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PRELIMINARY STUDY OF THE SERUM CHYMASE LEVELS IN PATIENTS WITH ARTERIAL HYPERTENSION

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ABSTRACT

Chymases are proteases stored in the cytoplasmic granules of mast cells. Under the influence of various stimuli, mast cell degranulation occurs and products such as tryptase, chymase and other mediators such as cytokines and chemokines, are released into the surrounding tissues and into the circulation, and promote vascular inflammation.

The aim of this study is to investigate the systemic level of chymase among patients with arterial hypertension on a therapy with angiotensin I converting enzyme (ACE) inhibitor. Serum level of chymase was detected among 30 patients with stage 1 essential arterial hypertension (AH) and 10 healthy controls, by commercially available enzyme-linked immunosorbent assay (ELISA) purchased from Cusabio Biotech co, following the manufacturer's instructions.

All hypertensive patients are being treated with Lisinopril and only 63.3% were with controlled hypertension. Serum level of chymase was significantly elevated in patients compared to healthy controls (99.6±159 vs. 9.34±10pg/ml; p=0.0076). However, the chymase levels between patients with controlled and uncontrolled hypertension was similar (95±186 vs. 107±104pg/ml; p=0.85). Increased level of total cholesterol and LDL was established among AH patients with uncontrolled hypertension compared to patients with controlled blood pressure (5.8±0.96 vs 4.5±1.1mmol/l;p=0.004 and 3.14±0.99 vs. 2.4±0.69 mmol/l; p=0.03 respectively).

Also, correlation analysis between chymase and total cholesterol, LDL-cholesterol and TG levels was performed. A weak positive correlation between chymase and total cholesterol was observed (R=0.359; p=0.051) in AH group. Our preliminary study demonstrated a significant elevation of serum chymase in AH patients independently of Lisinopril treatment.

Key words: chymase, arterial hypertension, total cholesterol, LDL

INTRODUCTION

Angiotensin (Ang) II plays an important role in cardiovascular homeostasis, not only in the systemic circulation but also at the tissue level, and is involved in the remodeling of the heart and vasculature under pathological conditions. Although alternative Ang II-forming pathways are known to exist in various tissues, the details of such pathways remain unclear (1). Several studies with angiotensin I converting enzyme (ACE) inhibitors have demonstrated a remarkable improvement in the morbidity and mortality rates of patients with primary hypertension and congestive heart failure. Since ACE inhibitor therapy not only improves systemic haemodynamics but also provides a better prognosis, the cardiac renin-angiotensin system is apparently one of the major targets of ACE inhibitor therapy (2). However, an estimated 20–40% of patients do not respond to and/or tolerate ACE inhibition (3). In addition to ACE, human chymase – a novel cardiac Ang II forming enzyme – has been identified in human hearts. Unlike in the rat heart, the major (80%) component of Ang II-forming activity in the left ventricle is due to human chymase (2). Various organs in different species have unique profiles regarding the pathways of Ang II formation. In humans, chymase-dependent Ang II formation was predominant in the lung, aorta, and heart (4).

Chymases are proteases stored in the cytoplasmic granules of mast cells. Mast cells are increasingly being recognized as effector cells in many cardiovascular conditions. Mast cell-derived enzymes such as tryptase and

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chymase can have detrimental effects on blood vessel structure, while mast cell-secreted cytokines and chemokines can maintain vascular inflammation (5).

Mast cells can be activated by various stimuli triggering numerous receptors on the mast cell surface. The most common mechanism is by cross-linking of IgE receptors. However, several endogenous and exogenous factors, including neurotensin, substance P and endothelin 1, inflammatory mediators, such as complement components and reactive oxygen species (ROS) are able to potentiate FcεRI-mediated activation or to activate mast cells directly in an FcεRI-independent manner (6).

