Chitotriosidase activity in sarcoidosis and some other pulmonary diseases

MARJETA TERČELJ¹, BARBARA SALOBIR¹, SAŠA SIMCIC², BRANKA WRABER², MIRJANA ZUPANCIC³ & RAGNAR RYLANDER⁴

¹Department of Respiratory Diseases and Allergy, University Medical Centre, Ljubljana, Slovenia, ²Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana, Slovenia, ³Laboratory Department, Children’s Hospital, University Medical Center, Ljubljana, Slovenia, and ⁴BioFact Environmental Health Research Center, Lerum, Sweden

Abstract

Background: Patients with sarcoidosis have elevated levels of several markers of inflammation. Particularly high levels have been reported for chitotriosidase. In this study, we evaluate whether determining chitotriosidase in serum would be useful in the diagnosis and clinical management of patients with sarcoidosis. Methods: Patients with newly diagnosed sarcoidosis and patients with asthma, fibrosis, asbestosis, lung cancer or chronic obstructive pulmonary disease (n = 190) were recruited from an outpatient department. Individuals with no disease (n = 26) served as controls. An X-ray was taken, diffusion capacity was measured and blood samples were taken for analysis of chitotriosidase, soluble receptor for interleukin-2, tumour necrosis factor alpha and angiotensin converting enzyme. In most patients with sarcoidosis, the analyses were done before and after regular treatment with corticosteroids over 6 months. Results: Some patients with sarcoidosis had markedly high activities of chitotriosidase, but activities above controls were also found among patients with asbestos, fibrosis and lung cancer. There were significant relationships between chitotriosidase and interleukin-2 receptor and angiotensin-converting enzyme. After treatment, chitotriosidase activity decreased in 52 of 69 patients. Conclusions: The results confirm that chitotriosidase activity is markedly increased in some cases of sarcoidosis. As increased activities are also found in other diseases, chitotriosidase cannot be considered a specific marker of sarcoidosis. In cases of sarcoidosis where high CTO activities are found, this enzyme could serve as a useful marker supporting the diagnosis of sarcoidosis when following the effects of treatment and in surveillance for recurrence of the disease.

Key Words: Asbestosis, asthma, chitotriosidase, fibrosis, inflammation

Introduction

Chitotriosidase (CTO) is a chitinase protein secreted by activated macrophages and polymorphonuclear leucocytes (PMN) (1–3). It comprises part of the innate immune system and has the capacity to hydrolase chitin, and is thus important in the body’s defence against chitin-containing agents such as insects and fungi (2). High activities of CTO were first reported in Gaucher’s disease (4) and later among patients with diseases such as Alzheimer, multiple sclerosis, thalassemia and atherosclerosis (2), all of which comprise lysosomal storage disorders and macrophage activation.

Regarding pulmonary disease, the alveolar macrophage is part of the primary defences in the lung, and inhalation of different agents may induce the secretion of a variety of cytokines and other mediators of inflammation. Previous studies have demonstrated significantly higher activities of CTO in serum among patients with active pulmonary sarcoidosis (5,6), but not in patients with tuberculosis (7) or pulmonary fibrosis (8). In broncho-alveolar lavage (BAL), however, there is no difference in CTO activity between subjects with sarcoidosis and idiopathic pulmonary fibrosis (8), suggesting macrophage activation limited to the airways among those with fibrosis.

To further explore the usefulness of measuring CTO among persons with sarcoidosis in the clinical setting, CTO in serum was analysed in patients with...
sarcoidosis and several other pulmonary diseases and those without disease, and compared to other markers of inflammatory pulmonary disease.

**Material and methods**

Patients were recruited from the outpatient clinic at the Department of Respiratory Diseases and Allergy of the University Medical Hospital in Ljubljana, Slovenia. The study was conducted with the full informed consent of all participating subjects and was approved by the Ethics Committee at the hospital.

