Serum copeptin as a diagnostic and prognostic biomarker of coronary artery disease among patients with type 2 diabetes mellitus

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Background
Diabetes is one of the major risk factors for coronary artery disease (CAD); hormones implicated in cardiac diseases may play a role in diabetes development. Increased activities of the arginine-vasopressin (AVP) system were shown to be associated with type 2 diabetes mellitus (T2DM). The aim of this study was to estimate the values of serum copeptin as a predictive biomarker of CAD and to assess the correlation between copeptin and cardiometabolic risk factors in patients with T2DM.

Patients and methods
The case–control study included 110 patients with T2DM and 80 age-matched and sex-matched control group. All the participants were subjected to B-mode ultrasonography of both common carotid arteries to measure carotid intima-media thickness (mm), echocardiography, and coronary arteriography. Serum copeptin levels were measured with a new sandwich immunoassay by using a human copeptin enzyme-linked immunosorbent assay kit.

Results
Patients with T2DM had significantly higher serum copeptin levels (7.64±1.98 pmol/l) compared with control groups (4.64±1.11 pmol/l). Serum copeptin levels were significantly higher in patients with CAD (8.64±2.55 pmol/l) compared with patients without CAD (6.36±0.86 pmol/l). Interestingly, copeptin was positively correlated with cardiometabolic risks. The area under the curve of serum copeptin levels in differentiating patient with T2DM from control was 0.768 ($P<0.001$) and differentiating patient with CAD from the nonischemic group was 0.818 ($P<0.001$).

Conclusion
The higher serum level of copeptin in patients with T2DM especially in the patient with CAD is strongly correlated with cardiometabolic risk factors.

Keywords:
arginine vasopressin, coronary artery disease, copeptin, type 2 diabetes mellitus

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Introduction

Cardiovascular disease (CVD) is the leading cause of death and disability worldwide [1,2]. Obesity and type 2 diabetes mellitus (T2DM) are considered as one of the major risk factors for coronary heart disease development [3,4].

Arginine-vasopressin (AVP) is a neurohormone released from the neurohypophysis for maintaining fluid homeostasis [5]. Copeptin is the metabolic product of AVP precursor, and it is more stable than AVP and has a long half-life [6]. Mounting evidence indicates that elevated copeptin was significantly associated with a higher risk of diabetes, independent of other risks.

Compelling evidence suggests that the primary prevention of CVD is dependent upon the ability to identify high-risk individuals long before the development of overt events. Biomarkers play a critical role in the definition, prognostication, and decision making regarding the management of cardiovascular events. Thus, the aim of this study was to estimate the values of serum copeptin as a predictive biomarker of coronary artery disease (CAD) and to assess the correlation between copeptin and cardiometabolic risk factors in patients with T2DM.

Patients and methods

A case–control study was conducted that included 110 patients with T2DM. The diagnosis of diabetes was done according to ADA 2017. The enrolled diabetic

patients were classified into two groups: 63 with CAD and 47 without CAD. Moreover, the study included 90 age-matched and sex-matched healthy individuals as a control group.

The patients with type 1 diabetes mellitus, patients with estimated glomerular filtration ratio less than 60 ml/min/1.73 m^2, patients with decompensated liver disease, patients with rheumatic valvular heart diseases, patients with decompensated heart failure, patients with previous myocardial infarction or patients with recent cerebrovascular events (such as brain infarction or hemorrhage) within the prior 6 months were excluded from this study. All patients were subjected to thorough history taking and full clinical assessment including blood pressure and anthropometric variables. BMI was calculated as weight in kg/height $(m)^2$. The patients underwent elective coronary angiography for suspected CAD in the Cardiology Department of Zagazig University Hospitals. All patients were investigated using a 12-lead standard ECG and echocardiography. Coronary arteriography was performed for all patients by the Judkins technique for assessment of the lesion's distribution and description. CAD was considered significant if there was at least 50% diameter stenosis in at least one coronary artery. The Ethical Committee of the Faculty of Medicine, Zagazig University approved our study protocol, and all participants provided written informed consent.

We evaluated our participants in the outpatient clinic of Diabetes and Endocrinology Unit, Internal Medicine Department, Zagazig University Hospitals. All participants provided written informed consent. The study has been conducted according to the guidelines of the Ethical Committee of the Faculty of Medicine, Zagazig University.

Blood sampling and laboratory assessments

Blood samples were drawn from all patients after an overnight fast and divided into two portions: 1 ml of whole blood was collected into evacuated tubes containing EDTA, for hemoglobin A1c (HbA1c), and 1 ml of whole blood was collected into evacuated tubes containing potassium oxalate and sodium fluoride (2 : 1) for fasting plasma glucose (FPG). Sera were separated immediately from 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). LDL cholesterol was calculated using the Friedewald formula [7]. 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 assessments of insulin resistance, and was calculated as FSI (μU/ml)×FBG (mg/dl)/405, and ⍰-cell function (HOMA-B) was calculated. C-peptide concentration was measured in serum by ELISA kit according to the manufacturer's instructions (Wkea Med Supplies Corp., Jilin, China) [8].

