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Usefulness of Serum Trypsin Level as an Independent Biomarker for Coronary Plaque Instability in a Chinese Population

Meixiang Xiang, M.D.^a, Jiusong Sun, Ph.D.^b, Yan Lin, M.D.^a, Jie Zhang, Ph.D.^b, Han Chen, M.D.^{a,b}, Dan Yang, M.D.^a, Jianan Wang, M.D.^a, and Guo-Ping Shi, D.Sc.^b

^a Department of Cardiology, the Second Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou 310009, China

^b Cardiovascular Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115, USA

Abstract

Trypsin and chymase are unique mast cell proteases that are essential in atherogenesis. This study establishes a link between serum trypsin and chymase levels and human coronary heart diseases (CHD) in a cohort of 270 subjects. Serum trypsin levels were significantly higher in patients with substantial CHD than in those without substantial CHD (substantial CHD vs. unsubstantial CHD: 7.81 ± 0.52 vs. 6.11 ± 0.51 ng/mL, $P=0.002$). After subgrouping the substantial CHD patients into those with acute myocardial infarction (AMI) and those with unstable or stable angina pectoris (UAP or SAP), we demonstrated that serum trypsin levels were nearly doubled in AMI patients as compared with unsubstantial CHD patients (11.13 ± 1.55 vs. 6.11 ± 0.51 ng/mL, $P<0.01$), and significantly higher than in UAP patients (7.19 ± 0.62 ng/mL, $P<0.05$) or SAP patients (6.80 ± 0.94 ng/mL, $P<0.05$). Although Spearman's correlation test showed that serum trypsin correlated significantly with age ($P=0.014$) and weakly with fasting glucose ($P=0.084$), total cholesterol ($P=0.071$), low-density lipoprotein ($P=0.063$), and triglyceride ($P=0.058$), serum trypsin levels remained significantly higher in substantial CHD patients than in unsubstantial CHD patients in a multiple linear regression test after adjusting for all these confounders ($P=0.008$). Serum chymase levels were also higher in AMI patients (27.64 ± 7.57 ng/mL) or UAP patients (24.62 ± 8.06 ng/mL) than in SAP patients (15.20 ± 0.81 ng/mL) or unsubstantial CHD patients (16.84 ± 0.56 ng/mL), although such differences were not statistically significant. Spearman's correlation test revealed that serum chymase levels correlated significantly only with fasting glucose levels ($P=0.019$), and CHD status did not affect chymase levels before and after adjusting for all confounders. Our observations suggest that serum trypsin is an independent biomarker for coronary plaque stability in this Chinese population.

Keywords

mast cell; trypsin; chymase; coronary heart disease

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Corresponding author: Guo-Ping Shi, D.Sc. Cardiovascular Medicine Brigham and Women's Hospital 77 Avenue Louis Pasteur, NRB-7 Boston, MA 02115 Tel.: 617-525-4358 Fax: 617-525-4380 gshi@rics.bwh.harvard.edu.

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1. Introduction

Mast cells participate in atherogenesis by releasing cytokines to induce vascular cell protease expression [1]; chemokines to recruit inflammatory cells [2,3]; and proteases to promote foam cell formation [4,5], angiogenesis [6], matrix degradation [7,8], vascular cell apoptosis [9,10], and latent protease and cytokine activation [11,12]. In addition to cysteine proteases and matrix metalloproteases, mast cells also express the neutral serine proteases tryptase and chymase, which are unique to mast cells. These mast cell-specific proteases were expressed highly in human atherosclerotic lesions [13-15]. Inhibition of tryptase or chymase blocks macrophage foam cell formation [5] and reduces atherosclerotic lesion sizes in monkeys and in hamsters [16,17]. Thus, we hypothesized that these mast cell proteases are also higher in the peripheral blood of patients with atherosclerosis. In this study, we collected 270 subjects who were diagnosed with acute myocardial infarction (AMI), unstable angina pectoris (UAP), and stable angina pectoris (SAP), as well as those without substantial coronary heart disease (unsubstantial CHD), and examined whether serum levels of these mast cell proteases are different among patients with different degrees of vascular complications.

