THE ROLE OF CHITOTRIOSIDASE ACTIVITY AS A PROGNOSTIC BIOMARKER IN SARCOIDOSIS

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ABSTRACT

Introduction: Chitotriosidase (ChT) is a chitinase that is massively expressed by lipid-laden tissue macrophages in human beings. The aim of this study was to compare ChT activities in the serum and bronchoalveolar lavage (BAL) fluid and serum ChT levels with serum angiotensin converting enzyme (ACE) levels in patients with newly diagnosed pulmonary sarcoidosis (SARC), pulmonary tuberculosis (TB) and interstitial lung disease (ILD), all of which create difficulties in the differential diagnosis in daily practice.

Materials and methods: ACE concentration and ChT activity were measured in pulmonary TB (n=28), SARC (n=37), and different types of ILD (n=23). ChT activities in the serum, BAL fluid and serum ACE concentrations were determined using ELISA kits.

Results: Serum ACE levels were significantly higher in the SARC patients than those in the TB and ILD patients (p<0.001). There were no differences among patients regarding ChT activities in serum and BAL fluid. ChT activities in the BAL fluid and serum did not correlate with serum ACE levels.

Conclusion: Our results indicate that, ChT cannot be considered as a specific marker of SARC since ChT activities were also increased in TB and ILD. ACE level seemed to be a more specific biomarker for SARC than ChT. Further studies are required to better understand the role of ChT and ACE concentrations in the pathogenesis of SARC and their involvement in fibrotic remodeling in certain types of diffuse lung diseases.

Key words: Sarcoidosis, pulmonary tuberculosis, interstitial lung disease, chitotriosidase, ACE.

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Introduction

ILD belongs to a heterogeneous group of lung disorders with different clinical courses and pathogenic mechanisms. Among these diseases, the most common ones are SARC, idiopathic pulmonary fibrosis (IPF) and pulmonary fibrosis associated with systemic sclerosis (SSc). The prognosis of these diseases depends on different developmental processes towards lung fibrosis. IPF is a rapidly progressive disease unresponsive to therapy1,2. SARC is a systemic granulomatous disease that primarily affects the lungs and lymphatic systems of the body. SARC occurs throughout the world and affects both sexes, all races and ages.

The epidemiology of SARC remains problematic for several reasons. These include lack of a precise, consistent case definition, variability in the methods of case ascertainment and disease presentation, lack of sensitive and specific diagnostic tests, resulting in under recognition and misdiagnosis of the disease, and the paucity of systematic epidemiologic investigations of its cause.

The disease shows a consistent predilection for adults less than 40 year of age, peaking in those aged 20 to 25 years. Most studies suggest a slightly higher disease incidence rate in women. In an only population-based incidence study of SARC in the United States, the incidence rates were 5.3 per 100,000 person-years for men and 6.3 per 100,000 person-years.
for women. On the basis of cumulative incidence estimates, the lifetime risk of SARC is 0.05% for U.S. whites and 2.4% for U.S. blacks. Estimates of the prevalence of SARC range from fewer than 1 case to 40 cases per 100,000, with an age-adjusted annual incidence rate of 35.5 per 100,000 for blacks and 10.5 per 100,000 for whites. Swedes, Danes, and U.S. blacks appear to have the highest prevalence rates in the world. SARC is rarely reported in Spain, Portugal, India, Saudi Arabia, and South America, partly because of the absence of mass screening programs and also because of the presence of other, more commonly recognized granulomatous diseases (TB, leprosy, fungal infection) which may obscure SARC recognition.

The clinical presentation of SARC depends on the extent and severity of the organ involved. Approximately 5% of cases are asymptomatic and detected incidentally by chest radiography. Systemic complaints of fever, anorexia, and arthralgias occur in 45% of cases. Pulmonary complaints such as dyspnea on exertion, cough, chest pain, and hemoptysis (rare) occur in 50% of the cases. SARC is classified into five stages based on the pulmonary findings on chest radiography: normal findings (stage 0), bilateral hilar lymphadenopathy (BHL) (stage 1) BHL and infiltrates (stage 2), infiltrates alone (stage 3) and fibrosis (Stage 4). In terms of ocular manifestations, about 30-60% of patients develop intraocular inflammatory signs with bilateral granulomatous uveitis as the most common presentation. The CD4/CD8 ratio of vitreous-infiltrating lymphocytes has a high diagnostic value in ocular SARC, comparable to that of the bronchoalveolar lavage (BAL) fluid in pulmonary SARC. Cardiac manifestations include heart blocks and sudden death. Corticosteroid therapy may be effective for ventricular arrhythmias in the early stage but less effective in the late stage. The introduction of cardiac MRI has improved the diagnosis of cardiac involvement in systemic SARC. Approximately 25% of patients may have noncaseating granulomas at autopsy however less than 5% have clinical cardiac disease.