Measurement of serum copeptin levels

The level of copeptin in samples was determined using a double-antibody sandwich ELISA kit supplied by Elabscience Co. Ltd (Donghu Hi-Tech Development Area, Wuhan, China).

Carotid ultrasonography

Ultrasonography of the carotid arteries was done in the Radiodiagnosis Department of Zagazig University Hospitals for elective patients. Carotid artery atherosclerosis was determined by two examiners for all patients across all six sites, using high-resolution B-mode ultrasound (M-Turbo; SonoSite, Washington, Bothell, USA), according to the protocol. The B-mode ultrasound examination is performed with a linear array transducer of 7.5–12 MHz on the selected patients in supine position with the head slightly extended and turned to the opposite direction of the common carotid artery (CCA) being studied. Both sides were imaged at three places, that is, at the proximal part, mid part, and the bulb. The means of the three maximum right and three maximum left far wall measurements were calculated for each CCA. On a longitudinal, two-dimensional ultrasound image of CCA, each anterior (near) and posterior (far) wall of the CCA was displayed as two bright white lines separated by a hypoechogenic space. The distance between the leading edge of the first bright line (the blood–intima interface) of the far wall and the leading edge of the second bright line (media–adventitia interface) indicates the intima-media thickness. A reading of more than 0.8 mm is considered to be abnormal and taken as the earliest marker of atherosclerosis [9].

Statistical analysis

Data were analyzed on the SPSS (version 21, SPSS Inc., Chicago, Illinois, USA). Pearson correlation was used to assess the association between serum copeptin, carotid intima-media thickness (CIMT), clinical, biochemical tests, and other studied metabolic

parameters. Receiver operating characteristic analysis was performed to assess the potential accuracy of serum copeptin, for the diagnosis of T2DM.

Results

Among the studied participants, in the control group, 55% were male and 45% were female, and their mean age was 49.94±5.56 years. In the diabetic group, 61% were male and 38% were female, and their mean age was 45.11±6.3 years. Control and T2DM groups were matched for age, ethnicity, and sex.

Clinical and biochemical characteristics of the studied groups

Patient with T2DM had significantly higher values of diastolic blood pressure, BMI, waist/hip ratio, postprandial plasma glucose, FPG, HbA1c, FSI, HOMAR-IR, triglycerides (TG), high-sensitivity C-reactive protein (hs-CRP), and CIMT than the control group ($P<0.05$). On the contrary, there was a significantly lower value of HDL in patients with T2DM compared with the control group ($P<0.001$; Table 1).

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

	Control group (*n*=80) (mean ±SD)	Patient with T2DM (*n*=110) (mean ±SD)	*P*
Systolic blood pressure (mmHg)	132.79±21.01	137.4±21.90	0.334
Diastolic blood pressure (mmHg)	76.65±7.5	80.48±8.5	<0.05*
BMI (kg/m^2)	22.47±9.05	37.31±5.89	<0.001*
Waist/hip ratio	0.97±0.389	1.46±0.55	<0.001*
Total cholesterol (mg/dl)	166.7±69.56	222.7±59.1	<0.001*
Triglycerides (mg/dl)	135.8±77.4	199.76±70.39	<0.001*
LDL cholesterol (mg/dl)	108.12±40.3	155.1±46.76	<0.001
HDL cholesterol (mg/dl)	48.9±10.69	35.1±16.8	<0.01*
Fasting plasma glucose (mg/dl)	82.96±4.62	140.9±30.19	<0.001*
Postprandial glucose (mg/dl)	114.6±15.19	173.84±56.86	<0.001*
Fasting insulin (μIU/ml)	5.3±3.28	11.48±5.19	<0.001*
HOMA-IR	1.51±0.43	3.71±2.35	<0.001*
HbA1c (%)	4.78±0.405	6.8±3.183	<0.001*
Uric acid (mg/dl)	4.03±1.926	7.95±2.95	<0.001*
CIMT (mm)	0.789±0.119	1.296±0.48	<0.001*
hs-CRP (μg/ml)	2.61±1.165	4.84±2.23	<0.001*
Serum copeptin (pmol/l)	2.44±1.23	5.21±1.16	<0.001*

CIMT, carotid intima-media thickness; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessments of insulin resistance; hs-CRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; T2DM, type 2 diabetes mellitus. *$P<0.05$.