2. Materials and methods

2.1. Patient selection

From July to December 2008, a total of 357 patients admitted to the Department of Cardiology at the Second Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou, China, were enrolled consecutively due to their clinical symptoms of precordial discomfort, chest pain, dyspnea, cardiac dysfunction (defined as ejection fraction less than 50% by echocardiography), increasing levels of myocardial enzyme, or electrocardiogram abnormalities including ST-T changes. The human study protocol was pre-approved by the Hospital Review Committee. All patients gave informed consent. Given the possible association of tryptase and chymase with autoimmunity, allergic diseases, and cancer, we excluded patients with the following conditions to limit the potential confounding effects: asthma (n=18), history of allergic diseases (n=21), arthritis (n=2), cancer (n=6), renal failure (n=14), chronic hepatic disease (n=21), rheumatic heart disease (n=2), valvular heart disease (n=2), or other cardiac diseases (n=1). The remaining 270 subjects received coronary angiography. Of these, 175 were diagnosed with substantial coronary heart disease (substantial CHD), with one or more main coronary arteries having at least 50% stenosis. The remaining 95 subjects, with less than 50% or without luminal narrowing of the main coronary artery, were selected as unsubstantial CHD controls. Of the 175 patients with substantial CHD, 139 had acute coronary syndrome (ACS), and 36 had stable angina pectoris (SAP). Among the 139 patients with ACS, 31 were diagnosed with acute myocardial infarction (AMI), and 108 were diagnosed with unstable angina pectoris (UAP). AMI was diagnosed by levels of creatine kinase-MB that were twofold more than the upper normal limit and by levels of troponin-I that were fivefold more than the upper normal limit; by precordial discomfort, chest pain, dyspnea, and other myocardial ischemic symptoms; or by ST-T changes in electrocardiography indicative of myocardial ischemia and/or infarction. UAP was diagnosed by the progression of ischemic symptoms less than three months before enrollment. SAP was diagnosed by invariable character of exertional precordial discomfort more than three months before enrollment.

2.2. Clinical information

Patient information — including age, height, weight, body-mass index (BMI), sex, history of hypertension, diabetes, and smoking (consuming tobacco for at least three years) — was

recorded. Blood samples from the peripheral vein at least 12 hours after fasting were collected to determine levels of serum total cholesterol, triglyceride, low-density lipoprotein (LDL), high-density lipoprotein (HDL), and fasting glucose, using standard laboratory procedures (AU5400, Olympus automated analyzer) at the clinical laboratory of the Second Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou, China. Arterial blood samples were extracted from the sheath in the radial or femoral artery during the interventional procedure for trypsin and chymase measurement. For AMI patients, blood was drawn at the time of primary percutaneous coronary intervention, within a few hours/days after onset. For UAP, SAP, or unsubstantial CHD patients, blood was drawn during hospitalization, at the time of the interventional procedure. All serum sample aliquots were stored at -80°C .

2.3. ELISA

Human serum trypsin and chymase levels were determined using ELISA. To detect serum total trypsin (both α - and β -trypsin), anti-trypsin polyclonal antibody (1:5000, Calbiochem, La Jolla, CA) was used for plate coating. After blocking the ELISA plate with 3% bovine serum albumin, purified human trypsin (Calbiochem) as standard and serum samples (1:1 in 1xPBS) were added to the plate, followed by 2 hours of incubation at room temperature. Mouse anti-human trypsin monoclonal antibody (1:3000, AbD Serotec, Raleigh, NC), which recognized both human α -trypsin and β -trypsin, was used as the detecting antibody for 1 hour at room temperature. The plate was washed, incubated with HRP-conjugated goat anti-mouse IgG (1:2000, KPL, Gaithersburg, MD), developed in TMB solution (Thermo Scientific, Waltham, MA), and read at 450 nm. To detect serum chymase, we coated a 96-well plate with mouse anti-human chymase monoclonal antibody (1:1000, AbD Serotec). The ELISA plate was then blocked with 3% bovine serum albumin. Human serum with known chymase level was used as the standard. Each serum sample was diluted (1:60) in 50 mM Tris-HCl, pH 8.0, boiled for 5 minutes, and incubated on a pre-coated plate overnight. Biotin-conjugated mouse anti-human chymase monoclonal antibody was used as the detecting antibody for 1 hour (1:4000, Millipore, Billerica, MA), followed by incubating the plate with peroxidase-conjugated avidin for 1 hour (1:2000, Sigma). The plate was developed with TMB solution (Thermo Scientific) and read at 450 nm. Serum collected from the artery or vein did not show any differences in trypsin and chymase levels (data not shown).

