Serum Gamma-Glutamyl Transferase Is Associated with Epicardial Fat Thickness in Middle Aged Women

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Accumulating data indicate that gamma-glutamyl transferase (GGT) is associated with cardiovascular morbidity and mortality. A positive correlation between GGT levels and various cardiometabolic risk factors has been previously found. The aim of this study was to investigate the relationship between GGT and epicardial fat tissue thickness (EFTT) a marker of visceral adiposity. A total of one hundred five middle-aged (40-60 years) women were included in this cross-sectional study. EFTT was measured using 2-dimensional echocardiography, on the free wall of the right ventricle perpendicularly to the aortic annulus. Biochemical parameters were determined with an automated biochemical analyzer. Values of GGT were logarithmically transformed (log-GGT) because of their skewed distribution. We found that log-GGT correlated with age, body mass index, abdominal circumference, EFTT, triglycerides, fasting plasma glucose and uric acid. In multivariate regression analysis, log-GGT levels were independently associated with EFTT (ß=0.27, p=0.004), postmenopausal status (ß=0.25, p=0.008) and triglycerides (ß=0.23, p=0.01). In conclusion, in middle-aged women, GGT independently correlated with EFTT and may be a marker of visceral adiposity and increased cardiovascular risk.

Keywords: Gamma-glutamyl transferase, epicardial fat tissue thickness, middle-aged women.

Gamma-glutamyl transferase (GGT) is a glycoprotein which catalyses the degradation of glutathione, an important antioxidant, leading to the generation of free radicals and low density lipoprotein oxidation [1]. In clinical practice, GGT is evaluated as a marker of hepatic diseases, cholestasis, and alcohol consumption. Besides the liver, GGT may be found in other organs such as the kidney, lungs and vascular endothelium [2]. Epidemiologic data indicate a strong association between GGT levels and all-cause and ischemic heart disease mortality [3]. A positive correlation between GGT levels and various classical cardiovascular risk factors including age, hypertension, smoking, arterial stiffness and male gender has been reported [4,5]. High values of GGT were associated with metabolic syndrome components, particularly obesity and high blood pressure [6].

Epicardial adipose tissue is placed in the pericardial cavity and surrounds the myocardium and coronary arteries. It has physiological roles in heart mechanical protection and may serve as an energy store of fatty acids. Excessive epicardial fat accumulation is a sign of visceral adiposity and a source of pro-inflammatory and pro-atherogenic mediators which may affect coronary vessels and myocardium [7,8]. Epicardial fat tissue thickness (EFTT) has been associated with an increased cardiovascular risk. It has been shown that EFTT has a strong predictive value for coronary heart disease, over and above usual measures of systemic adiposity, particularly body mass index (BMI) [9,10].

EFTT has been independently correlated with GGT in patients with angiographically proved coronary artery disease [11]. The association between GGT with EFTT in middle aged women has not yet been studied. The aim of this study was to investigate the relationship of GGT with EFTT and other markers of cardiovascular risk, in middle aged (pre- and postmenopausal) women, without known cardiovascular disease.

Experimental part

One hundred five middle aged (40-65 years) women were included. Exclusion criteria were: documented ischemic heart disease, heart failure or arrhythmia, infection, acute or chronic inflammatory diseases, cancer, chronic kidney or pulmonary diseases, endocrine and metabolic diseases other than obesity. There were also excluded subjects with

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history of viral hepatitis, cirrhosis, alcohol consumption (>20 g/day), or liver enzymes three times of the normal upper values, and women who were taking hormone replacement treatment. The study included patients evaluated between August 2018 and January 2019, at the 2nd Department of Internal Medicine from Cluj-Napoca. The study protocol was approved by the Ethical Committee of our institution and the participants signed the informed consent.

A complete clinical examination was performed in all subjects. BMI was calculated as the ratio between weight and the square of height. Waist circumference was measured using a tape, at the midpoint between the inferior costal border and the superior margin of the iliac crest. Women were considered postmenopausal if they have not had a menstrual period for more than one year. Peripheral blood pressures were measured with Arteriograph (Tensiomed, Budapest, Hungary) using an oscillometry based technique.

Laboratory measurements

Blood samples were taken in the morning after an overnight fast in siliconised tubes with sodium fluoride for fasting plasma glucose determination and without additives for the serum. Biochemical examinations were made using an automated chemistry analyzer - AU480 Chemistry Analyzer (Beckman Coulter Inc., Danaher Corp, Brea, California, USA). Fasting plasma glucose, GGT, aspartat aminotransferase, alanin aminotransferase, uric acid, C-reactive protein, total cholesterol, high-density lipoprotein cholesterol (HDL-c), low density lipoprotein cholesterol (LDL-c) and triglycerides (TG) were determined from serum samples.

