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Beta cell function as an assessment tool for cardiovascular risk in patients with metabolic syndrome

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Abstract

Background/Aim: Epicardial fat tissue (EFT) is considered a cardiovascular risk factor independent from visceral adiposity, obesity, hypertension, and diabetes. Fasting serum C-peptide is a known marker of endogenous insulin secretion and beta cell function. Our aim was to evaluate C- peptide levels in patients with metabolic syndrome (MetS) in relation to the EFT thickness.
Methods: Forty-five subjects with MetS without a history of coronary artery disease and 25 healthy volunteers were enrolled this prospective case-control study. We examined the laboratory values, including C peptide, insulin, and HOMA-IR after 8 hours of fasting. EFT thickness was measured by two-dimensional transthoracic echocardiography.
Results: The serum C peptide levels were significantly higher in patients with metabolic syndrome compared to the healthy controls [3.41(1.98) ng/ml vs 2.07 (1.39), $P<0.001$]. C peptide levels were correlated with BMI ($P=0.032$, r=0.281) and serum triglycerides ($P=0.023$, r=0.288). Patients with MetS had remarkably high EFT thickness [0.63(0.22) mm, $P=0.043$]. EFT thickness was correlated with age ($P=0.008$, r=0.397), weight ($P=0.042$, r=0.308) and C-peptide ($P=0.002$, r=0.460) in patients with MetS.
Conclusion: EFT thickness and elevated C-peptide are independent risk factors influencing atherosclerosis. The strong association between EFT thickness and C-peptide demonstrated herein indicates that EFT may play an important role in C peptide secretion, possibly contributing to the cardiometabolic risk in patients with MetS.

Keywords: Metabolic syndrome, C-peptide, Epicardial fat tissue, Cardiovascular risk

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Introduction

Over the last ten years, the metabolic health of individuals worsened due to sedentary lifestyle and change in nutritional habits. In developed and developing countries, regardless of age, over-feeding related health problems have become a pandemic [1]. This has resulted in an increased incidence of metabolic syndrome (MetS) related diseases such as diabetes, obesity, hyperlipidemia, hypertension, which all lead to cardiovascular diseases [2].

Evidence shows that insulin resistance, inflammation and endothelial dysfunction are the ethologic mechanisms of metabolic syndrome. We all know that several indexes and biological markers were investigated to predict the cardiovascular risk scale of metabolic syndrome and related conditions [3]. Early detection of risk factors allows us to assess personalized treatment strategies in metabolic syndrome.

C-peptide originates from the middle part of proinsulin and is not only a valid marker for beta cell function but also suggested for use as an indicator for cardiovascular risk in patients with metabolic syndrome. Although the role of C-peptide in triggering vascular complications is still unclear, numerous studies hypothesize that it might contribute to plaque development in patients with insulin resistance and type 2 diabetes [4].

Epicardial fat tissue (EFT) is the visceral fat lateral to the right ventricle and the anterior wall of the left ventricle, which shares the same microcirculation with the myocardium. Recently, EFT was shown to perform a high rate of white adipose tissue lipolysis and lipogenesis which makes it a metabolically active organ. EFT is a triglyceride storage and suggested to release high levels of free fatty acid into the near myocardium and microcirculation in case of metabolic stress [5]. Previous studies suggested that echocardiographic EFT thickness measurement can be an indirect way to demonstrate atherosclerosis and left ventricular diastolic dysfunction as well as predict high cardio-metabolic risk and should be a therapeutic target [6].

This study aimed to evaluate C-peptide level in terms of EFT thickness in a representative sample of patients with metabolic syndrome and further demonstrate the C-peptide level of patients with high cardiovascular disease risk.

Materials and methods

Subjects and settings

Forty-five subjects with MetS were selected at an internal medicine outpatient policlinic. Criteria used for the diagnosis of metabolic syndrome were the recommendations of the National Cholesterol Education Program, Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults -Adult Treatment Panel III (NCEP-ATP III). Metabolic syndrome was defined by the presence of at least three of these components: *1*) Increased waist circumference (>102 cm for men, >88 cm for women) *2*) Elevated triglycerides (≥150 mg/dL) or the use of triglyceride-lowering drugs *3*) Low HDL-cholesterol (<40 mg/dL in men, <50 mg/dL in women), *4*) Hypertension (≥130 / ≥85 mmHg) or use of antihypertensive drugs, and *5*) Fasting glucose (≥110 mg/dL) or the use of antidiabetic drugs [7]. Exclusion criteria were the presence of cardiovascular disease, congenital heart disease, heart valve disease, neoplastic, inflammatory, and infectious diseases.