2.4. Statistical analysis

All ELISA data were expressed as mean $\pm$ SE. For patient serum sample data, those with normal distribution and homogeneity of variance, independent sample *t* test, and one-way analysis of variance (ANOVA) LSD test were used for the comparison between two groups and among multiple groups, respectively. The non-parametric Kruskal-Wallis H test was used for multiple-group comparison with skewed data distribution or heterogeneity of variance. For the ranked data, Fisher's exact test and the Pearson chi-square test were used for the comparison between two groups and among multiple groups. To analyze the correlation of serum trypsin or chymase and other values, we used Spearman's correlation test. To examine the influence of potential confounders to serum trypsin and chymase levels, we adjusted for age, sex, HDL, LDL, triglyceride, total cholesterol, BMI, and fasting glucose using a multiple linear regression model. SPSS16.0 version was used for analysis, and $P < 0.05$ was considered statistically significant.

3. Results

3.1. Patients with substantial CHD had higher serum tryptase and chymase levels than unsubstantial CHD donors

In this study, all 270 in-hospital patients underwent coronary angiography, which confirmed that 175 of them had substantial CHD. Independent sample *t* test showed that patients with substantial CHD ($n=175$) were older (65.88 ± 0.76 vs. 57.49 ± 1.24 years, $P<0.001$) and had lower HDL levels than those without substantial CHD ($n=95$) (47.16 ± 0.94 vs. 50.47 ± 1.39 mg/dL, $P=0.044$; Table 1). We observed no significant differences in BMI, fasting glucose levels, total cholesterol, triglyceride, or LDL levels between the two groups. Fisher's exact test demonstrated that both sex ($P=0.030$) and history of diabetes mellitus ($P=0.059$), but neither smoking nor history of hypertension, affected CHD status (Table 1). Substantial CHD patients had substantially higher serum tryptase levels than unsubstantial CHD patients (7.81 ± 0.52 vs. 6.11 ± 0.51 ng/mL, $P=0.002$), as well as higher chymase levels (23.22 ± 5.15 vs. 16.84 ± 0.56 ug/mL, $P>0.05$; Table 1), although the difference did not reach statistical significance for chymase levels.

3.2. AMI patients had the highest serum tryptase levels among all groups

After grouping the substantial CHD patients with AMI, UAP, and SAP, we found that patients with AMI had the highest serum tryptase levels (11.13 ± 1.55 ng/mL) — substantially higher than those in UAP patients (7.19 ± 0.62 ng/mL, $P<0.05$) and SAP patients (6.80 ± 0.94 ng/mL, $P<0.05$), and nearly twice as high as those in unsubstantial CHD patients (6.11 ± 0.51 ng/mL, $P<0.01$) (one-way ANOVA LSD test). Chymase level changes showed the same tendency. AMI patients had the highest chymase levels, followed by UAP patients, SAP patients, and unsubstantial CHD patients (27.64 ± 7.57 vs. 24.62 ± 8.06 vs. 15.20 ± 0.81 vs. 16.84 ± 0.56 ng/mL, $P>0.05$; Table 2), although none of the differences reached statistical significance, suggesting that human plaque stability associates with serum tryptase levels but associates only weakly with chymase levels. The Pearson chi-square test did not reveal any differences in sex, smoking, hypertension, or diabetes mellitus between the subgroups (Table 2). Among the three substantial CHD subgroups, age remained significantly higher in all three groups than in unsubstantial CHD patients. AMI patients had substantially lower BMI (22.45 ± 0.57 kg/mm²) than did UAP patients (23.74 ± 0.29 kg/mm², $P<0.05$) or SAP patients (24.84 ± 0.55 kg/mm², $P<0.01$), and even lower than those of unsubstantial CHD patients, but did not reach statistical significance (22.45 ± 0.57 vs. 23.33 ± 0.35 kg/mm², $P>0.05$; Table 2). AMI patients also had the highest serum fasting glucose levels (114.81 ± 6.92 mg/dL), although they did not reach statistical significance when compared with UAP patients (97.20 ± 2.06 mg/dL, $P=0.013$), SAP patients (100.99 ± 5.58 mg/dL, $P=0.023$), and unsubstantial CHD patients (98.76 ± 2.34 mg/dL, $P=0.029$) (Table 2). $P<0.009$ was considered statistically significant (Mann-Whitney *U* test).