Many studies assume that chymase-dependent Ang II formation as a major molecular mechanism responsible for the ineffectiveness of ACE inhibition therapy. Company C et al. found that *in vivo*, Ang II is primarily generated by ACE under basal conditions, but in inflammatory conditions, the release of mast cell chymase amplifies local Ang II concentrations. According to these authors, AT(1) receptor antagonists may be more effective than ACE inhibitors for treating ongoing Ang II-mediated vascular inflammation (7). The data from several clinical trials of using a combination of two drugs acting on renin level, angiotensin I level, or its receptor level, for the treatment of hypertension are reviewed in a recent manuscript (Chrysant & Chrysant, 2014). At present, serious doubts exist regarding the effectiveness and safety of the combinatorial treatment of patients with hypertension, coronary heart disease, heart failure, or nephropathy with proteinuria (8).

It is supposed that chymase may be involved in structural remodeling of the cardiovascular system (9). Human chymase also induce activation of transforming growth factor-beta, a major stimulator of myocardial fibrosis (9, 10) and matrix metalloproteinase-9 (11). In addition, Uehara Y et al. suggested that arterial chymase may participate in the acceleration of lipid deposition in arterial walls exposed to high plasma cholesterol in hamsters and that inhibition of arterial chymase may retard the progression of atherosclerosis (12). These authors propose that high serum cholesterol may trigger upregulation of vascular chymase and facilitate the development of atherosclerosis (12).

In accordance with the above, the aim of the present study was to investigate the systemic level of chymase among patients with arterial hypertension on a therapy with ACE inhibitor and its correlation with the lipoprotein profile.

MATERIALS AND METHODS

Subjects: The study includes 30 patients with stage 1 essential arterial hypertension (AH) (systolic blood pressure of 140–159 mm Hg and/or diastolic blood pressure of 90–99 mm Hg, according to the Guidelines for the management of arterial hypertension). All patients were male, current non-smokers at age between 36 and 69 years. Patients with concomitant diseases of the cardiovascular system or diabetus mellitus were excluded.

All patients had already been diagnosed with hypertension and had been treated with the ACE-inhibitor drug Lisinopril for more than one year. We categorized blood pressure as controlled (systolic blood pressure of <140 mm Hg and diastolic blood pressure of <90 mm Hg) or uncontrolled (systolic blood pressure of ≥140 mm Hg or diastolic blood pressure of ≥90 mm Hg). 19 of 30 patients (63.3%) were with controlled hypertension.

The control group consisted of 10 healthy volunteers, age-sex-and-smoking status matched to the patient group.

Informed consent was obtained from all subjects and authorization was given by the Ethics Review Board of the Faculty of Medicine, Trakia University, Stara Zagora.

Serum level of chymase:
Quantitative determination of chymase in sera was performed by enzyme-linked immunosorbent assay (ELISA) using a commercially available human mast cell chymase ELISA kit purchased from Cusabio Biotech Co. (Wuhan, China) following the manufacturer's instructions. For determination of the chymase concentration (pg/ml), the optical density was measured at 450 nm with correction at 570 nm, according to the manufacturer's instructions and using a calibration curve based on the kit standard. The minimum detectable concentration of the chymase ELISA kit was less than 39 pg/ml.

Serum level of Total cholesterol (TC); LDL-cholesterol and triglycerides (TG):
Lipid profile levels were determined by enzymatic and colorimetric methods.

Total cholesterol values were determined by the Enzymatic Colorimetric Determination of Serum Cholesterol (CHOD-PAP) method. We used the following risk classification of Total Cholesterol: desirable <200 mg/dl (5.2 mmol/l); borderline high 200 – 239 mg/dl (5.2 – 6.2 mmol/l); high >240 mg/dl (6.2 mmol/l).

According to the National Cholesterol Education Program, if a person has no other risk factors, an LDL level can be evaluated as follows: Less than 100 mg/dL (2.59 mmol/L) – Optimal; 100-129 mg/dL (2.59-3.34 mmol/L) – Near optimal, above optimal; 130-159 mg/dL (3.37-4.12 mmol/L) – Borderline high; 160-189 mg/dL (4.15-4.90 mmol/L) – High; Greater than 189 mg/dL (4.90 mmol/L) – Very high.

