Serum Chitotriosidase Activity in Patients With Coronary Artery Disease

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Background  Atherosclerosis is considered to be an inflammatory disease in which the initial process is augmented infiltration of monocytes into the vessel wall and their subsequent differentiation from macrophages into lipid-laden foam cells. Chitotriosidase is one of the most quantitative proteins secreted by activated macrophages, so the aim of this study was to investigate the association of the level of serum chitotriosidase activity with atherosclerotic coronary artery disease (CAD).

Methods and Results  A total of 200 subjects undergoing coronary angiography were divided into 4 subgroups according to the number of diseased vessels and their serum chitotriosidase activity levels were measured. Serum chitotriosidase activity in patients with CAD was significantly higher than in normal control subjects (p<0.001). Serum chitotriosidase activity was also significantly associated with the extent of CAD as defined by the number of stenosed vessels (p<0.001).

Conclusion  Serum chitotriosidase activity can be considered a strong inflammatory marker of CAD. Moreover, plasma chitotriosidase activity may be also regarded as a quantitative indicator of disease extent, as well as being a marker of disease presence.  (Circ J 2008; 72: 71–75)

Key Words:  Atherosclerosis; Chitotriosidase; Coronary artery disease; Inflammation

Atherosclerosis is considered to be an inflammatory disease that is characterized by progressive deposition of lipids and fibrous matrix in the arterial wall. The initiation of atherogenesis involves activation of endothelial cells, which facilitates monocyte infiltration of the vessel wall. These monocytes differentiate into macrophages, which accumulate lipids from the circulation and remain in the vessel wall, thereby becoming so-called foam cells. Subsequent migration and proliferation of smooth muscle cells from the media into the neointima is observed, probably induced by growth factors and cytokines secreted by the activated endothelial cells and macrophages. Activated macrophages inside the atherosclerotic lesion play a central role in atherosclerosis and the formation of lipid-laden foam cells from macrophages represents a landmark for atherosclerosis. The molecular mechanisms involved in the anomalous behavior of macrophages in atherogenesis have only been partially disclosed. A more comprehensive understanding of atherogenesis necessitates more detailed knowledge of the proteins secreted in the vessel wall by the macrophages that take part in this pathological process.

Compelling evidence suggesting the central role of inflammation in the pathogenesis of atherosclerosis highlights the importance of macrophage activity indicators, such as chitotriosidase, in the diagnosis and prediction of coronary artery disease (CAD). Several markers of systemic inflammation and macrophage activation have already been found to be associated with the presence of complex lesions and the development of serious cardiovascular events. Chitotriosidase, a human chitinase member of family 18 glycosylhydrolase, is one of the most quantitative proteins secreted by activated macrophages, so its activity has been proposed as a biochemical marker of macrophage accumulation and activity in several lysosomal lipid storage diseases, such as sphingolipidoses (Nieman Pick, GM-gangliosidosis and Krabbe disease). Recently, some studies demonstrated a significant association between chitotriosidase activity and atherosclerosis. However, there is little information about the association of serum chitotriosidase activity with atherosclerosis and to our knowledge ours is the only second study to assess this relationship in patients with CAD. To our knowledge this is the first clinical study investigating the association in patients with stable ischemic heart disease and is also unique by having a control group composed of persons with angiographically documented strictly normal coronary arteries.

The primary aim of this study was to investigate the association of serum chitotriosidase activity with the presence and extent of CAD assessed by coronary angiography (CAG). Our secondary goal was to assess the relationship of high-sensitivity C-reactive protein (hsCRP) with the presence and angiographic severity of CAD.

Methods

Study Design and Subjects

The study was conducted in accordance with the Helsinki Declaration of 1975. The study protocol was approved by the Ethics Committee at Istanbul University, Cerrahpasa

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School of Medicine, and written informed consent was given by all patients. Plasma chitotriosidase activity and hsCRP levels were measured in 200 consecutive patients (176 men, 24 women) with suspected stable CAD who underwent CAG in the Cardiac Catheterization Laboratory for routine diagnostic purposes. Subjects had either a cardiac history or symptoms sufficient to warrant angiography. The indications were history of CAD, clinical evidence of angina pectoris, and suspect chest pain. All patients with myocardial infarction within 30 days, those with unstable angina who had anginal pain at rest within 30 days, those with a history of prior coronary revascularization were excluded. None of the subjects included in this study showed any evidence of ongoing systemic or cardiac inflammatory disease. Patients with diabetes mellitus, a history of recent clinical infection, concurrent major renal, hepatic and malignant disease were also excluded.

