in 7 sarcoidosis patients (16.6%) and in only one subject in the control group. *Conclusion:* The prevalence of anti-CCP antibodies in patients with sarcoidosis was found to be significantly lower than RA patients and similar with the healthy control group. This result shows that anti-CCP antibodies don't have an important role in the pathogenesis of sarcoidosis, but could be important in revealing the overlap syndromes of sarcoidosis-RA *(Sarcoidosis Vasc Diffuse Lung Dis 2014; 31: 198-202)*

Key words: sarcoidosis, anti-CCP antibodies, prevalence

Introduction

Sarcoidosis is a systemic disease characterized by the involvement of multiple tissues and organs and a non-calcified granulomatous reaction, which is not well understood (1). Although its pathogenesis is not clear, there appears to be a cellular immune system activation and a non-specific inflammatory response against some genetic and environmental factors (2). Proinflammatory cytokines produced by Th1 and macrophages trigger the inflammatory cascade, and granuloma formations occur as a result of tissue permeability, cellular influx and local cell proliferation (3). The indispensable pathological finding of sarcoidosis is non-calcified epithelioid cellular granulomas (4). Increased active CD4 T lymphocytes have been shown in tissues with sarcoidosis. These lymphocytes are shown to increase diffusely in the granulomatous lesion, while small

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numbers of CD8 T-Ly, B-cells, plasma and mast cells are identified on the outer part of the granuloma (5). Sarcoidosis is a chronic granulomatous disease that may present with different clinical findings. It may mimic a number of primary rheumatic diseases and/or develop concomitantly with these. The disease most frequently presents with bilateral hilar lymphadenopathy, infiltrations in the lungs and skin and eye lesions. Locomotor involvement is determined to be %15-25 (6). Two major joint involvement patterns have been identified: acute and chronic forms. Being the most common one, the acute form can be the first sign of sarcoidosis and may present with arthralgia, arthritis and/or periarthritis. Chronic sarcoid arthritis is usually accompanied by pulmonary parenchymal disease or other organ involvement, and occurs rarely and at a late stage of the disease (7). In sarcoidosis patients, serum levels of some biochemical indicators, such as serum angiotensin converting enzyme(ACE) and calcium, is elevated. Today, clinical, radiological and physiological parameters are used to determine the activity and severity of this disease, which can involve multiple systems, and serum indicators are screened accordingly.

The autoantibody developing against citrulline is called anti-cyclic citrullinated peptide (anti-CCP) (8). Anti-CCP antibodies are divided into two groups: anti-CCP1 and anti-CCP2. The sensitivity of the anti-CCP2 antibody test is approximately 64-89% and its specificity is quite high with a rate of 88-99% (9). Anti-CCP antibodies have a high predictive value in rheumatoid arthritis (RA) patients and are associated with disease severity. They have been found to be associated with erosive disease, joint deformities and extraarticular involvement in RA cases. There are studies and case reports in literature where the relationship between anti-CCP antibody positivity and disease is investigated in patients with psoriatic arthritis (PsA), systemic sclerosis (SSc) and Sjögren's syndrome (SjS) (10, 11, 12, 13). However, there are no studies investigating these parameters in sarcoidosis with locomotor involvement. The purpose of this study is to determine the prevalence of RF and anti-CCP antibodies, evaluate a possible correlation with clinical and laboratory findings and explore its significance as a criterion of activity in sarcoidosis patients where locomotor involvement is an important finding.

Materials and Methods

Forty-two patients presenting to the rheumatology outpatient clinic and diagnosed with sarcoidosis as a result of the examinations made and 45 RA patients diagnosed according to the ACR classification criteria were enrolled in the study. As control group, 45 healthy subjects of matching age and gender were included in the study. nformed consent forms were obtained from all patients. Diagnosis of sarcoidosis was made by demonstration of non-calcified granulomas with biopsies collected from different organs and tissues and histopathological examination. Other possible causes of granulomatous disease (bacterial, fungal infections) were excluded. Laboratory examinations were made for all sarcoidosis patients; routine biochemistry, acute phase reactants (ESR, CRP), serum ACE, serum calcium and serum 25-hydroxyvitamin D3 levels were checked. A thoracic CT scan was made to stage sarcoidosis. All cases were inquired in a detailed manner; they were given systemic and rheumatic care. Before the inclusion of subjects in the patient and control groups, their informed consent was obtained and enrollment forms were completed. Demographic, clinical, serological and radiological data of all patients were recorded. Anti-CCP antibody levels were measured by using a second-generation ELISA (enzyme-linked immunosorbent assay) method on the blood samples taken. An anti-CCP antibody level of >5 IU/ml was considered as significant. Rheumatoid factor (RF) IgM was determined with the nephelometry method. An RF level of >14 IU/ml was considered as significant.

