

Research Article

Serum Level of Anti-CCP in Patients with Active Pulmonary Tuberculosis in Iran

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Introduction

Tuberculosis (TB) is a major cause of human mortality and a worldwide public health problem. TB is a chronic, communicable disease caused by *M. tuberculosis* made distinctive by a destructive necrotizing granulomatous tissue response to the organisms. *Mycobacterium tuberculosis* causes two million deaths every year and every year 8 million of new cases of TB are diagnosed worldwide [1,2]. Both pulmonary TB and also cases of extra pulmonary disease are increasing [3]. About 10% of extra pulmonary TB affects the bones and joints [4]. Tuberculosis of bones and joints often presents as gradually worsening arthritis. Systemic and pulmonary symptoms are frequently absent, and the differential diagnosis must include other possible causes of septic osteoarticular disease, inflammatory arthritis. Osteoarticular tuberculosis rarely involves more than one joint, and this may help to differentiate it from other polyinflammatory diseases. Also, the radiographic changes of tuberculosis are slow to develop compared with those of pyogenic infections, and a reduction in the joint space is often a late occurrence [5]. Clinical confusion occurs especially when tuberculosis super infects joints previously involved by inflammatory arthritis [6].

TB arthritis most commonly manifests as a mono arthritis of the weight bearing joints [7], but oligo articular or poly articular presentation is not rare and these findings may mimics inflammatory diseases such as the spondyloarthropathies [8] or rheumatoid arthritis (RA), or both [9]. Also, the serum of patients with TB may contain rheumatoid factors (RFs) in up to 40% of cases [10]. Patients with TB, even those without evidence of a direct musculoskeletal/local involvement, may present with a variety of rheumatic symptoms and signs including arthralgia and arthritis. The presence of rheumatic manifestations can lead to challenges in making or excluding a diagnosis of rheumatoid arthritis (RA) in patients infected with TB. For distinguishing between true RA and TB rheumatic symptoms, the presence of RF is of little help and can be confusing or misleading. Anti-cyclic citrullinated peptide (Anti-CCP) was detected in the

serum samples of RA patients which show high diagnostic accuracy. Citrulline is an unusual amino acid created by de-imitation of arginine residues in several proteins by the action of peptidyl arginine deaminase (PAD). The antibodies are detected by ELISA, where a synthetic cyclic citrullinated peptide (CCP) is used as substrate [11].

Materials and Methods

50 serum samples of patients with active lung tuberculosis (29 men and 11 woman) diagnosed according to clinical symptoms, radiologic studies, and laboratory confirmation including direct smear of sputum and sputum cultures for *Mycobacterium tuberculosis* and consecutively recruited from the department of tuberculosis and pulmonary disease in Iran Mehr Hospital, in Tehran (Iran), an endemic area of tuberculosis Also 50 sex and age-matched healthy subjects are studies for serum anti-ccp levels. Among the TB patients, the variables recorded were age, gender, time from diagnosis, duration of symptoms, fever, hemoptysis, cough, myalgia and arthralgia and serum titer of anti-ccp. Anti-CCP antibodies were tested by commercial ELISA (Aeskulisa; Aesku. Diagnostics, Germany). Each of the tests was performed and evaluated by operators who were blinded to the patients' clinical data, and radiological and microbiological results. The assay was performed according to the manufacturer's instructions. To determine the precision of the assay, the variability (intra and inter-assay) was assessed by examining its reproducibility on three serum samples selected to represent a range over the standard curve. Analytical sensitivity was 1.0U/ml. Intra- and inter assay CVs were 0.4-8.9 % and 1.0-8.2 %, respectively. All moral standards in this study were compiled and the project was approved by the University's ethics committee.

Statistical analysis

Analysis was performed using the SPSS version 13, Student's t test and a 2 test to compare antibody titers or positivity rate, respectively, between patients with TB and controls. Pearson correlation coefficients were used to study the relationship between clinical

Table 1: Demographic, clinical characteristics and anti-CCP levels of patients with TB and healthy controls.

Characteristics	Patients with TB	Control Group
Age (years), mean (SD)	51.1(15.2)	47.4(15.7)
Sex (M/F)	29/21	30/20
Duration of symptoms (months), mean (SD)	4.4(1.6)	-
Fever, No(%)	36(72)	-
Cough, No(%)	39(78)	-
Hemoptysis, No(%)	39(78)	-
Rheumatologic symptoms, No(%)	6(12)	-
Serum Anti-CCP level (>15 IU)	17(34)	3(6)

measures and the level of anti-CCP. A value of $P < 0.01$ was considered significant.