The 200 subjects are classified into 4 groups: control group (n=53) comprised subjects with strictly normal coronary angiograms and shown as CAD (0); 1-vessel disease group (1VD group, n=52); 2-vessel disease group (2VD group, n=47); and 3-vessel disease group (3VD group, n=48). CAD was defined as at least 1 coronary artery with ≥50% luminal diameter stenosis. To evaluate the angiographic extent of CAD, the patients were classified according to the number of diseased vessels. Luminal narrowing ≥50% was defined as a significant lesion. Subjects with minor irregularities of the coronary vasculature or moderate diameter reduction (<50%) were excluded.

Information regarding age, medications, and medical history were obtained during interview with a physician. Serum lipid parameters were also determined.

Protocol for CAG

Selective CAG was performed by the Judkins technique through the femoral approach with 6F catheters. Stenosis severity was determined by visual estimation (in ≥2 orthogonal views) and angiographic findings were assessed by experienced cardiologists. Operators reading the angiograms were unaware of the results of any laboratory analyses. The number, location, and severity of lesions in each arterial segment were recorded.

Blood Sampling

After an overnight fast, blood samples were drawn at the time of catheterization, before administration of contrast agent or medications. Blood samples were left to clot and the serum was separated by centrifugation within 2h of sampling. All serum samples were stored at −70°C until analysis.

Biochemical Analysis

Quantification of Lipids

Total cholesterol (TC) and triglyceride (TG) levels were quantified enzymatically with a Beckman Synchron LX 20 analyzer (Boehringer Mannheim, Mannheim, Germany). High-density lipoprotein-cholesterol (HDL-C) was measured after precipitation of apolipoprotein B-containing lipoproteins with Mg++ phosphotungstate (Boehringer Mannheim). Low-density lipoprotein-cholesterol (LDL-C) was calculated by the Friedewald formula.

Chitotriosidase Enzyme Assay

Chitotriosidase activity was measured by incubating 5 μL serum with 100 μL of 22.0 μmol/L 4-methylumbelliferyl-(β-D-N,N‘,N“-triacetylchitotriose (MU-(1-GlcNAc)3; Sigma Chemical Co, St Louis, MO, USA) as the substrate in Mellvain’s phosphate-citrate buffer, pH 5.2 for 1h at 37°C (modified from Hollak et al14). Reaction was terminated by adding 120 μL 0.5 mol/L Na2CO3-NaHCO3 buffer, pH 10.7, and the fluorescence of 4-methylumbelliferone was measured with a fluorimeter (Titertek; excitation 355 nm, emission 460 nm).

hsCRP Assay

Serum concentrations of hsCRP were measured by the Behring BN II Nephelometer (DADE Behring, Marburg, Germany) and expressed as milligrams per liter.

Statistical Analysis

The statistical analyses were performed using SPSS statistical program version 12.0 (SPSS, Chicago, IL, USA). All values are expressed as mean±SD unless otherwise stated. Quantitative variables were compared with ANOVA 1-factor adjusted for age and sex. Hypothetical associations between chitotriosidase activity, hsCRP and plasma lipid levels were studied by ANOVA multivariate with the variables of sex, age and smoking as covariates. Comparisons of plasma chitotriosidase activity and hsCRP between studied groups were done by ANOVA 1-factor adjusted for age and sex. The Kruskal-Wallis test was used to compare more than 2 normally distributed means. Serum lipid levels were studied by ANOVA multivariate with the variables of sex and age. Multiple comparisons of chitotriosidase activity and number of diseased vessels were done by ANOVA 1-factor and the Bonferroni adjustment. A value of p<0.001 was considered statistically significant for all analyses.

