

Serum C peptide and carotid intima-medial thickness are independent markers of glucose intolerance among patients with ischemic cerebrovascular stroke

Nearmeen M. Rashad^a, Ghada M. Samir^a, Hanan M. Sabry^b,
Nesreen M. Mohy^c, Shereen M. El Shabrawy^d

Departments of ^aInternal Medicine, ^bNeurology, ^cRadiodiagnosis, ^dMedical Biochemistry, Faculty of Medicine, Zagazig University, Zagazig, Egypt

Correspondence to Nearmeen M. Rashad, MD, Department of Internal Medicine, Faculty of Medicine, Zagazig University, Zagazig, 44519, Egypt. Tel: +20 122 424 8642; e-mails: nrashad78@yahoo.com, n.rashad@zu.edu.eg.com

Received 18 October 2019

Accepted 7 April 2019

The Egyptian Journal of Internal Medicine
2019, 31:368–375

Background

Stroke is the most common cause of disability worldwide. C-peptide is co-secreted with insulin from beta cells of the pancreas, thus providing a better index of endogenous insulin production and pancreatic beta cell function. The objective of the current study was to explore serum C-peptide and carotid intima-media thickness in patients with ischemic stroke (IS) and to assess the association of serum C-peptide with vascular and metabolic risks.

Patients and methods

The case–control study included 50 healthy control and 150 patients with IS who were stratified into three subgroups according to their fasting plasma glucose, normal glucose tolerance (NGT), impaired glucose tolerance (IGT), and type 2 diabetes mellitus (T2DM). All the participants were subjected to B-mode ultrasonography of both common carotid arteries to measure carotid intima-media thickness (mm). Serum C peptide concentration was measured by enzyme-linked immunosorbent assay.

Results

Serum C peptide levels were higher in IS patients compared with control. Among the IS patients, C peptide was higher in T2DM compared with IGT and NGT and is associated with vascular and metabolic risks, after adjusting for the traditional risk factors. C-peptide was a statistically significance predictor of T2DM among IS patients by the logistic regression analysis test. In addition, homeostasis model assessment of insulin resistance (HOMA-IR), fasting plasma glucose, BMI, and fat mass index (FMI)% were independently correlated with C-peptide by. The diagnostic value of C-peptide by receiver operating characteristic curves was highly significant; the sensitivities and the specificities were 96 and 98%.

Conclusion

Serum peptide levels were higher in IS patients compared with the control. Among the IS patients, serum C peptide was higher in T2DM compared with IGT and NGT and is associated with vascular and metabolic risks.

Keywords:

C peptide, carotid intima-media thickness, glucose intolerance, stroke

Egypt J Intern Med 31:368–375

© 2019 The Egyptian Journal of Internal Medicine
1110-7782

Introduction

Emerging evidence demonstrated that stroke remains the third leading cause of death after heart diseases and malignancies and the most frequent cause of disability worldwide [1]. A growing body of evidence has corroborated that ischemic stroke (IS) is the most preventable disease. Interestingly, 90% of IS risk factors are treatable, in particular, hypertension, type 2 diabetes mellitus (T2DM), cardiac diseases, cigarette smoking, obesity, hyperlipidemia, physical inactivity, psychosocial stress, and depression [2].

Diabetes is one of the growing health problems worldwide. Even more importantly, there are currently 34 million patients with diabetes in the Middle East and North Africa [3]. Egypt was

ranked eighth worldwide for the number of people with T2DM and this number is expected to reach 7.8 million in 2025 [4].

T2DM is a risk factor for atherosclerosis which is responsible for coronary heart disease, peripheral arterial disease, and cerebrovascular disease [5]. Atherosclerosis is an important pathologic cause of cardiovascular (CV) and cerebrovascular diseases. Additionally, CV and cerebrovascular diseases are the leading causes of mortality in humans and can

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

have significant impacts on morbidity. Atherosclerosis starts with a preclinical increase in the thickness of the internal and medial membrane of the arterial wall, which has been related to higher coronary heart disease and stroke rates [6].

Noteworthy, there are few studies about the correlations between serum C peptide and carotid intima-media thickness (CIMT). The role of insulin resistance as a predictor of CV risk has been well documented. Numerous reports have described that serum insulin measurement gives a wrong value of insulin secretion because insulin after its secretion into the portal vein passes through the liver where ~50% of the delivered insulin is extracted. The measurement of C-peptide thus provides a better index of endogenous insulin production and pancreatic beta cell function than insulin measurements [7].

Omics studies have indeed demonstrated that C peptide is a single peptide chain of 31 amino acids with a molecular weight of 30 200 g/mol [8]. The objective of the current study was to explore serum C-peptide and CIMT in patients with ischemic CVS and to assess the association of serum C-peptide with vascular and metabolic risks.