Statistical Analysis

All statistical analyses were made by using SPSS version 9.0 (Chicago, II, USA). Prevalence was calculated for each group and comparisons for categorical variables were made with a Chi-square test. Continuous variables were compared with a Student's t-test. For all statistical tests, a p value of <0.05 was considered to be statistically significant.

Results

Forty-two sarcoidosis patients (10 males) were included in the study. Mean patient age was 45.2

years (20-70 years) and mean duration of disease was 3.5 years. An assessment of system and organ involvement showed that 20 (47.6%) patients had erythema nodosum, 3 (7.1%) had uveitis, 1 (2.3%) had myositis, 1 (2.3%) had neurosarcoidosis and 32 (76.2%) had arthritis. Of the 32 patients with arthritis 28 (87.5%) had involvement of the ankle joint, 3 had involvement of the knee joint and 1 had involvement of the wrist joint. None of the patients had cardiac involvement. Thoracic CT revealed stage 1 sarcoidosis in 12 (28.5%) patients, stage 2 sarcoidosis in 22 (52.4%) patients, stage 3 sarcoidosis in 4 (9.5%) patients and stage 4 sarcoidosis in 4 (9.5%) patients (Table 1). Histopathological verification of sarcoidosis was made by EBUS, mediastinoscopy and demonstration of non-calcified granulomas with a skin and axillary LAP biopsy. In laboratory analyses, 15 (35.7%) patients had elevated serum ACE, 6 (14.3%) had elevated serum calcium and 2 (4.7%) had elevated serum 25-hydroxy vitamin D3. In the tests made during initial application, elevated ESR was detected in 25 (59.5%) patients and elevated CRP in 23 (54.7%) patients.

Of the 45 patients enrolled in the study with a diagnosis of RA, 12 were males and 33 were females (%); their mean age was 48.4 years and mean duration of disease 9.2 years. Two sarcoidosis patients (4.7%) and 38 RA patients (84.4%) were found to be positive for anti-CCP antibodies while positivity for the antibody wasn't detected in the healthy control group. The two patients found to be positive for anti-CCP antibodies were also diagnosed with rheumatoid arthritis based on the ACR criteria (sarcoidosis-RA overlap syndrome). The sarcoidosis patiens with positive anti-CCP antibodies was nonsmoker. RF positivity was detected in 7 sarcoidosis patients (16.6%) and in only one person in the healthy control group.

Table 1. Demographic, clinical and laboratory features in patients with sarcoidosis.

Features	Patients, N=42 (%)
Age, mean, year	45.2 year
Disease duration, mean, year	3.5 year
Sex (woman/men)	32/10
Erythema nodosum	20 (47.6%)
Uveitis	3 (7.1%)
Myositis	1 (2.3%)
Neurosarcoidosis	1 (2.3%)
Arthritis	32 (76.2%)
Elevated serum ACE level	15 (35.7%)
Anti-CCP positivity	2 (4.7%)
RF positivity	7 (16.6%)
Stage 1	12 (28.5%)
Stage 2	22 (52.4%)
Stage 3/4	4/4 (9.5%)

Discussion

In this study, the prevalence of anti-CCP antibodies was found to be significantly lower in sarcoidosis patients than RA patients and similar with the healthy control group. The two patients found to be positive for anti-CCP antibodies were also diagnosed with rheumatoid arthritis based on the ACR criteria (sarcoidosis-RA overlap syndrome). RF positivity was detected in 7 sarcoidosis patients (16.6%) and in only one person in the healthy control group. Sarcoidosis is a chronic systemic inflammatory disease of unknown etiology, characterized by non-calcified granuloma formations. Sarcoidosis involves a hyperimmune response to an unknown agent which occurs through the activation of antigen-presenting cells such as Type 2 alveolar epithelial cells, alveolar macrophages and dendritic cells and CD4+Th1 lymphocytes (14). Monocyte-macrophage activation starts against an inflammatory agent that reaches the lungs by respiration and cytokines such as the tumor necrosis factor alpha (TNF-alpha) and interleukin-1 (IL-1) are released. In sarcoidosis, hypercalcaemia and hypercalciuria develops depending on the level of 1,25 dihydroxy vitamin D3 released with the 1-alpha hydroxylase enzyme activity from the alveolar macrophages in granulomas (15). As a result, it is thought that the calcium-dependent peptidyl arginine deiminase(PAD) enzyme is activated and citrullination occurs. Citrullination may develop in many systems of the body in case of physiological apoptosis and inflammation. Migration of monocytes and granulocytes to the inflammation may lead to a vicious circle by triggering PAD activation. HLA Class II-dependent T cell response and immune complex formation with the antibodies against citrulline plays a role in the pathogenesis of RA. Like RA, sarcoidosis also involves immune mechanisms with increased CD4(+) T lymphocyte response. In sarcoidosis, as disease activity decreases, CD4(+) Th1 rate decreases and CD8(+) T lymphocyte rate increases. Most interestingly, spontaneous