Physical and laboratory measurements

The collection of anthropometric data (weight, height, waist circumference) and measurement of systemic blood pressure were obtained by physical examination according to standard procedures. The BMI (body mass index) was calculated by dividing weight (kg) by height (m^2). Waist circumferences were measured in the horizontal plane midway between the lowest rib and iliac crest. Resting systolic and diastolic BP was measured using a standard mercury sphygmomanometer after a 5-min rest. Fasting venous blood samples were collected in the morning after at least 8 hours of fasting. Serum cholesterol, triglyceride, and high-density lipoprotein cholesterol (HDL-C) were measured by enzymatic colorimetric methods with commercially available kits (COBAS 311, Roche Diagnostics GmbH, Mannheim, Germany), and low-density lipoprotein cholesterol C (LDL-C) was calculated according to the Friedewald formula. Serum glucose measures were determined enzymatically using the hexokinase method (Roche Diagnostics GmbH, Mannheim, Germany). HbA1c was determined with a COBAS 311 analyzer using the particle-enhanced immunoturbidimetric method (Roche Diagnostics, Mannheim, Germany). Final results were expressed as percent HbA1c of the total Hb according to the protocol of the Diabetes Control and Complications Trial/National Glycohemoglobin Standardization Program (DCCT/NGSP). The particle-enhanced immunoturbidimetric method with a Behring Nephelometer BN-100 (Behring Diagnostic, Frankfurt, Germany) was used to measure C reactive protein (CRP). The sensitivity of the test was 0.1 mg/L. C-peptide levels were determined by electrochemiluminescence (ECLA) assay in a random-access analyzer (Cobas E411, Roche Diagnostics, Les Pennes-Mirabeau, Bouches-du-Rhône, France). The homeostasis model assessment of insulin resistance (HOMA-IR) score was calculated using the formula defined by Matthews et al. (1985): HOMA IR = [fasting insulin (mU/mL) × fasting glucose (mmol/L)]/22.5 [8].

Measurement of echocardiographic epicardial fat tissue

Two-dimensional transthoracic echocardiography was performed by a single cardiologist. EFT thickness was performed using the DICOM system. The free wall of the right ventricle was measured from the papillary muscle at end-diastole from the parasternal long-axis views of three cardiac cycles, using the aorta annulus for anatomic reference. The thickest point of EFT was measured, and the mean value of the EFT thickness was calculated.

Statistical analysis

Statistical analysis was performed with MedCalc Statistical Software version 12.7.7 (MedCalc Software, Ostend, Belgium; http://www.medcalc.org; 2013). Values were expressed as mean (standard deviation) or as percentages. The student t-test was used for the comparison of two independent and normally distributed variables. The Mann Whitney U test was performed for the comparison of independent and non-normally distributed variables. The chi-square test and Fisher Exact test were

performed to determine the differences between categorical variables. Correlations of variables were assessed with Pearson's and Spearman analysis. Statistical significance was assessed at *P*-value <0.05.

Ethics approval

Our study was approved by Ethics Committee of GOP Taksim Education and Research Hospital with the decision number 103 on 05.08.2020 and conducted per the Declaration of Helsinki. Informed consent forms were signed by each participant.

Results

The study group consisted of 45 patients (30 females, 15 males) and 25 (14 females, 11 males) healthy volunteers. The patient group included metabolic syndrome patients without a cardiovascular disease history, with a mean age of 55.7 (11.43) years. When parameters obtained from those with metabolic syndrome and healthy volunteers are compared, patients with MetS were higher values in terms of systolic blood pressure, weight, waist circumference, BMI, fasting glucose, HbA1c, insulin, HOMA, triglyceride, ALT, GGT, CRP (*P*<0.001 for all). Serum fasting C-peptide levels were higher in the group with metabolic syndrome compared to the healthy group [3.41 (1.9) vs 2.07 (1.39), *P*<0.001]. The patients with metabolic syndrome had higher EFT thicknesses [0.63(0.22)] than the healthy group [0.52 (0.11)], (*P*<0.001) (Table 1).