3.3. Serum tryptase is an independent biomarker for coronary plaque instability

As AMI patients had the oldest ages, the highest fasting glucose levels, and the lowest BMI, high tryptase and chymase levels may associate with those variables. To test this possibility, we performed Pearson's correlation test (for continuous variables with normal distribution and homogeneity of variance) and Spearman's correlation test (for continuous variables with not normal distribution or heterogeneity of variance, or binary data). Serum tryptase levels associated significantly with age ($P=0.014$), but weakly with triglyceride ($P=0.058$), LDL ($P=0.063$), total cholesterol ($P=0.071$), and fasting glucose levels ($P=0.084$) (Table 3). In contrast, serum chymase levels associated only with fasting glucose levels ($P=0.019$) (Table 4).

Although our data showed that AMI and UAP patients had significantly higher serum trypsin levels than SAP and unsubstantial CHD patients (Table 2), high serum trypsin levels may be confounded by other variables that significantly or even weakly associate with serum trypsin levels — including age, fasting glucose, LDL, triglyceride, and total cholesterol levels (Table 3). Moreover, age, HDL level, and sex distributions were also significantly different between substantial CHD and unsubstantial CHD patients (Table 1). Therefore, we performed a multiple linear regression test considering age, sex, BMI, HDL, fasting glucose, LDL, triglyceride, and total cholesterol levels as potential confounding factors in plaque instability. In this regression model, serum trypsin levels remained significantly higher in substantial CHD patients than in unsubstantial CHD patients ($P=0.008$), and serum chymase levels still did not reach significant differences between substantial CHD and unsubstantial CHD patients ($P=0.253$) (Table 5). Fasting glucose associated strongly with chymase ($P=0.019$) and weakly with trypsin ($P=0.084$). However, when considering age, sex, BMI, fasting glucose, HDL, LDL, triglyceride, total cholesterol, and trypsin and chymase levels as confounding factors, we found no significant differences in fasting glucose levels between substantial CHD and unsubstantial CHD patients ($P=0.248$) (Table 5), consistent with the conclusion from Table 1. These observations suggest that serum trypsin level, but not chymase or fasting glucose levels, is an independent biomarker of human plaque instability.

4. Discussion

In this study, we found that both serum trypsin and chymase levels were higher in patients with substantial CHD than in unsubstantial CHD patients, although chymase levels did not reach statistical significance. Further, patients with AMI had the highest trypsin and chymase levels, followed by UAP patients, SAP patients, and unsubstantial CHD patients (Tables 1 and 2). The significance of higher serum trypsin levels in substantial CHD patients than in unsubstantial CHD patients remained after adjustment for several important potential confounding factors (Table 5). These observations together suggest that mast cell trypsin is an important biomarker for atherosclerotic plaque vulnerability.

While few mast cells appear in the adventitia from normal human aortae, human atherosclerotic lesions contain an increased accumulation of mast cells — most located in the shoulder regions, where they are prone to rupture and erosion, and fewer in fibrous caps or lipid cores [13]. By staining mast cell granules, several studies demonstrated that a high percentage (~85%) of mast cells in the shoulder regions were active, whereas only one-fifth of mast cells were active in normal aortae [13,15,18]. These observations supported the idea that mast cells link to atherosclerotic plaque stability; our study was consistent with this concept. Patients with AMI had the highest serum trypsin and chymase levels — nearly double those in unsubstantial CHD patients from this Chinese population (Table 2). Indeed, in patients who died of AMI, high numbers of active mast cells accumulated at the site of atherosclerotic lesion erosion or rupture [14]. Activation of these mast cells correlated with intraplaque hemorrhage, endothelial cell apoptosis, vascular leakage, and leukocyte recruitment to plaques [19]. However, several studies from different countries yielded different conclusions. In Finland, Kervinen et al. found that serum trypsin levels were not significantly different among patients with AMI, UAP, and acute angina [20]. In the Netherlands, van Haelst and colleagues reported findings similar to those of the Finnish group. Furthermore, they did not find any differences in serum C-reactive protein levels among patients with AMI, patients with UAP, and those without myocardial ischemia [21]. In contrast, in the United States, Deliargyris et al. demonstrated higher serum trypsin levels in patients with significant CHD (> or =50% stenosis in > or =1 artery) than in those with non-significant CHD (<50% stenosis) [22]. In Poland, Filipiak et al. also reported that serum trypsin levels in patients with UAP, ST-elevation myocardial infarction (STEMI), and non-