Among other manifestations diabetes insipidus is the most frequently reported endocrine disorder, followed by hyperprolactinemia. Hormonal deficiencies associated with hypothalamic-pituitary SARC frequently include hypogonadism and diabetes insipidus. In addition to these, SARC is associated with fatigue and a high rate of psychiatric comorbidity.

Diagnosis of this disorder usually requires the demonstration of typical lesions in more than one organ system and exclusion of other disorders known to cause a granulomatous disease. Although granulomas may resolve with little sequelae, pulmonary fibrosis occurs in 20 to 25% of the patients with SARC and the pathogenesis of pulmonary fibrosis still remains uncertain.

Bronchoalveolar lavage (BAL) is an established diagnostic tool in evaluating inflammatory and immune processes of the lungs. There is no single cell type present in the BAL fluid that appears to be predictive for SARC. However, BAL fluid analysis can be very helpful in the differential diagnosis. Many granulomatous infections may mimic a sarcoid-like granulomatous reaction and should be considered in the differential diagnosis. Among others, Lyme disease (Borrelia burgdorferi), Q-fever (Coxiella burnetii), leishmaniasis (Leishmania spp), and tuberculosis (M. tuberculosis) with indicated etiological agents may present with clinical pictures resembling SARC.

The enzyme chitotriosidase (ChT, EC 3.1.4.12), also known as the macrophage chitinase, (the human analogue of chitinases found in non-vertebrate species), is one of the most abundant marker proteins secreted by activated macrophages and is not expressed in monocytes. Although the function of ChT in humans is still unknown, its expression has been shown to be increased in SARC in some studies.

ACE activity is commonly used as a marker for disease activity. Since no single biomarker with appropriate specificity, sensitivity and unequivocal diagnostic/prognostic significance has been found up to now, investigations have still been continued to find a better marker. SARC and TB share similar clinical characteristics and both are prevalent in Turkey. Differential diagnosis of both diseases necessitate detailed laboratory study, including biopsy. New clinical markers should be investigated in the clinical trials to simplify differential diagnosis. Therefore, the aim of this study was to compare ChT activity levels in serum and BAL fluid, and serum ChT levels with serum ACE levels in patients with SARC, TB and ILD, which create difficulties for differential diagnosis in daily practice.

**Material and methods**

**Patient Selection**

This is a prospective study conducted in the
Yedikule Chest Disease and Chest Surgery Education Hospital between June 2008 to May 2009. A total of 88 patients with the diagnosis of SARC, TB and ILD were included. The study was approved by the Institutional Ethics Committee and informed written consent was obtained from all participants.

Patients were divided into three groups as follows. Patients newly diagnosed as having pulmonary SARC during its stable phase were enrolled in the SARC Group. No patient was on current steroid treatment or received steroid treatment during the preceding 3 months. The diagnosis of SARC was confirmed by typical clinical presentation, histology, and high-resolution computed tomography findings.

In TB Group, the diagnosis of TB was based on the clinical presentation as well as radiological findings and then confirmed by sputum microscopy and culture for M. tuberculosis. None of the patients had received TB treatment before their enrollment to the study. In ILD Group, patients with various diffuse interstitial diseases were included. The final diagnoses of the patients in ILD Group were interstitial pneumonia of idiopathic pulmonary fibrosis (n=3), bronchiolitis obliterans–organizing pneumonia of various causes (n=4), non-specific interstitial pneumonia (n=2), pulmonary silicosis (n=1), asbestosis, histiocytosis X, desquamative interstitial pneumonia, progressive systemic sclerosis and acute ILD in 13 patients each. Again, none of these patients had received any treatment before their enrollment to the study.

All study patients were non-atopic, had no history of alcohol abuse and did not present with other significant co-morbidities. Patients on antilipidemic and antioxidant medications were excluded from the study. All patients underwent fiberoptic bronchoscopic examination, and BAL fluid samples were obtained for diagnostic purposes. All patients were periodically followed up by the same pulmonologist.

**Specimen collection and processing**

Bronchoscopy with BAL was performed in all patients for diagnostic purposes as previously reported. Blood samples were drawn from the forearms between 8.30 and 10.00 AM after a 12-hour overnight fasting period. For each patient, duplicate aliquots of each blood sample were collected in tubes with and without an anticoagulant (Sodium Ethylenediaminetetraacetic Acid-EDTA). After immediate centrifugation (3000 g, 10 min, 4°C) plasma samples were stored at -80 °C until the final analysis. All parameters from all samples were analyzed in a single batch after completion of patients’ enrollment. All parameters were measured twice at the beginning and at the end of each run.