Table 1: Clinical and biochemical characteristics of patients in the study

	Control (n=25) Mean(SD)	Metabolic syndrome (n=45) Mean (SD)	*P*-value
Age (years)	46.69 (8.1)	55.7 (11.43)	<0.001**
Gender			
Male	11(44%)	15(33.3%)	
Female	14(56%)	30(66.6%)	
SBP (mmHg)	119.23 (9.87)	134.89 (21.58)	<0.001**
DBP (mmHg)	70.96 (8.13)	75.34 (12.96)	0.176
WC (cm)	86.19 (11.31)	111.9 (13.36)	<0.001**
BMI (kg/m2)	25.77 (3.38)	34.02 (6.92)	<0.001**
Fasting glucose (mg/dl)	90.96 (11.12)	168.89 (93.2)	<0.001**
Hba1c (%)	5.52 (0.44)	8.18 (2.74)	<0.001**
Insulin (IU/ml)	7.46 (5.47)	28.22 (49.8)	<0.001**
C peptide (ng/ml)	2.07 (1.39)	3.41 (1.98)	<0.001**
HOMA-IR	1.62 (1.25)	3.41 (1.98)	<0.001**
TCH (mg/dl)	206.77 (38.36)	219.11 (46.74)	0.213
TG (mg/dl)	112.92 (54.9)	222.57 (167.99)	<0.001**
LDL-c (mg/dl)	130.19 (32.23)	130.7 (37.08)	0.953
HDL-c (mg/dl)	53.96 (13.94)	47.2 (9.3)	0.114
CRP (mg/dl)	2.46 (2.22)	5.75 (3.57)	<0.001**
EFT thickness (mm)	0.52 (0.11)	0.63 (0.22)	0.043*

BMI: body mass index, WC: waist circumference, TCH: total cholesterol, TG: triglycerides, HDL-c: high-density lipoprotein cholesterol, LDL-c: low-density lipoprotein cholesterol, CRP: C reactive protein, EFT: epicardial fat tissue. Mann-Whitney U, Student t and Fisher's Exact tests. Statistical significance: * *P*<0.05, ** *P*<0.001

The Pearson correlation analysis showed a statistically significant correlation between EFT thickness and C-peptide (*P*=0.002, r=0.460). EFT thickness was further correlated with age (*P*=0.008, r=0.397), weight (*P*=0.042, r=0.308), and waist circumference (*P*=0.035, r= 0.318) (Table 2). Serum fasting C-peptide levels were significantly correlated with triglycerides (*P*=0.023, r= -0.288) among the metabolic syndrome parameters (Table 3).

Table 2: Correlation of epicardial fat tissue thickness with other parameters

		Epicardial fat tissue
Age (years)	r	0.397[a,b]
	P	0.008
Weight (kg)	r	0.308[a]
	P	0.042
WC(cm)	r	0.318
	P	0.035[b]
Insulin (IU/ml)	r	0.111
	P	0.472
C peptide (ng/ml)	r	0.460[a,b]
	P	0.002
HOMA-IR	r	0.152
	P	0.324

[a] Spearman's rho correlation, [b] Pearson correlation, WC: waist circumference

Table 3: Correlations of C-peptide with metabolic syndrome characteristics

		C-peptide
Age (years)	r	0.113
	P	0.381
WC (cm)	r	0.181
	P	0.187
BMI (kg/m2)	r	-0.281
	P	0.032
Fasting glucose (mg/dl)	r	-0.162
	P	0.208
HDL-c (mg/dl)	r	0.236
	P	0.065
TG (mg/dl)	r	-0.288
	P	0.023
SBP (mmHg)	r	-0.021
	P	0.869

WC: waist circumference, BMI: body mass index, HDL-c: High density lipoprotein cholesterol, TG: Triglycerides, SBP: systolic blood pressure

Discussion

The prevalence of metabolic syndrome has gradually increased in the past decades, which brings cardio-metabolic and cardiovascular risks. In most countries, the prevalence of MetS ranges between 20% to %30 in the adult population, depending on the criteria [1]. Besides dyslipidemia, hyperglycemia, and hypertension, the patients with MetS are under the threat of increased proinflammatory and prothrombotic states. According to the International Diabetes foundation (IDF), patients with MetS have five-times increased risk for type 2 diabetes and 3 times increased risk for myocardial infarction or stroke [9].