Hypertension was described as the active use of antihypertensive drugs or values of blood pressure ≥140/90 mm Hg. Metabolic syndrome was defined according to the International Diabetes Federation (IDF) Criteria in patients having abdominal obesity (a waist circumference > 94cm in men and >88cm in women) and at least two of the following criteria: TG: ≥150mg/dL, reduced levels of HDL-c: <40mg/dL in men and 50mg/dL in women; increased blood pressure: systolic blood pressure ≥ 130mmHg or diastolic blood pressure ≥ 85mmHg; elevated fasting plasma glucose ≥ 100mg/dL [12]. Hyperlipidemia was defined in patients with TG concentration of 150 mg/dL or greater or an LDL-C concentration of 100 mg/dL or greater and/or taking cholesterol-lowering medication [13].

Transthoracic echocardiography

All subjects were examined by two-dimensional transthoracic echocardiography with an ALOKA αPROSOUND (Tokio, Japan) system equipped with a 3.5 Hz frequency transducer. With the patients lying in left lateral decubitus position, using long axis view, EFTT was identified as a hyperechoic space between the outer wall of the myocardium and the visceral layer of the pericardium, and was measured in end systole. The measurement was made perpendicularly on the aortic annulus. We calculated average values of three consecutive cardiac cycles [7]. Cardiac measurements of EFTT were done by an experimental physician. The intraobserver variability was tested in 10 patients examined twice one week apart. The correlation coefficient was 0.945.

Statistical analysis

Data distribution was tested using Kolmogorov-Smirnov test. All reported P values are two-sided and were considered statistically significant at $p < 0.05$. Data are expressed as mean±standard deviation (SD) or as number and percentage. Values of GGT were log transformed because of their skewed distribution. The t-test for independent samples or MannWhitney U-test were used to compare continuous variables. The evaluation of parameters' correlation was made by Spearman's test. Multivariable stepwise regression analysis was used to determine variables independently associated with log-GGT. SPSS 20.0 package was used for all statistical analyses.

Results and discussions

Clinical and laboratory parameters of the study group are listed in table 1. Mean age of participants was 54.7±5.9 years. There were 36 subjects (34.2%) with hypertension and among these 30 patients were treated with one or more antihypertensive drugs as follows: 19 (18%) angiotensin converting enzyme inhibitors, 18 (17.1%) diuretics, 17 (16.1%) beta-blockers, 7 (6.6%) angiotensin receptor blockers and an equal number of subjects took calcium channel blockers. Hyperlipemia was noted in 37 (35.2%) and 8 subjects (7%) were taking statins. The criteria for the diagnosis of the metabolic syndrome were identified in 34 patients (32.3%). There were not active smokers in the study group. Postmenopausal status was noted in 62 women (59%).

Bivariate analysis of correlation using Spearman rank test showed that both log-GGT and EFTT correlated with age, BMI, abdominal circumference, and TG. EFTT also correlated with LDL-c, while GGT significantly correlated with diastolic and mean blood pressure, fasting plasma glucose and uric acid (Table 2).

Postmenopausal women had significantly increased values of log-GGT and EFTT compared to premenopausal women (1.46±0.19 versus 1.29±0.22, p=0.02 and 5.11±0.11 versus 4.54±0.12 mm, p=0.002, respectively). Patients

with hypertension had increased log-GGT compared to patients without hypertension (1.47±0.20 versus 1.35±0.22, p=0.01) but not significantly increased EFTT (5±0.11 versus 4.80±0.12 mm, p=0.40).

Table 1
CHARACTERISTICS OF THE STUDY GROUP

Parameters	
Age (years)	54.7 ±5.9
Body mass index (kg/m²)	26.1±5
Abdominal circumference (cm)	84.6±10.6
Epicardial fat thickness (mm)	4.9±0.12
Left ventricular ejection fraction (%)	62.8±5.8
Aortic pulse wave velocity (m/s)	8.6±1.8
Systolic blood pressure (mmHg)	132±17.3
Diastolic blood pressure (mmHg)	76.5±11.1
Mean blood pressure (mmHg)	95±12.4
Low density lipoprotein cholesterol (mg/dl)	125.6±33.9
Triglycerides (mg/dl)	119±47.2
High-density lipoprotein cholesterol (mg/dl)	54.4±11.9
Fasting plasma glucose (mg/dl)	93.3±9.3
Gamma-glutamyltransferase (U/L)	28.3±15.1
Log-Gamma-glutamyltransferase	1.39±0.22
Aspartate aminotransaminase (U/L)	24.9±9.2
Alanine aminotransferase (U/L)	23.3±13.4
Uric acid (mg/dl)	4.46±1.24
C-reactive protein (mg/dl)	0.36±0.14