ST-elevation myocardial infarction (NSTEMI) were significantly higher (2~3 fold) than those in control subjects, who reported no history of ischemic heart disease [23]. Multivariate analysis revealed no influences of several common coronary plaque instability variables on tryptase levels, which is consistent with our findings. We currently do not have explanations for these discrepancies; all of these reports used the same radioimmunoassay from the same company (Pharmacia & Upjohn Inc., Bridgewater, NJ), with similar assay sensitivity to the sandwich ELISA we used in our current study. We speculate that race, culture, lifestyle, and genetic factors may affect mast cell tryptase expression and secretion.

Tryptase correlated strongly and positively with older age ($P=0.014$), and relatively weakly with fasting glucose, serum LDL, triglyceride, and total cholesterol levels ($P=0.05\sim0.08$). Serum lipoprotein levels are significantly lower in mast cell-deficient mice than in wild-type mice after consuming an atherogenic diet [24]. Mast cell tryptase degrades HDL [25] and promotes macrophage foam cell formation [5], but there is no evidence that tryptase directly regulates LDL, triglyceride, or total cholesterol production or degradation. Thus, it may be more reasonable to conjecture that the observed positive correlations between tryptase levels and LDL, triglyceride, or total cholesterol were due to CHD status. Age is a very interesting factor affecting serum tryptase levels. Recent studies demonstrated significant positive associations of serum tryptase levels with older age, both in a general population who were randomly enrolled to the study and in patients who had severe hymenoptera stings [26,27]. Our study is consistent with these studies. Older age associated with tryptase levels (Table 3) but not with chymase levels (Table 4). Age did not, however, affect the power of tryptase association between substantial CHD and unsubstantial CHD patients (Table 5).

Increased serum tryptase levels from activated mast cells in patients with unstable plaques (AMI and UAP) suggest that coronary plaques are important sources of this mast cell protease. Indeed, a large portion of mast cells in human atherosclerotic lesions is activated [13,15,18]. Beside atherosclerotic lesions, ischemic myocardium also contains activated mast cells and produces mast cell proteases [28,29], which may contribute in part to serum total tryptase levels. Histamine is another mast cell-specific product, and AMI and UAP patients also may have higher serum histamine levels than SAP patients or those with unsubstantial CHD. Although we did not test this hypothesis in this study, patients with ACS are known to have higher serum histamine levels than age- and sex-matched controls, and such increased histamine associates with enhanced oxidative stress [30]. In an animal model, stress-induced mast cell activation in atherosclerosis-prone mice also increased serum histamine levels [31].

Both in vitro and in vivo studies have suggested that chymase and tryptase participate in foam cell formation [4,5], latent protease activation [12,32,33], pericellular matrix protein degradation [7,8], vascular cell apoptosis [9,10,34; Shi, unpublished data], and angiogenesis [10,35]. However, the possible effects of serum tryptase or chymase on the coronary arteries have not been tested. As neutral proteases, both chymase and tryptase in the serum may participate in the activation of other protease zymogenes, as they do in atherosclerotic lesions *insitu*. Indeed, aortic cells often release both activated cysteinyl cathepsins and their pro-enzymes. The exact proteases to activate these pro-enzymes remain unknown. We have recently shown that mast cells release tryptase and chymase to promote vascular cell apoptosis *in vitro*. Similar activities may occur in the aortic lumen; thus, these mast cell proteases may destabilize the aortic wall and enhance the risk of plaque instability. But such speculations await experimental confirmation.