Results

The patients (mean and SD 51.1+/-15.2 years; range, 19-81 years) and healthy subjects (mean and SD 47.4+/-15.7 years; range, 23-85 years) ages were determined. The mean (SD) duration of TB related symptoms was 4.4 (1.7) months, 72% had fever, 78% had cough and 78% had hemoptysis. Rheumatic symptoms were relatively rare: arthralgia (2%), myalgia (4%), and eye and mouth dryness (1% and 3%, respectively). Mean (SD) levels of anti-CCP were significantly increased in patients with TB compared with controls: 29.8 (17.7) U/ml v 10.8 (7.9) U/ml ($p < 0.001$). Serum levels >15 U/ml were found in 17 (34%) patients compared with 2 (6%) controls ($p = 0.002$). Risk of pulmonary TB in subjects with anti-CCP serum level higher than 15U/ml is almost 12.5 fold persons lower than the cut-off point (odds ratio=12.4, CI 95%: 2.6-114.9). No relationship was found between the titer of anti-CCP and fever and any rheumatic symptom.

Table 1 summarizes the demographic, clinical characteristics, and anti-CCP levels of patients with TB and healthy controls.

Discussion

We sought to determine if anti-CCP was also associated with active pulmonary tuberculosis. The data presented here clearly indicate that anti-CCP can be found in the sera of unselected patients with TB. We found that 17 (34%) patients with TB have positive levels of anti-CCP. Although the presence of anti-CCP correlated with fever, it was not associated with symptoms and signs of arthritis.

Elkaya et al. showed significantly increased anti-ccp levels in tuberculosis patients in comparison with controls. In this study, 32 percent of patients in comparison with 2.6 percent of controls show serum levels above the upper normal limits. They found correlation between increased serum anti-ccp and a history of prolonged of fever, as we noted in the present study. As we revealed, no correlation was found between anti-ccp level and rheumatic symptoms in Elkayam study [12].

Patients with TB may have a variety of rheumatic symptoms and signs, including arthralgia and arthritis and TB may imitate inflammatory rheumatic diseases, such as RA [13]. Also the radiological abnormalities observed in tuberculosis arthritis may simulate RA [14]. Antibodies to CCP are present in the sera of a majority of patients with RA, only rarely in the sera of patients with

other diagnoses.

TB of the bones and joints, which constitutes a smaller part of extra pulmonary tuberculosis, is relatively rare in documented cases and can yield variable clinical manifestations. Many authors describe numerous cases of tuberculosis arthritis in patients presenting with monoarthritis [15-20]. Tuberculosis must always be considered in the differential diagnosis of chronic monoarthritis if devastating squal is to be avoided.

In recent years, various antibodies have been described in rheumatoid arthritis, and their clinical significance have been discussed. In particular, autoantibodies to citrullinated proteins such as fill grin and its circular form (cyclic citrullinate peptide: anti-ccp) are the most remarkable because of the reasonable sensitivity and high specificity in RA patients [21]. They are presenting most patients with RA and have been found to have a specificity of >90% [22]. Although, anti-CCP may present in the serum of patients with psoriatic arthritis and have questioned its specificity [23].

The presence of anti-CCP in a few infectious diseases, ashepatitic C has been studied. Although, rheumatic factor (RF) is present in the great majority of patients with hepatitis C arthritis and in a proportion of patients with other sub acute infections, particularly bacterial endocarditis, anti-CCP is negative in patients with HCV and measurement of anti-CCP may help in diagnosing RA in patients with chronic HCV infection [24].

Patients with active tuberculosis may have increased titers of various autoantibodies and serologic markers, such as rheumatic factor (RF), antinuclear antibody (ANA), anti cardiolipins, anti neutrophilic cytoplasmic antibody, anti-ds DNA, anti-Sm, anti-RNP and anti-Ro [25-27]. Genetic factors such as HLA and agene polymorphism of the citrullinating enzyme, peptidyl arginine deiminase, (that might express more stable mRNA and cause over-citrullination of proteins) might be associated with the breakage of self-tolerance and induction of autoimmunity against citrullinated proteins [20,28]. It is not clear whether the false positive anti-CCP reactivity seen in patients with TB is directed against citrullinated or non-citrullinated epitopes in the substrate for the CCP test [12].

Conclusion

A relatively high percentage of patients with active pulmonary tuberculosis show increased titers of anti-ccp which could impede diagnosis of articular TB. Therefore, it is recommended not to limit the diagnosis of arthritis types to anti-ccp test, and perform other assessments in order to differentiate articular TB.

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