Table 2
BIVARIATE CORRELATIONS OF GAMMA-GLUTAMYL TRANSPEPTIDASE AND EPICARDIAL ADIPOSE TISSUE WITH VASCULAR AND METABOLIC PARAMETERS

	Epicardial adipose tissue		Log-gamma glutamyl transferase	
	Rho	p	Rho	p
Age	0.23	0.01	0.27	0.005
Body mass index	0.34	<0.001	0.37	<0.001
Abdominal circumference	0.37	<0.001	0.40	<0.001
Systolic blood pressure	0.17	0.07	0.14	0.08
Diastolic blood pressure	0.09	0.32	0.08	0.07
Mean blood pressure	0.14	0.14	0.18	0.05
Low density lipoprotein cholesterol	0.27	0.007	0.14	0.16
Triglycerides	0.19	0.04	0.33	0.001
High-density lipoprotein cholesterol	-0.09	0.31	-0.10	0.26
Fasting plasma glucose	0.08	0.39	0.29	0.002
Gamma-glutamyltransferase	0.34	<0.001	-	-
Uric acid	0.16	0.10	0.30	0.002
C-reactive protein	0.02	0.81	0.11	0.32
Epicardial adipose tissue thickness	-	-	0.34	<0.001

Patients with metabolic syndrome had increased log-GGT and EFTT compared to those without metabolic syndrome (1.49±0.18 versus 1.34±0.22, p=0.001 and 5.40±0.12 versus 4.52±0.11 mm, p=0.001, respectively). Values of log-GGT and EFTT, according to the presence of metabolic syndrome components are listed in table 3.

In multivariable stepwise regression analysis, taking as dependent variable log-GGT and independent variables, parameters correlated with log-GGT in bivariate analysis, postmenopasal status, hypertension and metabolic syndrome, we found that log-GGT independently correlated with epicardial adipose tissue, postmenopausal status, and triglycerides (table 4).

When we replaced metabolic syndrome components with the presence of metabolic syndrome in the multivariate regression model, log GGT remained independently correlated with EFTT (B±SE=0.141±0.052, ß=0.26, p=0.007, 95%confidential interval for B: 0.039-0.2440) and also correlated with the presence of metabolic syndrome (B±SE=0.067±0.25;ß=0.25, p=0.008, 95% confidential interval for B:0.018-0.116).

Table 3
RELATIONSHIP OF log-GGT AND EFTT WITH COMPONENTS OF METABOLIC SYNDROME

	Log-gamma-glutamyl transferase		Epicardial fat tissue thickness	
	Mean (±SD)	p	Mean (±SD)	p
Increased abdominal circumference Yes No	 1.45±0.20 1.32±0.22	 0.004	 5.2±0.11 4.4±0.11	 <0.001
Increased systolic blood pressure Yes No	 1.40±0.24 1.30±0.21	 0.45	 5.0±0.13 4.8±0.11	 0.35
High triglycerides Yes No	 1.46±0.21 1.33±0.21	 0.003	 5.8±0.08 3.9±0.07	 <0.001
Low HDL-cholesterol Yes No	 1.38±0.21 1.41±0.24	 0.50	 4.7±0.12 0.48±0.11	 0.81

Table 4
MULTIVARIABLE STEPWISE REGRESSION ANALYSIS TO IDENTIFY INDEPENDENT PARAMETERS ASSOCIATED WITH GAMMA-GLUTAMYL TRANSFERASE

	Unstandardized B	95% CI for B	Standardized ß	p
Epicardial adipose tissue thickness	0.43	0.115-0.760	0.27	0.004
Postmenopausal status	0.21	0.40-0.202	0.25	0.008
Triglycerides	0.001	0.000-0.002	0.23	0.01

Abbreviations: CI=confidence

The main propose of this study was to assess the relationship between GGT and EFTT in middle aged women. Our results show an independent correlation between GGT and EFTT, suggesting that GGT may be a marker of visceral adiposity in women from this age group.