Both chymase and tryptase levels were higher in substantial CHD patients than in unsubstantial CHD patients, but such differences reached statistical significance only for

tryptase (Table 1). Despite the statistical insignificance of chymase, levels in AMI patients (27.64 ± 7.57 ug/mL) and UAP patients (24.62 ± 8.06 ug/mL) were substantially higher than those in SAP patients (15.20 ± 0.81 ug/mL) and unsubstantial CHD patients (16.84 ± 0.56 ug/mL) (Table 2). Although not tested, the difference between tryptase and chymase may result from mast cell subtype differences in human atherosclerotic lesions. In humans, mast cells contain two subtypes: those containing tryptase (MC_T) and those containing both tryptase and chymase (MC_{TC}) [36], although subtype MC_T may be converted into subtype MC_{TC} under certain conditions [37]. The relative percentage of each subtype depends on the type of connective tissue. In human urea choroids, for example, 96% of mast cells were MC_{TC} , whereas 63% of mast cells were MC_T in ciliary muscles [38]. In human atherosclerotic lesions, both MC_T and MC_{TC} have been reported [39,40]. Between these two subtypes, MC_{TC} may reach up to 80~90% in advanced human atherosclerotic lesions [40]. Although the exact percentage of these different subtypes of human mast cells in the current population is unknown, the presence of both subtypes may account for the significance of tryptase differences and the insignificance of chymase differences between substantial CHD patients and unsubstantial CHD patients. Alternatively, this study selected only 270 patients. A larger sample size would help to make a more accurate statement on whether chymase levels are significantly higher in patients with AMI or substantial CHD than in unsubstantial CHD patients. Such speculation merits further investigation.

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Clinical data and serum tryptase and chymase level comparisons between patients with substantial and unsubstantial coronary heart disease (CHD).

Table 1

Variables	Unsubstantial CHD (Mean $\pm$ S.E.) (n=95)	Substantial CHD (Mean $\pm$ S.E.) (n=175)	t value	P value*
Age (year)	57.49 $\pm$ 1.24	65.88 $\pm$ 0.76	6.082	0.000
Body-mass index (kg/mm ²)	23.33 $\pm$ 0.35	23.74 $\pm$ 0.24	0.990	0.323
Fasting glucose (mg/dL)	98.76 $\pm$ 2.34	101.12 $\pm$ 2.14	0.697	0.487
Total cholesterol (mg/dL)	159.46 $\pm$ 3.35	163.17 $\pm$ 3.39	0.779	0.437
Triglyceride (mg/dL)	157.51 $\pm$ 10.99	167.92 $\pm$ 10.57	0.632	0.528
High-density lipoprotein (mg/dL)	50.47 $\pm$ 1.39	47.16 $\pm$ 0.94	-2.023	0.044
Low-density lipoprotein (mg/dL)	89.32 $\pm$ 2.83	94.69 $\pm$ 2.73	1.264	0.207
Tryptase (ng/mL)	6.11 $\pm$ 0.51	7.81 $\pm$ 0.52	3.127	0.002
Chymase (ng/mL)	16.84 $\pm$ 0.56	23.22 $\pm$ 5.15	0.910	0.364

Unsubstantial CHD (n=95)		Substantial CHD (n=175)		P value**	
0***	1***	0***	1***		
Sex	40	55	50	125	0.030
Smoking	63	32	108	67	0.509
Hypertension	36	59	54	121	0.280
Diabetes mellitus	85	10	140	35	0.059

* Independent sample t test.

** Fisher's exact test. $P < 0.05$ was considered statistical significant.

*** Sex: 0-female, 1-male; Smoking: 0-non-smoker, 1-smoker; Hypertension: 0-no, 1-yes; Diabetes mellitus: 0-no, 1-yes.

Table 2

Clinical data and serum tryptase and chymase level comparisons among substantial CHD subgroups and unsubstantial CHD.