All patients with sarcoidosis were newly diagnosed using established criteria (9) and had not been treated with corticosteroids or other anti-inflammatory agents. For sarcoidosis and fibrosis, care was taken not to include patients with clinical signs of pulmonary infection. Most patients with sarcoidosis were examined before and at 6 months after conventional treatment with corticosteroids (oral prednisolone 20–40mg/day for 1 month and on alternate days 12–16mg for the rest of the period). Controls were recruited from subjects who came to the outpatient department, but after investigation were found to have had symptoms due to upper respiratory infection or reflux. None of the inflammatory tests conducted in connection with the CTO were positive for infection. Table I gives a breakdown of the diagnosis of the subjects.

The examinations comprised an X-ray with severity grading of granulomas in accordance with a numerical score (0–4) judged by size and extension of the infiltrates. The X-ray images were read by an experienced radiologist with no knowledge of the patient’s characteristics.

Freshly drawn venous blood samples were collected in tubes containing EDTA (to obtain plasma) and tubes without anticoagulant (to obtain serum). After centrifugation, plasma and serum samples (completely depleted of blood cells) were immediately stored at –20°C. Soluble IL-2 receptor (sIL-2R) in serum or plasma was quantitated immediately with an ELISA commercial kit (Milenia Biotech, Badnauheim, Gemany). Angiotensin-converting enzyme in serum (sACE) was determined using a colorimetric method (10).

Table I. Different diagnosis in study cohort.

<table>
<thead>
<tr>
<th>Disease</th>
<th>n</th>
<th>Age mean</th>
<th>% females</th>
</tr>
</thead>
<tbody>
<tr>
<td>No disease</td>
<td>26</td>
<td>46.9</td>
<td>27</td>
</tr>
<tr>
<td>Sarcoidosis-treated</td>
<td>88</td>
<td>47.5</td>
<td>52</td>
</tr>
<tr>
<td>Teated</td>
<td>81</td>
<td>47.5</td>
<td>51</td>
</tr>
<tr>
<td>Asthma</td>
<td>35</td>
<td>41.2</td>
<td>58</td>
</tr>
<tr>
<td>Fibrosis</td>
<td>21</td>
<td>66.9</td>
<td>56</td>
</tr>
<tr>
<td>Asbestosis</td>
<td>18</td>
<td>57.6</td>
<td>31</td>
</tr>
<tr>
<td>Lung cancer</td>
<td>15</td>
<td>64.3</td>
<td>1 (n)</td>
</tr>
<tr>
<td>COPD</td>
<td>8</td>
<td>54.0</td>
<td>7 (n)</td>
</tr>
</tbody>
</table>

Table II. Chitotriosidase activity (nmol/h/mL) in serum of groups of patients with different pulmonary diseases.

<table>
<thead>
<tr>
<th>Disease</th>
<th>n</th>
<th>Mean</th>
<th>SD</th>
<th>Median</th>
<th>Min</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>No disease</td>
<td>25</td>
<td>33</td>
<td>17</td>
<td>33</td>
<td>2</td>
<td>68</td>
</tr>
<tr>
<td>Sarcoidosis</td>
<td>88</td>
<td>777</td>
<td>670</td>
<td>629</td>
<td>12</td>
<td>3483</td>
</tr>
<tr>
<td>Asthma</td>
<td>35</td>
<td>48</td>
<td>49</td>
<td>29</td>
<td>2</td>
<td>243</td>
</tr>
<tr>
<td>Fibrosis</td>
<td>21</td>
<td>302</td>
<td>285</td>
<td>243</td>
<td>2</td>
<td>837</td>
</tr>
<tr>
<td>Asbestosis</td>
<td>18</td>
<td>245</td>
<td>284</td>
<td>63</td>
<td>15</td>
<td>825</td>
</tr>
<tr>
<td>Lung cancer</td>
<td>14</td>
<td>198</td>
<td>181</td>
<td>150</td>
<td>4</td>
<td>513</td>
</tr>
<tr>
<td>COPD</td>
<td>8</td>
<td>80</td>
<td>65</td>
<td>62</td>
<td>9</td>
<td>231</td>
</tr>
</tbody>
</table>