Serum triglyceride levels were determined by the Enzymatic-colorimetric, End point (GPO-PAP) method. We used the NCEP (American National Cholesterol Education Program) classification for serum triglyceride levels according to the risk of developing coronary heart diseases: normal < 150 mg/dL (1.7 mmol/L) ; borderline high 150-199 mg/dL (1.7-2.2 mmol/L); high 200-499 mg/dL (2.3-5.6 mmol/L) ; very high ≥ 500 mg/dL. (5.6 mmol/L).

Statistical analysis: Statistical analysis was carried out using statsoft software, version 6. Differences in serum levels of chymase between patients groups and controls were assayed by the non-parametric Mann–Whitney U test. Spearman rank correlation was used to measure the degree of association between levels of chymase and lipid profiles: total cholesterol (TC); LDL-cholesterol, triglycerides (TG). In all cases P value less than 0.05 (two-tailed) was considered significant.

RESULTS AND DISCUSSION

Our study demonstrated the significantly elevated serum level of chymase in patients compared to healthy controls (99.6±159 vs. 9.34±10pg/ml; p=0.0076) **(Figure 1).** As expected, the serum chymase level in the control group was very low, while in condition of hypertension the serum chymase level increased more than tenfold. These results showed that chymase has a role in the pathophysiology of hypertension and are in agreement with other studies that showed a significant elevation of tissues chymase in various cardiovascular diseases. (13, 14)

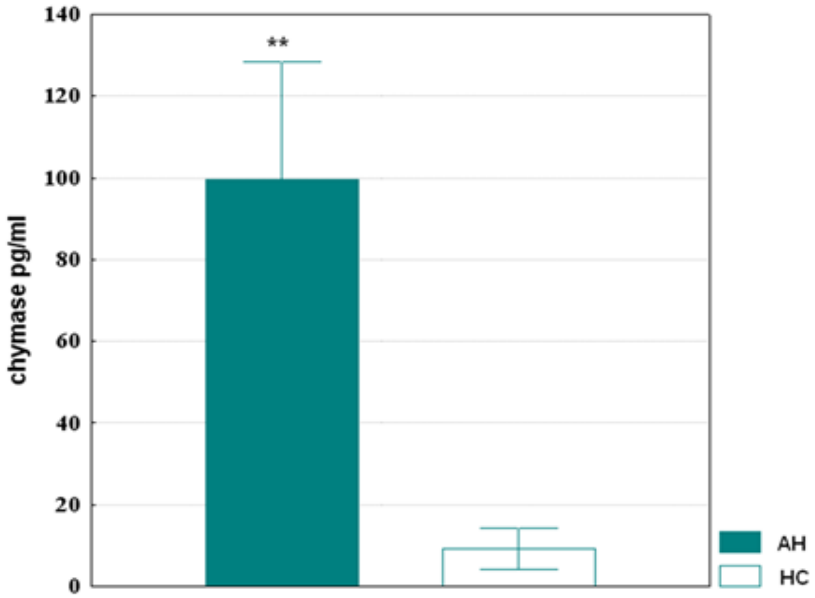

Figure 1. Serum chymase in hypertensive patients (AH) compared to healthy controls (HC) p<0.01

When the total patients group was subdivided into two subgroups according to the therapy response: patients with controlled hypertension (n=19) and patients with uncontrolled hypertension (n=11), we observed similar serum levels of chymase in both subgroups **(Figure 2)**. The chymase level was 95±186pg/ml in patients with controlled blood pressure and 107±104pg/ml (p=0.85) in patients with uncontrolled hypertension. Both patients subgroups showed a significant incensement of serum chymase compared to the control group (p=0.038 and p =0.0007 respectively). Probably, the chymase does not participate in the control of blood pressure by ACE inhibitors as Lisinopril. However, one limitation of our current result is the relatively small number of patients with uncontrolled hypertension. In addition, it is also possible local level, tissue chymase or enzyme activity to be influenced by treatment with Lisinopril. Here, we present that the systemic quantity of chymase remained unchanged after therapy with an ACE inhibitor.