remission is seen in many patients in this disease (16). It is thought that anti-CCP antibody positivity may not have been detected in patients experiencing spontaneous remission since serum analysis was made at a time of decreased disease activity. Concomitant RA was detected in the two sarcoidosis patients with anti-CCP positivity. While this suggests that anti-CCP antibodies don't play an important role in the pathogenesis of sarcoidosis, genetic factors and environmental factors like smoking may also be involved, in addition to the analysis being made at a time when the disease is not active.

Anti-CCP antibodies developing against citrulline proteins detected in the inflammatory synovium are synthesized from local plasma cells (17). B-cells synthesizing anti-CCP antibodies were found in the synovial fluid of anti-CCP-positive patients, and these proteins were understood to be a part of the humoral immune response (18, 19). In RA patients, anti-CCP antibodies are associated with erosive and extraarticular disease, and have a high specificity and sensitivity (20). In addition to RA, other rheumatic diseases were also investigated. Anti-CCP antibodies were investigated in patients with PsA and found to be associated with radiological erosion and polyarticular involvement (11). In anti-CCP-positive patients with primary Sjögren's syndrome (SjS), the relationship between synovitis and anti-CCP positivity was found to be significant but it was not significant for extraglandular involvement (13). This might be related to the overexpression of PAD 4 in synovial tissues. In another study, anti-CCP antibodies and RF were determined in the sera of 30 patients with RA and 22 patients with HCV-related polyarthropathy. Anti-CCP antibodies were positive in 83.3% of patients with RA and in 4.5% in patients with HCV and polyarthropathy. RF was positive in 90% of RA patients and in 81.1% of HCV patients with polyarthropathy. The authors concluded that anti-CCP antibodies are reliable laboratory markers to differentiate between RA and HCV-related polyarthropathy(21). In a study, it was thought that cases with concomitant systemic sclerosis (SSc) and RA could have a separate disease involving generalized sclerosis, severe seropositive polyarthritis and pulmonary fibrosis, and anti-CCP antibody levels were found to be significantly higher in concomitant SSc and RA than primary SSc patients (12). In our study, anti-CCP positivity was detected in 2 cases with overlap syndrome of sarcoidosis+RA while no patient followed up only for diagnosis of sarcoidosis had antibody positivity. This suggests that anti-CCP antibodies have no role in the etiopathogenesis of sarcoidosis, but could be an important marker for the early diagnosis of overlap syndrome with RA.

They are autoantibodies developing against antigenic determinants on the Fc fragment of the RF IgG molecule. While RF is usually associated with RA, it may be seen in other diseases and normal people too. In literature, low-titer RF positivity has been reported in sarcoidosis (15, 16). In our study, the RF positivity in sarcoidosis patients was found to be higher than the control group. In another study, anti-CCP antibody positivity was found to be associated with symmetric polyarthritis in PsA patients. In this study, anti-CCP antibodies were significantly higher in RA patients positive for Rheumatoid factor (RF) than RF-negative RA and PsA patients (10). Anti-CCP and RF were found to be comparable for sensitivity whereas anti-CCP was much more specific in terms of specificity. Anti-CCP antibodies were seen to have a very important diagnostic effect in RF-negative patients (22, 23).

Our study has certain limitations. The limited number of patients represents a significant limitation in terms of reaching a general conclusion about the relationship between anti-CCP antibodies and sarcoidosis. Studying these antibodies in the BAL fluid would make a significant contribution to the etiopathogenesis of sarcoidosis.

In conclusion, it was determined that the prevalence of anti-CCP antibodies is lower in sarcoidosis patients than RA patients and higher than the control group. Sarcoidosis can mimic many rheumatic diseases. In sarcoidosis cases presenting with serious joint involvement, RA should be watched for in differential diagnosis, and checking anti-CCP levels can be useful in the early diagnosis and treatment of a disease such as RA which is a cause of morbidity and mortality.

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