Patients and methods

A cross-sectional study included 150 unrelated patients with IS recruited from the Internal Medicine and Neurology Departments, Zagazig University Hospitals. The patients were stratified into three subgroups according to their fasting plasma glucose (FPG) based on the American Diabetes Association Criteria, reported in 2017: those with normal glucose tolerance (NGT; 30 patients), those with impaired glucose tolerance (IGT; 31 patients), and 39 patients with type 2 diabetes in addition to 50 healthy controls. All participants were matched, as regards age, gender, and ethnic origin.

The patients were chosen with the following inclusion and exclusion criteria. The inclusion criteria were focal or global neurological deficit lasting greater than 24 h on initial neurological evaluation. Computed tomography (CT) scan of the brain showed evidence of cerebral ischemia. However, the exclusion criteria were nonischemic etiology such as hemorrhagic stroke (patients with intracerebral hemorrhage and subarachnoid hemorrhage) and patients who had received drugs known to affect the level of C peptide. Patients with a history of respiratory disease, cancer, severe hepatic, renal diseases, acute

illness, hormonal therapy, any active inflammatory diseases, alcoholism, carotid artery surgery, and chronic heart failure were excluded from the study.

All patients in the study were subjected to the following: thorough history taking and full clinical assessment including general and neurological examination. Stroke severity within 72 h of onset of symptoms was assessed using the National Institute of Health Stroke Scale [12]. CT scan of the brain was performed for each patient to exclude intracranial hemorrhage and to diagnose cerebral infarction including its site and size. If CT scan was negative, it was repeated after 72 h. The size of the lesion was calculated according to the formula $0.5 \times A \times B \times C$ [where A and B are the largest perpendicular diameters measured on CT and C is the slice thickness (10 mm)] [9].

All scans were performed on a Siemens Somatom Balance scanner (Siemens Company, Berlin, Germany). The ethics committee of the Faculty of Medicine, Zagazig University, approved our study protocol, and all participants signed a written informed consent.

Blood sampling

Blood samples were drawn from all participants after an overnight fast and were divided into two portions: 1 ml of whole blood was collected into evacuated tubes containing EDTA, for HbA1c and 1 ml of whole blood was collected into evacuated tubes containing potassium oxalate and sodium fluoride (2 : 1) for FPG. Sera were separated immediately from the remaining part of the sample and stored at -20°C until analysis. We measured FPG using the glucose oxidase method (Spinreact, Girona, Spain). Total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides were measured by routine enzymatic methods (Spinreact). Low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald formula [10]. Fasting serum insulin (FSI) levels were estimated by an enzyme-linked immunosorbent assay kits (Ray Bio, Norcross, Georgia, USA). Insulin resistance was assessed using the homeostasis model assessment method, homeostasis model assessment of insulin resistance (HOMA-IR), and was calculated as $\text{FSI} (\mu\text{U/ml}) \times \text{FBG} (\text{mg/dl}) / 405$ and β -cell function (HOMA-B) was calculated. C peptide concentration was measured in serum by enzyme-linked immunosorbent assay kit according to the kit instructions (Wkea Med Supplies, Changchun, Jilin, China).

Dual-energy X-ray absorptiometry

The accurate and precise values of the body composition parameters were estimated from the

DXA scan of the total body, which included fat mass (FM), fat-free mass (FFM); additionally, the FM index (FMI; FM/height²) and the FFM index (FFMI/height²) were calculated.

Carotid ultrasonography

Ultrasonography of the carotid arteries was done in the Radiodiagnosis Department of Zagazig University Hospitals to elective patients. Carotid artery atherosclerosis was determined by one examiner for all patients across all six sites, using high-resolution B-mode ultrasound (M-Turbo; SonoSite, Washington, Bothell, USA), according to the protocol [9]. A transverse scan was followed by a longitudinal circumferential scan at 12 well-defined segments, the near and the far walls of the right and left common carotid, bulb, and internal carotid arteries from three imaging angles: anterior, lateral, and posterior. CIMT was measured at the far walls of the distal 1 cm of each common carotid artery at three imaging angles, and the mean and maximum CIMT values were measured by the leading edge-to-edge method. The mean maximum value of CIMT in each study participant (described as mean CIMT) was calculated based on the average of maximum CIMT values within the 12 arterial wall segments [10].

Statistical analysis

Statistical analyses were performed using the statistical package for the social sciences for Windows (version 19; SPSS Inc., Chicago, Illinois, USA). Data were expressed using the descriptive statistic (mean±SD) and were analyzed statistically by paired *t*-test or analysis of variance test. Pearson's correlation coefficient was used to assess the association between serum C-peptide, CIMT, clinical, biochemical tests, and other studied metabolic parameters in the elderly. Receiver operating characteristic (ROC) analysis was performed to assess the potential accuracy of serum C-peptide, the area under the curve (AUC), and the cutoff values for the diagnosis of T2DM among the elderly. A linear regression analysis was done to detect the main predictors of serum C-peptide values in the IS group. Logistic regression analysis was performed to determine the predictor marker associated with T2DM among the elderly patients. We considered *P* to be significant at less than 0.05 with a 95% confidence interval (CI).