In our study, we found high serum C-peptide levels in patients with MetS who do not have any cardiovascular event history. Studies showed that MetS is strongly associated with inflammation, insulin sensitivity, endothelial dysfunction, and oxidative stress [10-11]. To identify the presence and progression of these factors in MetS, several circulating factors including dyslipidemia components, C-peptide, bilirubin, fibrinogen, uric acid, reactive oxygen species, antioxidants were studied [12]. Although the exact mechanism remains unclear, C-peptide was suggested as an indicator of insulin resistance by two possible pathways. First, the elevated levels of pro-inflammatory cytokines in muscle tissue promotes the increase of triglycerides and insulin resistance by the regulatory effect of C-peptide [13]. Second, inactive hexamer insulin may be activated by the presence of C-peptide, which further increases glucose utilization [14]. We found no correlations between C-peptide and HOMA-IR in our patient group, which can be explained by the ongoing antidiabetic treatment of the patients with type 2 diabetes.

Among the metabolic syndrome parameters, we found that C-peptide was closely associated with serum triglyceride level only. Marx et al. showed that in patients with diabetes, C-peptide may cause early atherosclerotic lesions to progress through a collection of inflammatory cells and proliferation of

smooth muscle cells [15]. However, Patel et al. [16] suggested that fasting serum C-peptide levels predict cardiovascular and overall death better than serum insulin and its derived measures of insulin resistance, even in the non-diabetic population. Similar results in the literature suggest that C-peptide is a predictor of cardiovascular disease and overall mortality, possibly due to the increase atherogenic factors [17].

Epicardial fat tissue is normally present in humans and mammals. In lean humans it has been shown to express and secrete beneficial cytokines like adiponectin, which have vasodilatory properties [18]. However, in the presence of cardio-metabolic disorders like insulin resistance and obesity, adiponectin decreases and TNF-α increases, which leads up to arterial vasoconstriction, inflammation, and endothelial dysfunction [19]. EFT is thought to interact with coronary arteries by secreting cytokines to the interstitial fluid, the arterial wall and finally the endothelium. Besides this paracrine effect, it is hypothesized that EFT releases free fatty acids and cytokines directly into the vaso vasorum, which is called the vasocrine effect [20]. In previous reports, EFT was reported as an independent risk factor for athero-thrombotic cardiac events [21]. Furthermore, EFT thickness measurement was suggested as a predictive tool, even for the assessment of asymptomatic individuals [22]. In this report, the mean EFT thickness was 0.63 (0.22) mm in patients with metabolic syndrome, which was significantly higher than that in healthy controls. Although some studies revealed the relationship between EFT, insulin and HOMA-IR, the mechanism of interactions with glucose metabolism remains unclear [21]. We found no correlations between HOMA-IR and EFT thickness; however, a positive correlation existed between C-peptide and EFT thickness.

Visceral adipose tissue and EFT serve as a buffer for free fatty acids in metabolically healthy individuals. In the presence of obesity and type 2 diabetes, they both become thicker and dysfunctional [23]. It is noteworthy that the volume of visceral adipose tissue correlates with the volume of EFT, and thus, echocardiographic assessment has a crucial role in the cardiovascular risk assessment of patients with MetS and obesity [24]. Recent studies on the patients with MetS showed that EFT thickness could be a better indicator of visceral obesity rather than waist circumference [25]. Our study revealed that EFT thickness was positively correlated with waist circumference but did not correlate with other indices of obesity.

The major aim of the present report is to clarify the association of C-peptide with EFT. The determined correlation between C-peptide and EFT suggests that C-peptide could be used as a laboratory indicator of EFT thickness, which gives an insight about the cardio-metabolic risk of the patients with metabolic syndrome. Moreover, recent studies demonstrate the regulatory role of C-peptide on low-grade inflammation and its biological importance [26].

This study has some limitations. First, postprandial C-peptide levels were not included. Second, the ongoing antidiabetic, antihypertensive treatment of the patients in metabolic syndrome group caused an underestimation of the correlations considering insulin, HOMA-IR and C-peptide and blood pressure. Third, due to cross-sectional design of the study, a causal relationship could not be determined.

Conclusion

In this study, we determined high levels of serum fasting C-peptide and increased EFT thickness in patients with MetS. Furthermore, a clear positive association was found between C-peptide and EFT thickness in patients of MetS. However, longstanding observational studies are needed to understand the value of both C-peptide and EFT thickness measurement in predicting cardiovascular events in MetS.

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