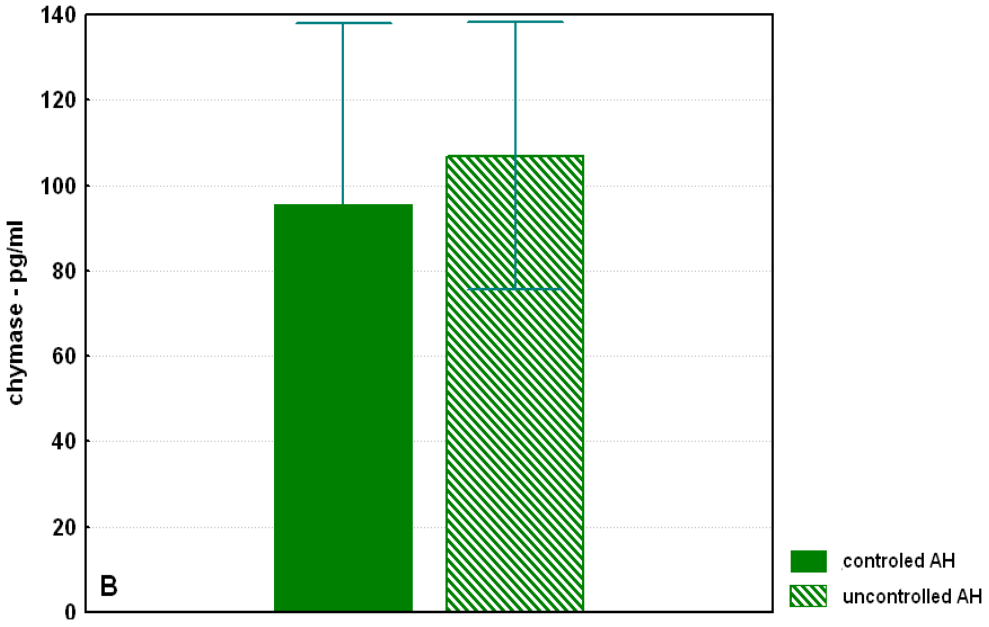

Figure 2. Serum chymase in patients with controlled and uncontrolled hypertension

Taking into consideration that uncontrolled high blood pressure, uncontrolled high levels of LDL-cholesterol and smoking are three important risk factors for cardiovascular disease and stroke, we investigated the correlation between serum levels of chymase and total cholesterol (TC); LDL-cholesterol and triglycerides (TG) among patients with controlled and uncontrolled blood pressure. The results presented on **Figure 3** demonstrated a significant increase in the levels of total cholesterol and LDL-cholesterol among AH patients with uncontrolled hypertension compared to patients with controlled blood pressure (5.8±0.96 vs 4.5±1.1mmol/l; p=0.004 and 3.14±0.99 vs. 2.4±0.69 mmol/l; p=0.03 respectively). The level of triglycerides was higher in patients with uncontrolled AH than in patients with controlled AH, without reaching statistical significance.