Results

Clinical and biochemical characteristics of the studied groups

There were significantly higher values of systolic and diastolic blood pressure in the IS group compared with the control group. In addition, the values of BMI, waist

circumference, waist/hip ratio, FMI%, FFMI%, FPG, postprandial blood glucose, FSI, HOMA-IR, HbA1c, LDL, TG, and CIMT were significantly higher in the IS group compared with the control group. On the other hand, patients with IS had significantly lower values of HOMA-β and HDL (*P*>0.05) (Table 1).

Clinical, anthropometric, and laboratory parameters in the IS group as shown in Table 2

The T2DM group had significantly higher values of systolic blood pressure, diastolic blood pressure, BMI, waist circumference, waist/hip ratio, FMI%, FFMI%, FPG, postprandial blood glucose, FSI, HOMA-IR, HbA1c, TG, and CIMT than the NGT group (*P*<0.05*). On the other hand, there were significantly lower values of HOMA-β. Regarding IGT, there were significantly higher values of BMI, waist circumference, FMI%, FFMI%, diastolic blood

Table 1 Clinical, anthropometric, and laboratory characteristics of all studied subjects

	Control group (mean±SD) (n=50)	IS group (mean±SD) (n=150)	<i>P</i>
Age (years)	39.5±4.24	38.7±2.86	0.402
Systolic blood pressure (mmHg)	132.79±21.01	137.4±21.90	< 0.05*
Diastolic blood pressure (mmHg)	76.65±7.5	85.48±8.5	< 0.001*
BMI (kg/m ²)	26.57±3.05	36.31±7.89	< 0.001*
Waist circumference (cm)	85.02±8.8	136.99±8.89	< 0.001*
Waist/hip ratio	0.79±0.076	1.23±0.11	< 0.001*
FMI%	6.44±0.40	12.97±1.70	< 0.001*
FFMI%	20.09±1.61	24.65±2.87	< 0.001*
Total cholesterol (mg/dl)	166.76±18.87	175.9±69.36	0.352*
Triglycerides (mg/dl)	115.62±16.75	146.76±70.39	< 0.001*
LDL cholesterol (mg/dl)	121.22±26.79	152.1±46.76	< 0.001*
HDL cholesterol (mg/dl)	54.82±4.26	34.9±16.8	< 0.01*
Fasting plasma glucose (mg/dl)	77.62±6.276	123.9±30.19	< 0.001*
Postprandial glucose (mg/dl)	124.6±11.19	173.84±56.86	< 0.001*
FSI (μU/ml)	7.4±1.82	13.18±6.37	< 0.001*
HOMA-IR	1.57±0.40	5.7±4.14	< 0.001*
HOMA-β	118.6±37.1	85.8±27.9	< 0.001*
HbA1c (%)	4.58±1.105	6.82±1.183	< 0.001*
C-peptide (ng/ml)	1.12±0.213	3.23±1.21	< 0.001*
CIMT (mm)	0.7±0.519	1.296±0.48	< 0.001*

CIMT, carotid intima-media thickness; HOMA-IR, homeostasis model assessment of insulin resistance; HbA1c, hemoglobin A1c cholesterol; HDL-C, high-density lipoprotein-cholesterol; IS, ischemic stroke; LDL-C, low-density lipoprotein. **P*<0.05.

Table 2 Laboratory and anthropometric parameters in patients with ischemic stroke stratified according to fasting blood glucose

	NGT (mean±SD) (n=45)	IGT (mean±SD) (n=56)	T2DM (mean±SD) (n=49)	P ₁	P ₂
Age (years)	39.5±12.8	39.3±14.83	37.6±9.2	0.941	0.469
BMI (kg/m ²)	30.5±1.94	35.3±4.13	43.7±8.49	<0.001*	<0.001*
Waist circumference (cm)	114.1±7.24	132.1±15.43	160.2±17.145	<0.001*	<0.001*
Waist/hip ratio	1.01±0.064	1.17±0.137	1.43±0.153	0.128	<0.001*
FMI%	10.1±0.64	11.83±1.382	15.79±3.06	<0.001*	<0.001*
FFMI%	20.38±1.29	23.54±2.75	27.95±5.42	<0.001*	<0.001*
Systolic blood pressure (mmHg)	129.5±21.34	132.2±22.04	144.3±15.59	0.515	<0.001
Diastolic blood pressure (mmHg)	74.2±8.108	78.83±8.14	79.18±7.022	<0.001*	<0.05
Total cholesterol (mg/dl)	165.3±56.9	159.5±63.6	202.7±56.46	0.620	0.521
Triglycerides (mg/dl)	118.8±50.37	140.3±77.21	177.8±77.81	0.105	<0.01*
LDL cholesterol (mg/dl)	139.5±48.61	157.9±60.21	157.1±54.5	<0.001*	0.792
HDL cholesterol (mg/dl)	33.8±8.68	38.32±14.83	31.8±6.875	<0.001*	0.227
Fasting plasma glucose (mg/dl)	91.7±5.82	114.1±26.05	162.7±33.5	<0.001*	<0.001*
Postprandial glucose (mg/dl)	126.1±15.25	172.6±50.26	244.3±53.9	<0.001*	<0.001*
FSI (μU/ml)	17.7±2.307	18.5±2.99	20.16±1.44	<0.001*	<0.001*
HOMA-IR	4.1±0.283	5.27±1.49	8.16±2.1	<0.001*	<0.001*
HOMA-β	137.3±20.1	95.81±37.1	84.7±21.71	<0.001*	<0.05*
HbA1c (%)	5.7±0.307	6.5±0.99	8.16±1.443	<0.001*	<0.001*
C-peptide (ng/ml)	3.7±0.199	4.03±0.288	3.2±0.995	<0.001*	<0.001*
CIMT (mm)	1.08±0.35	1.24±0.496	1.56±0.49	0.058	<0.001*