The CTO activity in serum was determined using the 22μM 4-methylumbelliferyl-β-D-N,N',N''-triacetylchitotriosiose (Sigma) in citrate phosphate buffer (pH 5.2). Five microlitres of serum was incubated with 100μL substrate for 1h at 37°C. The reaction was stopped with 2.5mL of 0.3M glycine/NaOH buffer (pH 10.6). Fluorescent 4-methylumbelliferone was read at excitation 365nm and emission 465nm using a Perkin-Elmer fluorimeter and CTO activity was expressed as nmol/h/mL (4).

Differences between groups were evaluated using the t-test and correlations between different parameters using the Spearman rank-order test. Significance was determined as p<0.05.

**Results**

**All diseases**

Table II reports the activity of CTO in the different categories of disease examined. The group average CTO activity was significantly higher than that of the controls in sarcoidosis, asbestosis, fibrosis and lung cancer (p=0.001, p=0.005, p=0.018 and p=0.040, respectively). The activity in sarcoidosis was significantly higher than in fibrosis or asbestosis (p=0.001 and p=0.001, respectively). For patients with asthma and COPD, the average activities were not significantly different from those of controls.

**Patients with sarcoidosis**

CTO activities >100 (twice the highest value for controls) were found in 79 of the 88 patients with sarcoidosis (91%). Activities above 100 were found in 18 (21%). The activity of CTO was related to the X-ray grading (r²=0.404, p=0.001), to the levels of sIL-2R (r²=0.553, p=0.001) and less to the levels of SACE (r²=0.264, p=0.017).

Table III gives the different parameters before and after treatment among patients with sarcoidosis who had CTO determination both before and after treatment. After treatment with corticosteroids, CTO activity decreased in 51 out of 68 (75%) patients. The results regarding CTO activity in different diseases are summarized in Figure 1.
Chitotriosidase in sarcoidosis

A slight elevation of CTO was found in BAL (8). Slightly elevated activities were also found among subjects with asbestosis and lung cancer, but not among subjects with asthma or COPD. There was a significant decrease in CTO among most of the patients with sarcoidosis after treatment with corticosteroids.

The relation between CTO and sIL-2R concurs with that of previous reports (11) and indicates that low CTO activities reflect a less active stage of sarcoidosis, as has been suggested previously (6,7). In studies on T-cells from BAL in sarcoidosis patients, stimulation of the cells increased the number of II-2+ cells (12). sIL-2R as well as CTO can thus be considered as a marker of the inflammatory stage of sarcoidosis. We also found a relation between CTO and sACE, as has been reported in a previous publication (7). The range of sACE values among patients with sarcoidosis in this study was 0.03 to 0.98, which is considerably less than the range for CTO (12–3483).

CTO is selectively expressed and released upon specific stimuli by human PMNs and macrophages, and granulocyte macrophage colony-stimulating factor (GM-SCF) is important in this process (3). As the role of CTO in the innate immune system is likely to be defence against chitin-containing organisms such as insects and fungi (2), the considerably higher activities among subjects with sarcoidosis could reflect a specific reaction to chitin, a major component of fungi. It has previously been suggested that fungi may be a causative agent in some cases of sarcoidosis (13,14). A high activity of CTO might thus reflect a defence reaction to chitin in the fungal cell wall, a process that can be initiated without the presence of an infection. The high activities of CTO in serum of patients with sarcoidosis could reflect a more general involvement of the body in comparison to fibrosis, which would be confined to the airways and the lung (8). In this connection it is of interest that β-glucan, which is a major constituent of fungi and has the capacity to induce granulomas, becomes widespread in the body after peroral administration (15).