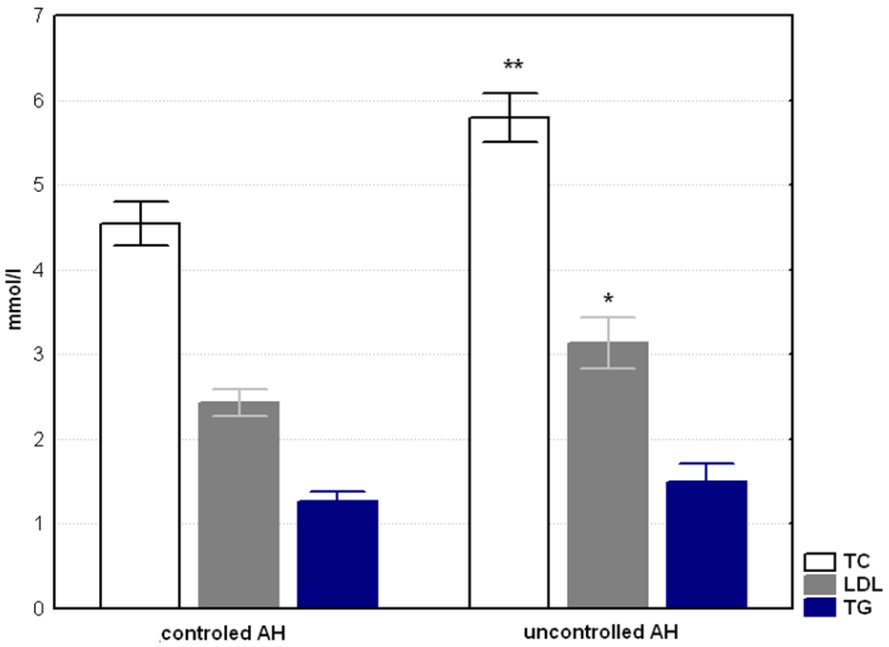

Figure 3. Total cholesterol (TC); LDL-cholesterol and triglycerides (TG) in patients with controlled and uncontrolled hypertension; *p<0.05; **p<0.01

The correlation analysis between chymase and total cholesterol, LDL-cholesterol and TG levels was also performed **(Figure 4).** A weak positive correlation was observed between chymase and total cholesterol (R=0.359) with borderline significance (p=0.051) in AH group.

Except serum total cholesterol levels, no other significant correlation was found in a univariate analysis between chymase and the investigated parameters of the lipid profile. Our results are in general agreement with other previous studies concerning chymase activity (12, 15). Uehara et al. (2000) had showed that serum total and LDL cholesterol concentrations significantly correlated with chymase activity in homogenates of human internal thoracic artery (13). However, larger sample-based investigations are needed to clarify the relationship found in our present study.

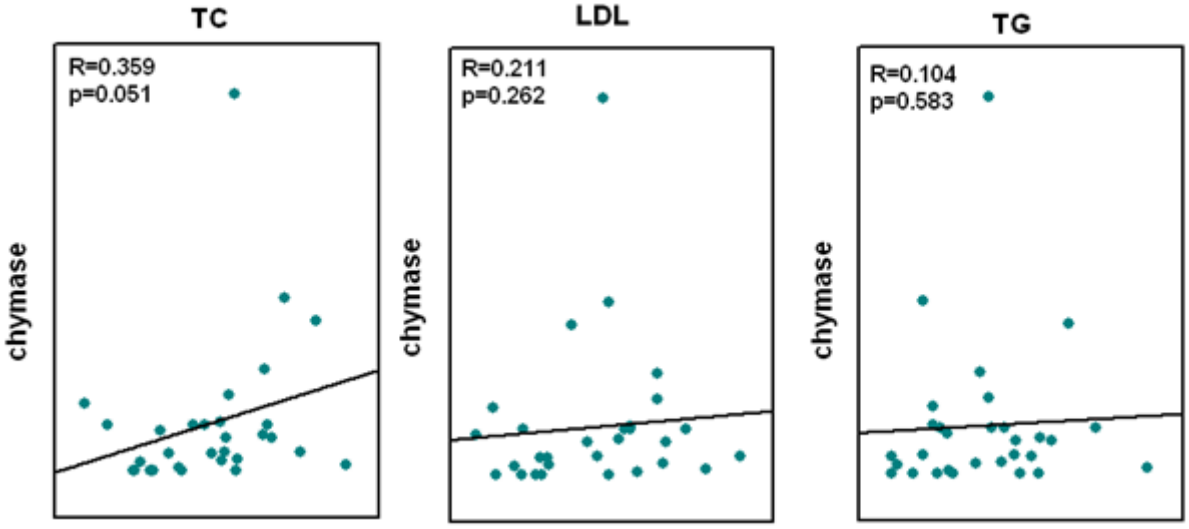

Figure 4. Correlation analysis between chymase and total cholesterol (TC); LDL-cholesterol, triglycerides (TG)

CONCLUSION

Our preliminary study demonstrated a significant elevation of serum chymase in AH patients independently of resistance of the hypertension treated with an ACE inhibitor - Lisinopril.

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ABBREVIATIONS

ACE-angiotensin I converting enzyme
AH- arterial hypertension
Angiotensin II- Ang II
ROS-reactive oxygen species
ELISA-enzyme-linked immunosorbent assay
TG- triglycerides
TC- total cholesterol

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