CIMT, carotid intima-media thickness; HOMA-IR, homeostasis model assessment of insulin resistance; HbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; IGT, impaired glucose tolerance; IS, ischemic stroke; LDL-C, low-density lipoprotein cholesterol; NGT, normal glucose tolerance; T2DM, type 2 diabetes mellitus. *P₁<0.001 when compared IGT with NGT. *P₂<0.05 when compared T2DM to NGT.

Table 3 Pearson's correlation between C peptide and carotid intima-media thickness (mm), and other parameters of patients with ischemic stroke

Variables	C-peptide (ng/ml)		CIMT	
	r	P	r	P
Systolic blood pressure (mmHg)	0.252	<0.001*	0.576	<0.01
Diastolic blood pressure (mmHg)	0.235	<0.001*	0.272	<0.001
BMI	0.628	<0.001*	0.399	<0.001*
Waist circumference (cm)	0.791	<0.001*	0.521	<0.001*
Waist/hip ratio	0.927	<0.001*	0.496	<0.001*
FMI%	0.787	<0.001*	0.495	<0.001*
FFMI%	0.462	<0.001*	0.297	<0.001*
Total cholesterol (mg/dl)	0.086	0.226	0.176	<0.001*
Triglycerides (mg/dl)	0.289	<0.001*	0.311	<0.001*
LDL cholesterol (mg/dl)	0.206	<0.001*	0.116	0.102
HDL cholesterol (mg/dl)	-0.612	<0.001*	-0.375	<0.001*
Fasting plasma glucose (mg/dl)	0.555	<0.001*	0.553	<0.001*
Postprandial glucose (mg/dl)	0.562	<0.001*	0.558	<0.001*
FSI (μU/ml)	0.991	<0.001*	0.624	<0.001*
HOMA-IR	0.808	<0.001*	0.656	<0.001*
HOMA-β	-0.630	<0.001*	-0.395	<0.001*
HbA1c	0.602	<0.001*	0.653	<0.001*

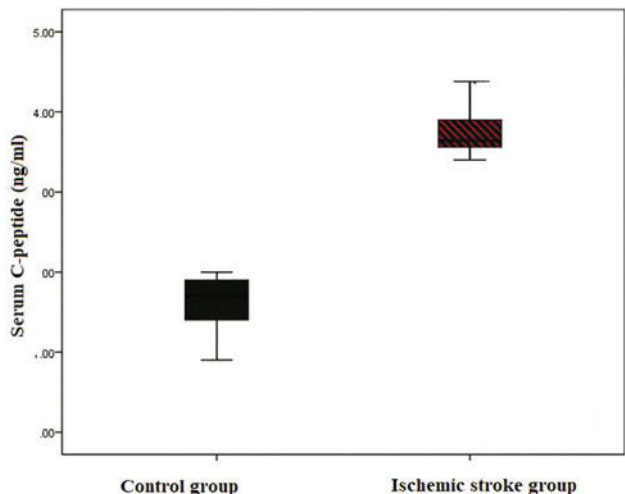
CIMT, carotid intima-media thickness; FSI, fasting serum insulin; HOMA-IR, homeostasis model assessment of insulin resistance; HbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; IGT, impaired glucose tolerance; IS, ischemic stroke; LDL-C, low-density lipoprotein cholesterol; NGT, normal glucose tolerance; T2DM, type 2 diabetes mellitus. *P<0.05.

pressure, FPG, postprandial blood glucose, FSI, HOMA-IR, HbA1c, LDL, and CIMT, postprandial blood glucose, and HbA1c compared with the NGT group ($P<0.05$). On the contrary, there was a significantly lower value of HDL and HOMA-β in IGT group compared with the NGT group ($P>0.05^*$) (Table 2).