### Table 1 Clinical Characteristics and Laboratory Findings Stratified by Number of Diseased Vessels

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Gender, % male</th>
<th>Age, years</th>
<th>Current smokers, %</th>
<th>TC (mg/dl)</th>
<th>HDL-C (mg/dl)</th>
<th>LDL-C (mg/dl)</th>
<th>TG (mg/dl)</th>
<th>TC/HDL-C</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAD (0)</td>
<td>53 (26.5%)</td>
<td>58±9.7</td>
<td>20</td>
<td>146±5.6</td>
<td>40±2.9</td>
<td>79±3.5</td>
<td>128±10.9</td>
<td>3.92±0.19</td>
<td></td>
</tr>
<tr>
<td>1VD</td>
<td>52 (26%)</td>
<td>58±10.5</td>
<td>23</td>
<td>130±7.07</td>
<td>35±1.47</td>
<td>92±5.31</td>
<td>107±8.93</td>
<td>3.72±0.12</td>
<td></td>
</tr>
<tr>
<td>2VD</td>
<td>47 (23.5%)</td>
<td>60±10.5</td>
<td>29</td>
<td>146±6.6</td>
<td>35±2.06</td>
<td>90±5.66</td>
<td>109±8.31</td>
<td>4.58±0.29</td>
<td></td>
</tr>
<tr>
<td>3VD</td>
<td>48 (24%)</td>
<td>61±10.3</td>
<td>25</td>
<td>131±8.17</td>
<td>33±1.64</td>
<td>75±26.88</td>
<td>120±11.73</td>
<td>4.03±0.32</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean±SD (range) or percentage of total subjects in each group. p value indicates significance levels between groups. CAD (0), control group without coronary artery disease; 1VD, 1-vessel disease group; 2VD, 2-vessel disease group; 3VD, 3-vessel disease group; NS, not significant (p>0.05); TC, total cholesterol; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; TG, triglycerides.
Results

Description of Subjects

The study population comprised 200 subjects (176 men, 24 women) undergoing CAG, who were classified into 4 groups according to the number of diseased vessels: 52 patients had 1VD, 47 patients had 2VD, 48 had 3VD and the other 53 had strictly normal angiograms and constituted the CAD-free control group. The characteristics and the laboratory findings of the groups are detailed in Table 1. No statistical differences between the groups in relation to age, TC, TG, LDL-C, HDL-C and TC/high-density lipoprotein ratio were found.

Serum Chitotriosidase Activity

Serum chitotriosidase activities are presented as box whisker plots for each of the 4 studied groups (Fig 1). A statistically significant stepwise increase in serum chitotriosidase activity level was observed according to the number of ≥50% stenosed vessels (Table 2). Serum chitotriosidase activities were 111±10.8 mmol·ml⁻¹·h⁻¹, 138±12.02 mmol·ml⁻¹·h⁻¹ and 179±11.4 mmol·ml⁻¹·h⁻¹ for patients with 1VD, 2VD and 3VD, respectively. Serum chitotriosidase activity in the control subjects was 83±10.3 mmol·ml⁻¹·h⁻¹. The variables of TC, LDL-C, HDL-C, TG, and age did not correlate with serum chitotriosidase activity. There was a significant difference in serum chitotriosidase activity between subjects with and without CAD (p<0.001). Differences in serum chitotriosidase activity were also statistically significant between the study groups based on the number of diseased vessels (p<0.001).

hsCRP

Serum concentrations of hsCRP are presented as box whisker plots for each of the 4 study groups (Fig 2). hsCRP levels for the 3 subgroups with different numbers of diseased vessels are shown in Table 2. Serum concentrations of hsCRP were 2.97±0.31 mg/L, 3.97±1.05 mg/L, 9.08±2.09 mg/L and 12.25±3.46 mg/L for patients with 1VD, 2VD and 3VD, respectively. Serum chitotriosidase activity in the control subjects was 83±10.3 mmol·ml⁻¹·h⁻¹. The variables of TC, LDL-C, HDL-C, TG, and age did not correlate with serum chitotriosidase activity. There was a significant difference in serum chitotriosidase activity between subjects with and without CAD (p<0.001). Differences in serum chitotriosidase activity were also statistically significant between the study groups based on the number of diseased vessels (p<0.001).
peripheral vascular diseases. Some studies have revealed that macrophages are activated and produce inflammatory markers such as chitotriosidase and hsCRP. Among the markers of inflammation, hsCRP is the most extensively studied and it is well known to be associated with the presence of atherosclerotic plaque.17-20 It belongs to the chitinase inflammatory family, a group of enzymes with the capacity to hydrolyze chitin. This enzyme is involved in the degradation of chitin-containing pathogens, with an unknown function in humans.21

We also assessed the relationship between hsCRP concentration and the number of diseased vessels. Statistical differences were found between patients with 1VD, 2VD and 3VD, respectively (Table 2). Mean hsCRP concentration in the control group was 2.97±0.31 mg/L. The variables of TC, LDL-C, HDL-C, TG and age did not correlate with hsCRP concentration. Differences were statistically significant between subjects with 2VD and 3VD vs control subjects (p<0.001).

We also assessed the relationship between hsCRP concentration and the number of diseased vessels. Statistical differences were found between patients with 1VD, 2VD and 3VD (p<0.05) (Fig 2). There was a parallel increase in serum chitotriosidase activity and hsCRP in association with the number of diseased vessels, but the statistical significance was more prominent for serum chitotriosidase activity (p<0.001 vs p<0.05). Backward linear regression analysis showed a significant correlation between chitotriosidase activity and hsCRP in the study groups (p<0.05) (Fig 3).