Variables	Unsubstantial CHD (n=95)	AMI (n=31)	UAP (n=108)	SAP (n=36)	P value ^d
Age (year)	57.49 ± 1.24	71.06 ± 1.87**	65.39 ± 0.90***	62.89 ± 1.73***	0.000 ^a
Body-mass index (kg/mm ²)	23.33 ± 0.35	22.45 ± 0.57	23.74 ± 0.29 [#]	24.84 ± 0.55***	0.017 ^a
Fasting glucose (mg/dL)	98.76 ± 2.34	114.81 ± 6.92	97.20 ± 2.06	100.99 ± 5.58	0.052 ^b
Total cholesterol (mg/dL)	159.46 ± 3.35	155.48 ± 5.68	165.12 ± 4.49	164.33 ± 8.39	0.798 ^a
Triglyceride (mg/dL)	157.51 ± 10.99	129.81 ± 8.81	172.28 ± 10.34	190.09 ± 41.75	0.217 ^a
High-density lipoprotein (mg/dL)	50.47 ± 1.39	48.23 ± 2.32	46.60 ± 1.16	47.91 ± 2.24	0.205 ^a
Low-density lipoprotein (mg/dL)	89.32 ± 2.83	89.35 ± 4.92	96.91 ± 3.64	92.79 ± 6.46	0.392 ^a
Tryptase (ng/mL)	6.11 ± 0.51	11.13 ± 1.55**	7.19 ± 0.62**	6.80 ± 0.94 [#]	0.001 ^a
Chymase (ng/mL)	16.84 ± 0.56	27.64 ± 7.57	24.62 ± 8.06	15.20 ± 0.81	0.603 ^a
	Unsubstantial CHD (n=95)	AMI (n=31)	UAP (n=108)	SAP (n=36)	P value ^{c,d}
	0 1	0 1	0 1	0 1	
Sex	40 55	12 19	30 78	8 28	0.066
Smoking	63 32	19 12	67 41	22 14	0.902
Hypertension	36 59	9 22	34 74	11 25	0.696
Diabetes mellitus	85 10	24 7	89 19	27 9	0.156

^a one way ANOVA LSD test (normal distribution and homogeneity of variance) ;

^b Kruskal-Wallis test (abnormal distribution or heterogeneity of variance);

^c Pearson Chi-Square test.

^d P<0.05 was considered significant between the groups.

* P<0.05 vs. Unsubstantial CHD;

** P<0.01 vs. Unsubstantial CHD;

[#] P<0.05 vs. AMI;

P<0.01 vs. AMI.

Table 3

Variables associated with serum tryptase levels in all subjects (n=270).

	Correlation Coefficient	<i>P</i> value [*]
Age (year)	0.149	0.014
Body-mass index (kg/mm ²)	0.025	0.680
Fasting glucose (mg/dL)	0.107	0.084
Total cholesterol (mg/dL)	0.113	0.071
Triglyceride (mg/dL)	0.118	0.058
High-density lipoprotein (mg/dL)	−0.078	0.214
Low-density lipoprotein (mg/dL)	0.116	0.063
Sex	−0.047	0.443
Smoking	0.000	0.997
Hypertension	−0.074	0.227
Diabetes mellitus	0.058	0.341

^{*} Spearman's correlation test.

Table 4

Variables associated with serum chymase levels in all subjects (n=270).

	Correlation Coefficient	P value
Age (year)	0.003	0.963 [*]
Body-mass index (kg/mm ²)	−0.036	0.554 [*]
Fasting glucose (mg/dL)	0.145	0.019 ^{**}
Total cholesterol (mg/dL)	0.046	0.460 [*]
Triglyceride (mg/dL)	0.014	0.825 [*]
High-density lipoprotein (mg/dL)	−0.012	0.848 [*]
Low-density lipoprotein (mg/dL)	0.058	0.352 [*]
Sex	−0.064	0.295 ^{**}
Smoking	−0.072	0.238 ^{**}
Hypertension	−0.060	0.325 ^{**}
Diabetes mellitus	0.026	0.676 ^{**}

* Pearson's correlation test;

** Spearman's correlation test.

Multiple linear regression model analysis of serum trypsin, chymase and fasting glucose levels between substantial CHD and unsubstantial subjects.

Table 5

Variable	β	SE	t	P Value	95% C.I.
Trypsin (ng/mL) *	0.355	0.133	2.675	0.008	0.093-0.616
Chymase (ng/mL) *	2.572	2.245	1.146	0.253	-1.850-6.994
Fasting glucose (mg/dL) **	-0.034	0.029	-1.158	0.248	-0.091-0.024

* Adjusted for age, sex, body-mass index, fasting glucose, HDL, LDL, triglyceride, and total cholesterol levels.

** Adjusted for age, sex, body-mass index, HDL, LDL, triglyceride, total cholesterol, and trypsin and chymase levels.