Pearson's correlation between serum C-peptide and CIMT with other parameters

There was a positive correlation between C-peptide and systolic blood pressure, diastolic blood pressure, BMI, waist circumference, waist/hip ratio, FMI%, FFMI%, FPG, postprandial blood glucose, FSI, HbA1c, LDL, TG, as well as HOMA-IR. Also,

Figure 1



Comparison of serum C-peptide (ng/ml) in the studied groups.

there was a positive correlation between CIMT and systolic blood pressure, diastolic blood pressure, BMI, waist circumference, waist/hip ratio, FMI%, FFMI%, FPG, postprandial blood glucose, FSI, HbA1c, TG, as well as HOMA-IR ($P<0.001^*$), while there were significant negative correlations between C-peptide and CIMT with HDL and HOMA- β ($P<0.001^*$) (Table 3).

Comparison of serum C-peptide (ng/ml) in the studied groups

Patients with IS had significantly higher values of serum C-peptide compared with the control group ($P<0.001^*$) (Fig. 1). In addition, the T2DM group had significantly higher values of serum C-peptide compared with IGT and NGT ($P<0.001^*$) (Fig. 2).

Pearson’s correlation between serum C-peptide and CIMT

There was a positive correlation between serum C-peptide and CIMT in the studied groups ($r=0.625$, $P<0.001$). These data are represented in Fig. 3.

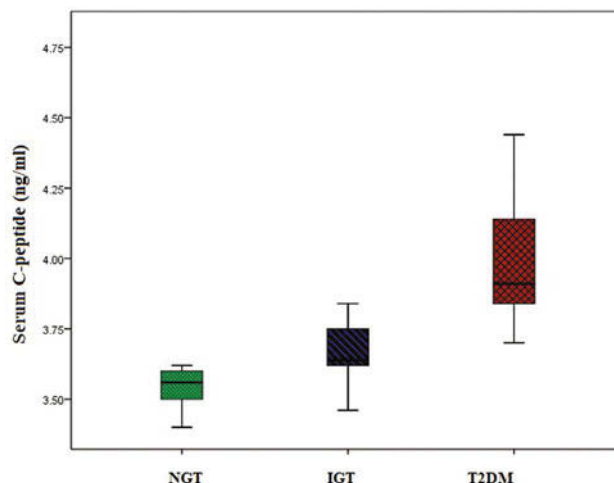
Linear regression analyses in IS patients

Linear regression analysis test was done to assess the main independent parameters associated with serum C-peptide. Our results showed that HOMA-IR, FPG, BMI, and FMI% were independently correlated with C-peptide (Table 4).

Logistic regression analysis evaluating the association of CIMT (mm) and serum C-peptide (ng/ml) with T2DM among patients with IS

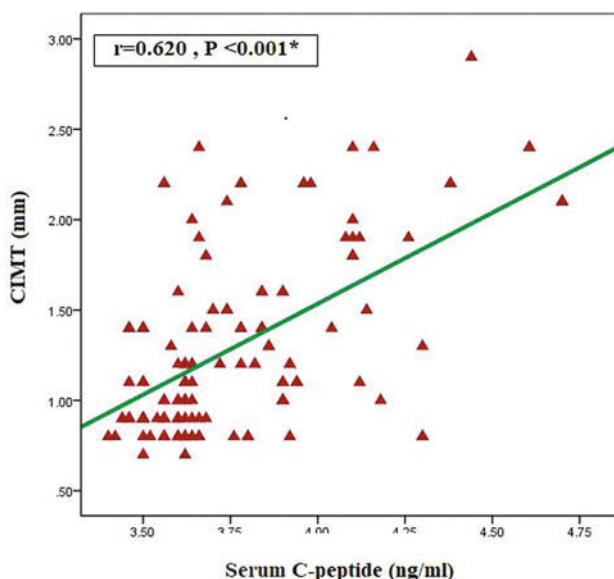
After adjusting for the traditional risk factors, logistic regression analysis test was done to evaluate the predictor of T2DM among the IS patients. C-

Figure 2



Comparison of serum C-peptide (ng/ml) in ischemic stroke groups.

Figure 3



Correlation between carotid intimamedia thickness (mm) and serum C peptide in the studied groups.

peptide was a statistically significant predictor of T2DM among the IS patients ($P<0.05$) (Table 5).

The accuracy of serum C-peptide in discriminating IGT from NGT by ROC analysis

We further investigated the potential diagnostic value of serum C-peptide by the ROC curves presented in Fig. 4. In IS, when we discriminate IGT from NGT, the cutoff value was 3.71 and the AUC were 0.939 (95% CI=0.903–0.975). Additionally, the sensitivities and the specificities were 96 and 97.7%. Thus, serum C-peptide could be a useful diagnostic test to discriminate IGT from NGT.

