Evaluation of chitotriosidase and high sensitive C reactive protein levels in patients with mucopolysaccharidoses

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Mucopolysaccharidosis, inflammation, chitotriosidase, hs-CRP

**ABSTRACT • Background and Aims:** Mucopolysaccharidosis is a lysosomal storage disease caused by deficiency of the relevant lysosomal enzyme. It has been recently shown that inflammatory pathways have been playing major role in the pathogenesis of mucopolysaccharidoses. Chitotriosidase is secreted mainly by active macrophages and is synthesized both in normal healthy conditions and also in inflammatory processes. High sensitive C reactive protein is an inflammatory biomarker found in high levels in cardiovascular disorders and inflammation. In this study, the aim was to evaluate the chitotriosidase and high sensitive C reactive protein levels in patients with mucopolysaccharidoses. **Method:** Thirty-six patients with mucopolysaccharidoses and 29 healthy children as control group were included into the study. Plasma chitotriosidase and high sensitive C reactive protein levels were studied in blood samples. Chitotriosidase was analyzed by spectrofluorometry and high sensitive C reactive protein was analyzed by nephelometry. **Results:** Plasma chitotriosidase levels were found to be significantly increased in patients with mucopolysaccharidoses with respect to the control group (p ≤0.05 was significant). There was no statistically significant difference for high sensitive C reactive protein levels between the patient and control groups. **Conclusions:** Chitotriosidase seems to be an important marker for patients with mucopolysaccharidosis showing inflammatory response as in other lysosomal diseases.

**INTRODUCTION**
Mucopolysaccharidoses (MPS) are lysosomal storage diseases caused by deficiency of enzymes that are responsible for degradation of glycosaminoglycans (GAG). In the pathogenesis of MPS, intralysosomal storage of GAGs can induce inflammatory pathway activation that leads to increase inflammatory mediators and oxidative stress mediators. Chitotriosidase (CHIT1) is first mammalian chitinase and is produced by a wide range of organisms including bacteria, fungi, insects, plants and mammals for nutrition, morphogenesis and defense against pathogens and also plays roles in tissue remodeling, cell migration, and atherogenesis (1,2).
CHIT1 has been suggested as an important biomarker for the support of the diagnosis of Gaucher’s disease (2). The elevation of CHIT1 may reflect a particular state of activation of macrophages and disease activation. Elevated levels of CHIT1 are also seen in some other lysosomal storage diseases such as Niemann Pick, Fabry disease, Wolman, Krabbe, GM1 gangliosidosis, Tay-Sachs and Sandhoff, metachromatic leukodystrophy, lysosomal acid lipase deficiency, fucosidosis, cystinosis and also galactosialidosis (3). CHIT1 has also been implicated in the pathogenesis of many human diseases through the improper induction of inflammation. Some inherited or acquired conditions related with inflammation and macrophage activation including infections (fungal and bacterial infection, malaria), atherosclerosis, sarcoidosis, nonalcoholic fatty liver disease or steatohepatitis, and some neurodegenerative diseases such as Alzheimer’s disease and ischemic cerebrovascular dementia (4-9) have increased levels of CHIT1.

High sensitive C reactive protein (hs-CRP) is an inflammatory biomarker found in high levels in cardiovascular disorders and inflammation.

In this study, the aim was to evaluate the CHIT1 and hs-CRP levels in patients with MPS as inflammation plays an important role in the pathogenesis of lysosomal disorders.

**MATERIALS and METHODS**

**Patients**

Patients diagnosed with either enzymatic or molecular analyses were included into the study. All patients signed informed consent and ethical approval was taken from Gazi University Ethics Committee.

A total of 36 MPS patients and 29 healthy children as control group were included into the study. Any patient who was suspected to have an infection by physical examination and/or laboratory investigation was excluded from the study. Having an other chronic disease was also another exclusion criteria.

**CHIT and hs-CRP levels**

Plasma CHIT1 activity was studied by spectrofluorometric method. Ten microliter of plasma was mixed with 100 μL of 0.022 mmol/L 4-MU-C3 in Citrate/Phosphate buffer, 0.1/0.2 mol/L, pH 5.2 and incubated at 37 °C for 15 min. The reaction was stopped with 2 mL of 0.5 mol/L Carbonate/Bicarbonate buffer, pH 10.7. 5 μl plasma was incubated for 1 h with 100 μl of 22 μmol/L 4 methyllumbelliferyl –β-D-N,N2,N22-triacetylchitotriose in citrate phosphate buffer pH 5.2 at 37°C. Fluorescence activity was measured by FP 6200. Enzyme activities were calculated based on a calibration curve of 4-methylumbelliferone (4-MU) for each assay (10). hs-CRP was studied by nephelometric method mentioned by Robert et al (11).