**Biochemical analysis**

Assay of serum ACE activity: Serum ACE activity was measured in duplicate aliquots, using a human enzyme-linked immunosorbent assay (ELISA) in accordance with the manufacturer's instructions (Quantikine, Human ACE Immunoassay, R&D Systems, Minneapolis, USA). The coefficients of intra- and inter-assay variations were 4.1% (n=10) and 4.7% (n=10), respectively.

Assay of plasma and BAL fluid ChT activity: ChT activities in plasma and BAL fluid samples were measured in duplicate aliquots, by a fluorimetric method using 4-methylumbelliferyl-β-D-N,N',N''-triacetylchitotriose as a substrate in accordance with the manufacturer's instructions (Sigma-Aldrich, Inc., USA). Hydrolysis of ChT was performed in an acidic environment (pH=5.0) at 37 °C. The coefficients of intra- and inter-assay variations were 4.9% (n=10) and 6.5% (n=10), respectively.

**Statistical analysis**

The Kruskal-Wallis analysis of variance test was used to examine significant intergroup differences and Mann-Whitney U test was used for intergroup comparisons of significant values. Correlation analysis was performed using Pearson’s correlation test. A P<0.05 was considered significant. Statistical analyses were performed using Statistical Packages for the Social Sciences (SPSS). (SPSS for Windows 10.0; SPSS, Chicago, IL). Data were presented as mean±standard deviation (SD).

**Results**

Eighty-eight patients met the inclusion criteria for the study. SARC group consisted of 37 patients (M/F= 8/29) with a mean age of 45.46±12.75 years. TB group consisted of 28 patients (M/F= 19/9) with a mean age of 37.29±13.50 years. ILD group had 23 patients (M/F= 10/13) with a mean age of 54.30±11.98 years. The groups were statistically different in terms of age, and this could be secondary to inclusion criteria regarding disease characteristics. However, in all the groups, the mean age corresponds well to the values reported in the literature for each specific disease. TB is generally seen in the young population in Turkey, which explains the lowest mean age (37 years) found in
the TB group. The mean age in the SARC group was 45.6 years, which is in accordance with the domestic statistics. Lastly, the mean age in the ILD group was 54.3 years and this value also corresponds well to the domestic demographic data defined for this disease.

One of the reasons why the sample sizes was different was that some patients who were initially included in the TB group were replaced to the SARC group since they had the final diagnosis of SARC. Another reason was that samples of some patients in the ILD group were lost due to technical error.

Clinical measurements and biochemical parameters related to all patients are outlined in Table 1.

<table>
<thead>
<tr>
<th></th>
<th>SARC Group (n=37)</th>
<th>TB Group (n=28)</th>
<th>ILD Group (n=23)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>45.46±12.75</td>
<td>37.29±13.50</td>
<td>54.30±11.98</td>
</tr>
<tr>
<td>Male/Female</td>
<td>29-Aug</td>
<td>19/9</td>
<td>13-Oct</td>
</tr>
<tr>
<td>Non-smoker</td>
<td>26 (70.3%)</td>
<td>7 (25%)</td>
<td>12 (52.2%)</td>
</tr>
<tr>
<td>Ex-smoker</td>
<td>3 (8.1%)</td>
<td>3 (10.7%)</td>
<td>1 (4.3%)</td>
</tr>
<tr>
<td>Smoker</td>
<td>8 (21.6%)</td>
<td>18 (64.3%)</td>
<td>10 (43.5%)</td>
</tr>
<tr>
<td>Serum ACE (ng/ml)</td>
<td>301.51±97.32</td>
<td>213.40±61.08a***</td>
<td>205.97±83.78“**”</td>
</tr>
<tr>
<td>Serum ChT (mU/L)</td>
<td>195.59±111.57</td>
<td>196.50±150.32</td>
<td>216.43±115.70</td>
</tr>
<tr>
<td>BAL ChT (mU/L)</td>
<td>28.27±28.50</td>
<td>37.34±35.49</td>
<td>27.39±32.26</td>
</tr>
</tbody>
</table>

Table 1: Clinical measurements and biochemical parameters in the study groups.