**Discussion**

Laboratory and prospective clinical studies3,14-16 have recently reinforced the inflammatory theory proposed by Ross et al.17 which stated that each step of the molecular and cellular responses leading to atherosclerosis is an inflammatory process in which activated macrophages seem to play a central role. Macrophages are present in all phases of atherogenesis and have been shown to be markers of atherosclerotic plaque formation.15 In the present study we analyzed 2 markers of activated macrophages in CAD: chitotriosidase and hsCRP. Among the markers of inflammation, hsCRP is the most extensively studied and it is well documented by several studies that high levels of hsCRP are associated with elevated risk of coronary, cerebral and peripheral vascular diseases18-21. Some studies have revealed an association between hsCRP and the severity and extent of CAD, but others have not found a significant correlation.22-25

Chitotriosidase is one of the most quantitative proteins secreted by activated macrophages and considered a strong marker of macrophage activity.26 It belongs to the chitinase family, a group of enzymes with the capacity to hydrolyze chitin. This enzyme is involved in the degradation of chitin-containing pathogens, with an unknown function in humans.21 It is synthesized as a 50-kDa protein composed of a 39-kDa N-terminal catalytic domain, a hinge region and a C-terminal chitin-binding domain. It is mostly secreted, but partly processed into a 39-kDa form that accumulates in lysosomes. In the tissues the 39-kDa form is abundant, whereas the secretory 50-kDa chitotriosidase is predominantly found in the blood stream.22 Chitotriosidase in humans is synthesized exclusively by activated macrophages and its enzymatic activity is elevated in the serum of patients with diseases in which macrophages are activated. A small number of studies have also demonstrated an association between macrophage chitotriosidase expression and atherosclerosis, suggesting a possible role as an atherosclerotic marker.1-13 Boot et al. documented that chitotriosidase activity is increased up to 55-fold in extracts of atherosclerotic tissue, demonstrating a clear association between chitotriosidase expression and lipid-laden macrophages in the atherosclerotic human vessel wall.23 Plasma chitotriosidase was also found to be associated with the extent of plaque in mice fed a high-fat, atherogenic diet.24 Artieda et al.11 investigated the association of serum chitotriosidase activity with atherosclerosis in 153 patients with atherothrombotic stroke and in 124 patients with unstable angina pectoris. The control group comprised subjects without evidence of CAD. The researchers revealed that serum chitotriosidase activity was increased in both the atherothrombotic group and in patients with CAD. Serum chitotriosidase activity was more prominent in stroke patients in comparison to patients with ischemic heart disease, suggesting more widespread atherosclerosis.25 In contrast to our findings, they found a correlation between age and serum chitotriosidase activity. Despite using a similar method of measurement, the serum chitotriosidase activity levels were relatively low in the study population of Artieda et al in comparison with the present patient population. This difference can be explained by differences in chitotriosidase activity observed between different ethnic populations, which may be attributable to distinct genotype distributions. These genetic variations are responsible for the recessive inherited deficiency in chitotriosidase activity and are found in individuals from various ethnic origins.28,29 In a related study, Artieda et al. also showed that chitotriosidase activity predicts the risk of new adverse cardiovascular events in a follow-up period of 4 years.30 Canudas et al. reported that serum chitotriosidase activity is not associated with lipid levels before and after treatment with statins, which suggests that plasma lipid level alteration does not affect macrophage chitotriosidase expression level in vivo and is in accordance with our study, showing no correlation between blood lipid levels and chitotriosidase activity.

To our knowledge our work is the first clinical study investigating the association of serum chitotriosidase activity with atherosclerosis in humans with stable CAD. Our findings support previous suggestions that inflammation is a crucial factor in atherogenesis and CAD progression. High levels of inflammation-related hsCRP and chitotriosidase activity were found to be associated with atherosclerosis and CAD progression. Both serum chitotriosidase activity and elevation of hsCRP level were related to the presence and extent of CAD. Additionally, serum chitotriosidase activity seems to be superior to hsCRP as a reference marker of inflammation, in respect of its ability to predict CAD. We believe that the relationship between serum chitotriosidase activity and atherosclerosis represents a new opportunity for using serum chitotriosidase activity as a marker for CAD.
Although our findings require further confirmation in other studies, serum chitotriosidase activity shows remarkable potential as a quantitative indicator of disease extent, besides being a marker of disease presence.

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References