Table 4 Linear regression analyses in patients with ischemic stroke to test the influence of the main independent variables against serum C-peptide (ng/ml) (dependent variable)

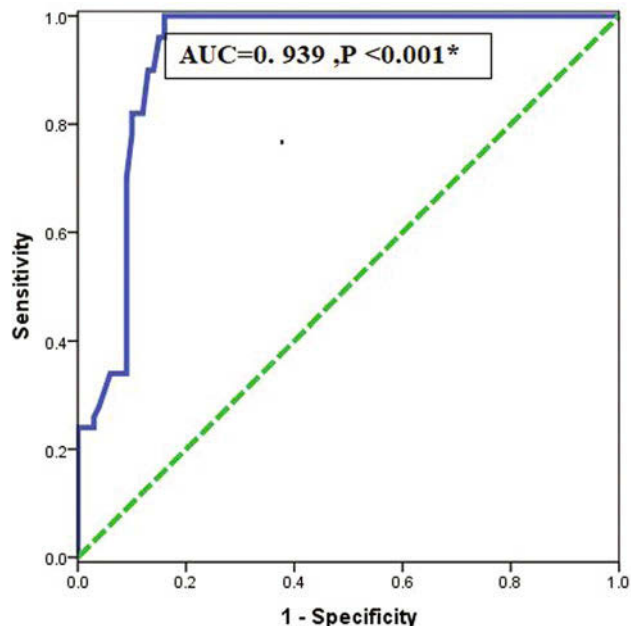
Model	Unstandardized coefficients		Standardized coefficients β	t	P	95% CI	
	B	SE				Lower bound	Upper bound
Constant	3.747	0.047		79.578	<0.001*	3.654	3.840
HOMA-IR	0.484	0.019	3.822	25.713	<0.001*	0.447	0.521
Fasting plasma glucose	-0.023	0.001	-3.041	-19.81	<0.001*	-0.025	-0.021
HDL cholesterol	0.000	0.001	-0.009	-0.420	0.675	-0.001	0.001
BMI	-0.016	0.007	-0.427	-2.274	<0.05*	-0.030	-0.002
FMI%	0.047	0.019	0.499	2.534	<0.05*	0.010	0.084

CI, confidence interval; HDL, high-density lipoprotein. * P <0.05.

Table 5 Logistic regression analysis evaluating the association of carotid intimamedia thickness (mm), and serum C-peptide (ng/ml) with type 2 diabetes mellitus among patients with ischemic stroke

Variables	B	SE	Wald	P	Odds	95% CI	
						Lower	Upper
CIMT (mm)	-0.190	0.597	0.102	0.750	0.827	0.256	2.664
C-peptide (ng/ml)	8.186	1.479	30.657	<0.001*	3591.4	198.041	65 129.13
Constant	-31.394	5.307	34.995	<0.001*	0.000		

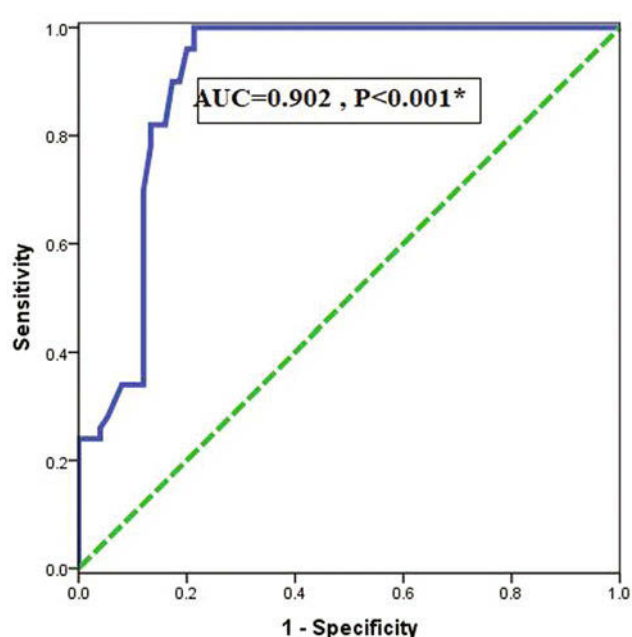
CI, confidence interval; CIMT, carotid intima-media thickness. * P <0.05.

Figure 4

Receiver operating characteristic curve: for serum C-peptide for discriminating impaired glucose tolerance from normal glucose tolerance.

The accuracy of serum C-peptide for discriminating T2DM from IGT by ROC analysis

We further investigated the potential diagnostic value of serum C-peptide by ROC curves as presented in Fig. 4. In IS, when we discriminate IGT from NGT, the cutoff value was 3.73 and the AUC was 0.902 (95% CI=0.884–0.996). Additionally, the sensitivities and the specificities were 96 and 98%. Thus, serum C-peptide could be a useful diagnostic test to discriminate T2DM from IGT (Fig. 5).

Figure 5

Receiver operating characteristic curve: for serum C-peptide for discriminating type 2 diabetes mellitus from impaired glucose tolerance.

Discussion

There was great evidence that the higher incidence of DM and its complications represent a major burden to public health. Therefore, there is a great interest in identifying asymptomatic individuals at risk who would be candidates for more intensive and evidence-based medical interventions [11].