Some subjects with sarcoidosis had low values of CTO in the range of the controls (9 out of 88). This finding is similar to that of a previous report on CTO in Gaucher’s disease (4) and could reflect the presence of an inherited enzyme deficiency in these individuals (16). The number of persons with this deficiency is different in different population groups, thus suggesting that this enzyme is ancient in the evolution and has become redundant in certain environments (2). The role of the enzyme deficiency in the risk of sarcoidosis and in relation to fungal exposure needs to be further explored. In clinical work on suspect cases of sarcoidosis, however, genetic characteristics are not explored, which means that the CTO test has a relatively low sensitivity for the disease. If a value of 1000 is taken

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>Before</th>
<th>6 months</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTO</td>
<td>68</td>
<td>824 (639)</td>
<td>378 (391)</td>
<td>0.001</td>
</tr>
<tr>
<td>X-ray</td>
<td>68</td>
<td>1.9 (1.0)</td>
<td>1.2 (0.9)</td>
<td>0.001</td>
</tr>
<tr>
<td>sIL-2R</td>
<td>57</td>
<td>567 (494)</td>
<td>352 (278)</td>
<td>0.002</td>
</tr>
<tr>
<td>sACE</td>
<td>65</td>
<td>0.42 (0.18)</td>
<td>0.31 (0.16)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Table III. Different markers of sarcoidosis before and after treatment with corticosteroids (mean and SD in parentheses). Paired sample test.

Comments

The major finding in this investigation was the markedly higher activities of CTO in some patients with sarcoidosis and slightly increased levels among patients with fibrosis, asbestosis and lung cancer, but not among those with asthma or COPD. Treatment with corticosteroids caused a decrease in the CTO activity in the majority of patients.

The study had certain limitations. For technical reasons, the different inflammatory parameters could not be analysed among all patients initially recruited and in certain diagnostic groups the number of patients was small.

In spite of this, we could confirm the findings of earlier studies reporting that many patients with active sarcoidosis have highly increased activities of CTO in serum (5–7). In the present study, we could also show the effect of treatment. The average CTO activities in this study were markedly higher than those reported in previous studies, possibly because of treatment – in this study none of the patients received medication. No account of medication is given in the previous reports, but their CTO activities were close to the activities after treatment in the present study. We also found slightly elevated activities among patients with fibrosis, which has previously only been reported in

Figure 1. CTO (nmol/h/mL) in different pulmonary diseases.
as a threshold clearly higher than values found in other diseases, the specificity in this material would be 100% but the sensitivity only 26%.

Subjects with asthma had activities of CTO similar to those among control subjects. It has earlier been reported that another member of the chitinase family – chitinase3-like 1 or YKL-40 – is increased among asthmatics (17); a promoter for the gene coding this chitinase has been reported to be more frequent among persons with asthma (18). YKL-40 has no enzyme activity, which suggests that exposure to a chitin-containing agent like fungi elicits a chitinase response that is different from the response initiated by the inflammation among asthmatics.

The results from this and previous studies demonstrate that CTO activity is high in fibrosis, asbestosis and in a number of other diseases involving macrophage activation (1–3). High activities have also been related to an increased risk of cardiovascular disease (19). CTO cannot therefore be considered a specific marker for sarcoidosis and is of little use in the diagnostic procedure of suspect cases unless the value is very high. Once the diagnosis has been established, however, determinations of CTO could be of value in following the efficiency of the treatment. In surveillance of patients with regressed sarcoidosis, determinations of CTO as a means of detecting recurrences of the disease could decrease the number of X-ray examinations. These concepts should be tested in further clinical trials.

In conclusion, a high activity of CTO found in some patients with sarcoidosis decreased after treatment. Although CTO is not specific for sarcoidosis, it could be a useful clinical tool for both diagnostic and patient surveillance purposes among patients who have high values when the diagnosis is made.

Acknowledgement

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Disclosures

None of the authors have any relationships with commercial organizations relevant to the study, nor any other disclosures to make.

References