**Statistical Analyses**

Results were expressed as mean and standart deviation. Student t test was used for all comparisons between two groups. Statistical results were expressed as significance of p value. Differences were considered significant when p ≤ 0.05.

**RESULTS**

Mean age of MPS patients were 8,18 year (range 1,5-18 years) and mean age of control group was 7,53 year (range 1-18 years).

There were 2 MPS I, 5 MPS II, 10 MPS III, 5 MPS IV, 13 MPS VI and 1 MPS VII patient (Table 1).

When plasma CHIT1 activity was compared with control group, plasma CHIT1 activity was found to be significantly increased in MPS patients. Mean CHIT1 level was 148,20 nmol/h/ml for MPS patients and was 84,87 nmol/h/ml (p ≤0.05) for the control group (Figure 1).

CHIT1 levels were also compared between enzyme replacement therapy (ERT) treated and non-treated MPS patients and there was no statistically significant difference (Figure 2).
Mucopolysaccharidosis is a group of multisystemic diseases characterized by GAG accumulation in several tissues or organs. In humans, CHIT1 is mostly expressed by mature monocyte-derived macrophages in blood and tissue macrophages in liver, spleen, lung, central nervous system (10-15) and to a lesser extent by leucocytes (16).

No significant difference was found for hs-CRP levels between MPS patients and control group. Mean hs-CRP level was 0.03 mg/dl with a standard deviation of 0.0224 in MPS patients and 0.03 mg/dl in control group, with a standard deviation of 0.247 and (p>0.05) (Figure 3).

No significant difference was found for hs-CRP levels between ERT treated and non-treated groups (p>0.05) (Figure 4).

**Table 1** MPS subtypes in the study

<table>
<thead>
<tr>
<th>MPS Subtypes</th>
<th>Number</th>
<th>ERT-Treated</th>
<th>ERT-Non-treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPS I</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>MPS II</td>
<td>5</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>MPS III</td>
<td>10</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>MPS IV</td>
<td>5</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>MPS VI</td>
<td>13</td>
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<td>0</td>
</tr>
<tr>
<td>MPS VII</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

MPS: Mucopolysaccharidosis. ERT: Enzyme replacement therapy.

**DISCUSSION**

Mucopolysaccharidosis is a group of multisystemic diseases characterized by GAG accumulation in several tissues or organs. In humans, CHIT1 is mostly expressed by mature monocyte derived macrophages in blood and tissue macrophages in liver, spleen, lung, central nervous system (10-15) and to a lesser extent by leucocytes (16).
Figure 2  Chitotriosidase levels in treated and non-treated groups.

Figure 3  hs-CRP levels in MPS and control groups.
There are studies showing different results in the literature. Sayeth et al. studied CHIT levels in lysosomal storage diseases. Among them, increased levels of CHIT1 activity were found in two MPS IV and two MPS VI patients (21).

Kurt et al. showed that in MPS patients, CHIT1 activity was not increased compared to control group (24-26).

hs-CRP is a circulating form of CRP which is more stable than CRP and that has a long half life up to 20 hours (27-30).

There is no other study that looked for hs-CRP levels in patients with mucopolysaccharidosis.

The results of this study suggest that inflammatory process is also present in patients with MPS reflected by increased CHIT1 levels.

When chitin or its particles are cultured with macrophages, macrophages activate and can lead to the production of some cytokines (17) which then induce subsequent pathways of inflammation. It has been shown that in MPS models there were increased levels of some (18). CHIT1 is expressed in acute and chronic inflammatory events (12,19).

Simonaro at al. showed that in MPS patients treated with ERT decreased systemic tumor necrosis factor (TNF)-alpha release can be seen (20) due to cessation of inflammation but in this study, CHIT1 levels were found higher in ERT treated group than the non ERT group although not statistically significant. This might be due to macrophage activation from early GAG deposition that has already initiated the inflammatory process.

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