We also report direct correlations of GGT levels with age, BMI, abdominal obesity, fasting plasma glucose and uric acid. GGT was increased in patients with hypertension and metabolic syndrome compared to those without these diseases. Our results are in line with previous studies which found that GGT associated with various cardiovascular risk factors. GGT levels have been correlated with abdominal circumference, BMI [14] uric acid [15], hypercholesterolemia, high triglycerides, smoking, low HDL and high fasting glucose [4]. GGT has been shown to be increased in metabolic syndrome and was correlated with individual components of metabolic syndrome [16,17]. In a prospective study, Lee et all reported that baseline values of GGT were associated with the onset of metabolic syndrome after 8 years of follow-up [3]. The correlation between GGT values and hypertension has been also previously documented. In prospective investigations GGT was predictive for new onset hypertension [18,19], possibly contributing to the pathogenesis of hypertension.

There are few data regarding the relationship between GGT and EFTT. A study which included patients with coronary artery disease found increased GGT and EFTT in patients compared to healthy controls, while GGT activity significantly and independently correlated with EFTT [11].

GGT is an enzyme present on the membrane of numerous cells and also in the serum. Its most important physiological role is the extracellular hydrolysis of glutathione, which is an essential antioxidant in humans. Glutathione is transformed in cysteine which, subsequently, is taken up by the cells and used in resynthesis of intracellular glutathione. Thus, GGT plays an important role in oxidative stress regulation [1,20].

Epicardial adipose tissue surrounds the heart and encases coronary arteries. It is considered the visceral adiposity for the heart, having the same embryologic origin as mesenteric and omental fat. In physiologic conditions, epicardial fat protects the heart from mechanical stress and may release anti-inflammatory cytokines, endothelium protective and anti-oxidant substances [21,22]. However, in metabolic diseases, such as obesity, metabolic syndrome or diabetes mellitus, epicardial adipose tissue increases and becomes dysfunctional producing pro-inflammatory and proatherogenic molecules [23,24,25,26].

The mechanisms behind the association of GGT and EFTT are not elucidated. However, several hypotheses can be speculated. Both GGT and EFTT have been found to be linked to various metabolic disturbances, including obesity and metabolic syndrome, as mentioned before. These conditions are associated with increased oxidative stress and insulin resistance [27]. Increased oxidative stress and insulin resistance have been associated with both increased GGT and EFTT. The increase in oxidative stress induces overconsumption of glutathione and may lead to stimulation of GGT synthesis in order to correct glutathione levels [28]. Epicardial adipose tissue is a component of visceral obesity. It has been shown that obesity is associated with an increased oxidative stress [29]. Epicardial adiposity has more markers of oxidative stress than subcutaneous fat tissue [30]. In patients with metabolic syndrome EFTT was associated with systemic oxidative stress [31] and is supposed to play a more important role in the stimulation of systemic oxidative stress than subcutaneous fat tissue. Another possible link between GGT activity and EFTT is insulin resistance which has been previously associated with both, higher levels of GGT [32,33] and increased amount of epicardial fat [22].

Both GGT and epicardial adiposity, besides being associated with various factors involved in the pathogenesis of atherosclerosis, may also directly contribute to the development of cardiovascular disease. It has been reported that GGT may occur at the level of the atherosclerotic plaques where it may promote atherogenesis via LDL oxidation [34]. Epicardial adiposity due to its close proximity with coronary arteries may accelerate atherosclerosis via local production of inflammatory cytokines [9,35,36] Adipokines secreted by epicardial adipose tissue may alter myocardial metabolism and contractility [35]. Via complex mechanisms including fatty atrial infiltration or fibrosis [35,37] and disturbed NADPH activity [38], EATT may be a trigger of atrial fibrillation.

Another important finding of our study is the independent association between GGT activity and postmenopausal status. These results are in line with one previous study which reported increased values of GGT in post-menopausal compared with pre-menopausal women, together with increased oxidative stress and reduced antioxidant defense [28].

Study limitations. One first limitation is the cross-sectional analysis of the data which does not permit a cause – effect evaluation. A second limitation is the relatively small sample size and thus larger studies are needed to confirm our results. A third limitation is the echocardiografic measurement of EFTT which is a less sensitive and specific than three-dimensional magnetic resonance imaging and multidetector computerized tomography determination [39]. However, the echocardiographic measurement of EFTT is considered a simple, accurate and reproducible method [23]. A forth limitation refers to the fact that we used a single blood sample for the determination of GGT and this may not allow for long term analysis.

Conclusions

We found direct correlations between GGT activity and various markers of metabolic and cardiovascular risk. Serum GGT levels associated independently with epicardial fat thickness suggesting that GGT may be an indicator of visceral obesity and increased cardiovascular risk in middle-aged women.

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Manuscript received: 9.05.2019