Discussion

Plasma ChT activity is commonly used and recommended for optimization of the clinical management of SARC. In several clinical investigations in SARC, plasma ChT activity has been shown to be a good marker for monitoring patients who have been treated with corticosteroids or any other medical treatment(12-20). Although we measured ChT activity in SARC, pulmonary TB and ILD, this activity was not found to be greater in SARC than in TB and ILD in this study. Since not all newly diagnosed SARC patients received steroid treatment, this group of patients was not classified as cases with active or inactive disease states. All the patients enrolled were recently diagnosed and no one in the groups was receiving any treatment.

Serum ChT activity of patients with SARC was first evaluated by Grosso et al. in 2004, and a significantly higher activity of this chitinase was recorded in sera of patients with SARC compared with those of controls(20). The idea to detect this enzyme in this particular granulomatous lung disease sprang from evidence of direct involvement of activated macrophages in the pathogenesis of SARC and granuloma formation(10). Tercelj et al. in 2009 reported that some patients with SARC had markedly higher ChT activities, but activity levels above controls were also found among patients with asbestos, fibrosis and lung cancer(9).

After treatment, ChT activity decreased in 52 of 69 patients. In their study, increased activities were also found in other diseases, and so it was concluded that ChT cannot be considered a specific marker of sarcoidosis. Cakir et al. showed that compared to healthy individuals, patients with pulmonary TB had elevated serum ChT levels. Of note, the elevated ChT levels returned to normal after completion of the standard 6-month antituberculous treatment. They also suggest that elevated serum ChT activity in pulmonary TB might be a
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marker of the disease extent, severity, and response to antituberculous treatment.

In our study, the ILD group was compared to TB and SARC groups. This study is the first study which compares ChT activity levels in the serum, and BAL fluids in these three groups and also with ACE values. Although the average serum ChT activity was higher in the ILD group than in the other two groups, this did not reach a statistical significance.

Recent studies have shown increased ChT activity in patients with fibrotic lung diseases. Our patients with hypersensitivity pneumonia-a granulomatous disease constituted 26% of the ILD group. According to the American Thoracic Society (ATS) and the European Respiratory Society (ERS) criteria, patients with clinical, and radiologic diagnosis of idiopathic pulmonary fibrosis (IPF)/ILD constitute 56% of the ILD group. As a result, in accordance with the recent literature, it can be suggested that the ChT enzyme activity can increase in patients with granulomatous and fibrotic lung diseases. The results of this study confirm that ChT activity is markedly higher only in some cases of SARC. The BAL fluid ChT activity was also found to be the highest in the TB group. Enhanced activation of alveolar macrophages involved in granuloma formation is a common feature of both of these granulomatous lung disorders, which have different etiologies.

According to the results, because of the wide statistical margin of both BAL fluid and serum levels of ChT in each group, it can be concluded that the ChT enzyme levels can not be used as a biomarker for SARC. However, as seen in the literature reviews, studies done in Mediterranean countries have shown that there is a genetic defect which leads to ChT enzyme deficiency. As a Mediterranean country, this fact should be taken into consideration in relevant studies performed in Turkey. In a recent study of Bargagli et al. (7), ChT concentrations proved to be a biomarker with good sensitivity and specificity that is easily detected in serum. However, the enzyme activity was not determined in this study. Further research is needed to confirm its prognostic value and its role in clinical practice and to determine its potential for differentiating SARC from other interstitial and granulomatous lung diseases.

ACE levels have been widely used for determining SARC activity. Although serum ACE levels have attracted a particular level of interest, diagnostic sensitivity and specificity of ACE for sarcoidosis are not perfect. In the present study, as a major disease activity marker, the serum ACE concentrations were found to be significantly higher in the SARC patients than in the TB and ILD patients, which confirms the hypothesis that this enzyme could be a marker of SARC. Bargagli et al. reported that BAL fluid and serum ChT activities were correlated with the radiological stages of the disease and also serum ACE levels. The prognostic value and sensitivity of ChT (but not ACE concentrations) was also correlated with radiological stage of the disease. However, chitinase was more sensitive as its levels were elevated in the serum and BAL fluids of all patients with progressive disease and in more than 85% of all SARC patients. ACE levels seems to be more specific for SARC than for ChT. The difference between ChT and ACE is that ChT is produced by both neutrophils and activated macrophages, whereas ACE is produced by only macrophages.

In conclusion, ACE is known to be the best-known marker of SARC, both ACE concentrations and ChT activity need to be evaluated by further investigations. Since elevated ChT activities are also found in TB and ILD, ChT can not be considered as a specific marker of SARC. Based on the only ChT activity levels, it is still not easy to evaluate the activity of SARC in clinical practice. Further research is needed to better understand the role of ChT in the pathogenesis of SARC and its involvement in fibrotic remodelling in certain diffuse lung diseases.

References