Carotid intima-media thickness (CIMT) measured by an ultrasound is a noninvasive, safe, low-cost, reproducible, and well-validated marker of preclinical atherosclerosis [12–14]. Interestingly, recent studies have suggested that increased CIMT is independently linked to CV risks [13–15]. Thus CIMT could be used as a diagnostic marker of risk for CV events [16].

Mounting evidence indicates that the role of insulin resistance as CV risk has been well documented. In fact, it is indeed for an accurate test to assess the insulin levels. Thus, the objective of the current study was to explore serum C-peptide and CIMT in patients with ischemic CVS and to assess the association of serum C-peptide with vascular and metabolic risks.

As expected our results have shown that there were significantly higher values of obesity indices, metabolic, and vascular risks in the IS group compared with the control. Even more importantly, serum C-peptide levels were significantly higher in the IS group compared with the control.

Concordance with our findings, Li and colleagues observed that serum C-peptide level was strongly associated with stroke in nondiabetic participants and is significantly associated with obesity indices [17].

Similar to our findings, Hirai and his colleagues detected that serum C-peptide levels were associated with increased all-cause mortality. Regarding women, serum C-peptide levels were associated with ischemic heart disease mortality. However, as regards men, serum C-peptide levels were associated with stroke mortality [18].

The results presented herein are innovative as this study performs a robust evaluation of serum C-peptide and CIMT as diagnostic markers of preclinical atherosclerosis. Noteworthy, our results confirmed that serum C-peptide and CIMT were significantly higher in T2DM compared with the NGT group. Interestingly, they were positively correlated with other vascular and metabolic risks.

Similar to our findings, Marx and colleagues observed that among patients with T2DM, the C-peptide level has been reported to be significantly correlated with CIMT [19].

Recently published studies have highlighted the reason for the increased risk of death among people with high C-peptide levels as they detected the involvement of C-

peptide in atherogenesis [20]. The interesting finding of experimental studies which were conducted to assess the atherogenic role of C-peptide found that C-peptide colocalizes with intimal monocytes, macrophages, and CD4-positive lymphocytes and that, *in vitro*, the C-peptide induces monocyte and T-cell chemotaxis [21].

Notably, it was shown that C-peptide activated the proliferation of vascular smooth muscle cells through the activation of Src kinase, phosphoinositide 3-kinase, and extracellular signal-regulated protein kinases 1 and 2 [22].

We in this study attempted to pierce out the independent correlation with C-peptide. Our results showed that HOMA-IR, FPG, BMI, and FMI% were independently correlated with C-peptide by linear regression analysis test.

Evidence also suggests that elevated C-peptide levels have been associated with atherogenic risk factors, including increased levels of triglycerides and high blood pressure [23].

Meanwhile, according to Kim *et al.* [24], there was a strong correlation between C-peptide and triglyceride level, HDL, leptin levels, and BMI.

The results of the current study have shown after adjusted for the traditional risk factors that C-peptide was a statistically significant predictor of T2DM among IS patients by logistic regression analysis test.

Similar results have been confirmed by Wang and colleagues and they observed that C-peptide is correlated with clinical coronary artery disease independent of the traditional CV risk factors, such as age, hypertension, smoking status, dyslipidemia, glycemic control, and insulin resistance, in T2DM patients [25].

However, the growing evidence show that C-peptide levels are associated with atherogenic risk factors in both diabetic and nondiabetic patients. The finding of the current study show that the potential diagnostic value of serum C-peptide by ROC curves was highly significant; the sensitivities and the specificities were 96 and 98%. Thus, serum C-peptide could be a useful diagnostic test discriminating T2DM from IGT.

Conclusion

The results of the current study found higher levels of C-peptide in IS patients compared with control. Even more importantly, among the IS patients, serum C-

peptide was higher in T2DM compared with IGT and NGT and was associated with vascular and metabolic risks.

Financial support and sponsorship

Nil.

Conflicts of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

- Bunevicius A, Iervasi G, Bunevicius R, Appel LJ, Braun LT, Chaturvedi S, *et al.* Neuroprotective actions of thyroid hormones and low-T3 syndrome as a biomarker in acute cerebrovascular disorders. *Expert Rev Neurother* 2015; 15:315–326.
- Goldstein LB, Bushnell CD, Adams RJ, *et al.* Guidelines for the primary prevention of stroke: a guideline for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke* 2011; 42:517–584.
- Adler AI, Stevens RJ, Neil A, Stratton IM, Boulton AJM, Holman RR. UKPDS 59: Hyperglycemia and other potentially modifiable risk factors for peripheral vascular disease in type 2 diabetes. *Diabetes Care* 2002; 25:894–899.
- International Diabetes Federation Diabetes Atlas. *Diabetes care* (2nd ed.). International Diabetes Federation: Brussels. 2003.
- Held C, Hjemdahl P, Eriksson SV, Björkander I, Forslund L, Rehnqvist N. Prognostic implications of intima-media thickness and plaques in the carotid and femoral arteries in patients with stable angina pectoris. *Eur Heart J* 2001; 22:62–72.
- Acevedo M, Kråmer V, Tagle R, Arnaiz P, Corbalán R, Berríos X, *et al.* Cardiovascular risk factors among young subjects with high carotid intima-media thickness. *Rev Med Chil* 2011; 139:1322–1329.
- Ferranini E, Cobelli C. The kinetics of insulin in man. *Role Liver Diabetes Metab Rev* 1987; 3:365–397.
- Ashby TD, Frier BM. Circulating c-peptide measurement and clinical application. *Annals Clin Biochem* 1981; 18:125.
- Castillo J, Davalos A, Noya M. Progression of ischaemic stroke and excitotoxic amino acids. *Lancet* 1997; 349:79–83.
- Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without the use of the preparative ultracentrifuge. *Clin Chem* 1972; 18:499–502.
- Wright JT Jr, Williamson JD, Whelton PK, Snyder JK, Sink KM, Rocco MV, *et al.* A randomized trial of intensive versus standard blood pressure control. *N Engl J Med* 2015; 373:2103–2116.
- Tardif JC, Heinonen T, Orloff D, Libby P. Vascular biomarkers and surrogates in cardiovascular disease. *Circulation* 2006; 113:2936–2942.
- Lorenz MW, Markus HS, Bots ML, Rosvall M, Sitzer M. Prediction of clinical cardiovascular events with carotid intima-media thickness: a systematic review and meta-analysis. *Circulation* 2007; 115:459–467.
- De Groot E, van Leuven SI, Duivenvoorden R, Meuwese MC, Akdim F, *et al.* Measurement of carotid intima-media thickness to assess the progression and regression of atherosclerosis. *Nat Clin Pract Cardiovasc Med* 2008; 5:280–288.
- Lorenz MW, Polak JF, Kavousi M, Mathiesen EB, Volzke H, Bots ML, *et al.* PROG-IMT Study Group. Carotid intima-media thickness progression to predict cardiovascular events in the general population (the PROG-IMT collaborative project): a meta-analysis of individual participant data. *Lancet* 2012; 379:2053–2062.
- Lorenz MW, Gao L, Ziegelbauer K, Norata GD, Empana JP, Schmidtman XX, *et al.* Predictive value for cardiovascular events of common carotid intima media thickness and its rate of change in individuals at high cardiovascular risk - Results from the PROG-IMT collaboration. *PLoS One* 2018; 13:e0191172.
- Li Y, Meng L, Li Y, Sato Y. Associations of serum C-Peptide level with body fat distribution and ever stroke in nondiabetic subjects. *J Stroke Cerebrovasc Dis* 2014; 23:e163–e169.
- Hirai FE, Moss SE, Klein BE, *et al.* Relationship of glycemic control, exogenous insulin, and C-peptide levels to ischemic heart disease mortality over a 16-year period in people with older-onset diabetes: the Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR). *Diabetes Care* 2008; 31:493–497.
- Marx N, Silbernagel G, Brandenburg V, *et al.* C-peptide levels are associated with mortality and cardiovascular mortality in patients undergoing angiography: the LURIC Study. *Diabetes Care* 2013; 36:708–714.
- Walcher D, Aleksic M, Jerg V, Burgmaier M, Kleber M, Grammer T, *et al.* C-peptide induces chemotaxis of human CD4-positive cells: involvement of pertussis toxin-sensitive G-proteins and phosphoinositide 3-kinase. *Diabetes* 2004; 53:1664–1670.
- Marx N, Walcher D, Raichle C, Aleksic M, Bach H, Grüb M, *et al.* C-peptide colocalizes with macrophages in early arteriosclerotic lesions of diabetic subjects and induces monocyte chemotaxis in vitro. *Arterioscler Thromb Vasc Biol* 2004; 24:540–545.
- Walcher D, Babiak C, Poletsek P, Hombach V, Zieske A, Homma S, *et al.* C-Peptide induces vascular smooth muscle cell proliferation: involvement of SRC-kinase, phosphatidylinositol 3-kinase, and extracellular signal-regulated kinase 1/2. *Circ Res* 2006; 99:1181–1187.
- Haban P, Simoncic R, Zidekova E, Ozdin L. Role of fasting serum C-peptide as a predictor of cardiovascular risk associated with the metabolic X-syndrome. *Med Sci Monit* 2002; 8:CR175–CR179.
- Kim ST, Kim BJ, Lim DM, Song I, Jung J, Lee K, *et al.* Basal C-peptide level as a surrogate marker of subclinical atherosclerosis in type 2 diabetic patients. *Diabetes Metab J* 2011; 35:41–49.
- Wang L, Lin P, Ma A, Zheng H, Wang K, Li W, *et al.* C-peptide is independently associated with an increased risk of coronary artery disease in T2DM subjects: a cross-sectional study. *PLoS One* 2015; 10:e0127112.