Clinical and biochemical characteristics of patients with type 2 diabetes mellitus

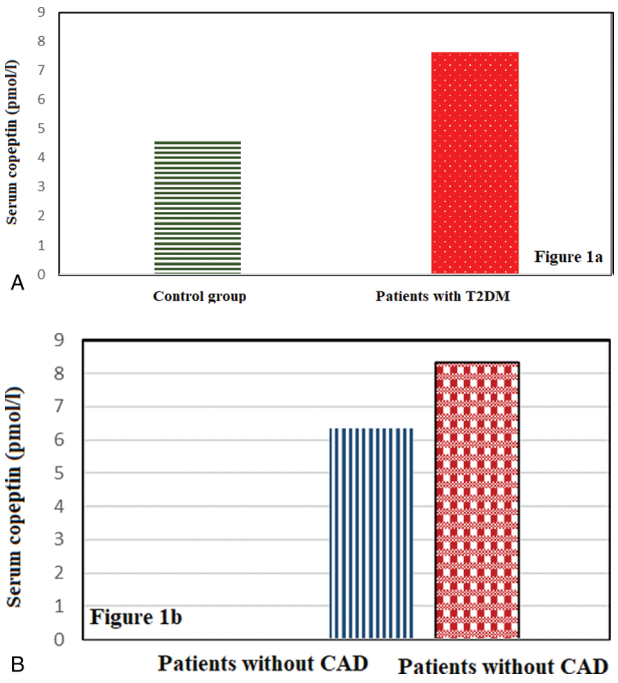
Among patients with T2DM, patients with CAD had significantly higher values of systolic blood pressure, diastolic blood pressure, postprandial plasma glucose, FPG, HbA1c, FSI, HOMAR-IR, TG, hs-CRP, and CIMT than normal glucose tolerance (NGT) group ($P<0.05$). On the contrary, there were significant higher values of FPG, postprandial plasma glucose, FSI, HOMAR-IR, HbA1c, and hs-CRP in impaired glucose tolerance group compared with NGT group ($P<0.05$). On the contrary, there was a significantly lower value of HDL in the impaired glucose tolerance group compared with NGT group ($P<0.001$; Table 2).

Table 2 Laboratory and anthropometric parameters in obese patients stratified according to fasting plasma glucose

	Without CAD (*n*=63) (mean ±SD)	With CAD (*n*=47) (mean ±SD)	*P*
Systolic blood pressure (mmHg)	122.6±17.45	147.6±16.9	<0.001*
Diastolic blood pressure (mmHg)	75.06±7.64	79.56±7.25	<0.05*
BMI (kg/m^2)	32.55±13.34	31.03±5.1	0.492
Waist/hip ratio	1.39±0.571	1.43±0.21	0.492
Total cholesterol (mg/dl)	170.5±56.9	180.1±72.1	0.521
Triglycerides (mg/dl)	117.04±47.7	176.1±96.2	<0.01*
LDL cholesterol (mg/dl)	134.7±44.5	137.3±42.5	0.792
HDL cholesterol (mg/dl)	42.5±12.03	38.6±13.76	0.227
Fasting plasma glucose (mg/dl)	91.5±7.05	158.3±31.7	<0.001*
Postprandial glucose (mg/dl)	128.8±26.3	249.6±55.2	<0.001*
Fasting insulin (μIU/ml)	5.63±0.81	15.29±3.96	<0.001*
HOMA-IR	1.26±0.18	5.93±1.71	<0.001*
HbA1c (%)	5.68±0.365	8.02±1.245	<0.001*
CIMT (mm)	0.97±0.296	1.64±0.534	<0.001*
hs-CRP (μg/ml)	3.16±1.72	6.04±1.98	<0.001*

CAD, coronary artery disease; CIMT, carotid intima-media thickness; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessments of Insulin resistance; hs-CRP, high-sensitivity C-reactive protein; IGT, impaired glucose tolerance; LDL, low-density lipoprotein; NGT, normal glucose tolerance; T2DM, type 2 diabetes mellitus. *$P_1<0.001$ when compared IGT with NGT. *$P_2<0.05$ when compared T2DM with NGT'.

Figure 1



(a): Serum copeptin (pmol/l) levels in the studied groups. (b) Serum copeptin (pmol/l) levels in patients with type 2 diabetes mellitus.

Table 3 Pearson correlations between copeptin (pmol/l) and other parameters of studied groups

Variables	Copeptin (pmol/l)	
	r	*P*
Sex	0.029	0.754
Age (years)	0.085	0.347
Systolic blood pressure (mmHg)	0.938	<0.001*
Diastolic blood pressure (mmHg)	0.029	0.854
BMI	0.014	0.971
Waist/hip ratio	0.088	0.386
Total cholesterol (mg/dl)	0.076	0.452
Triglycerides (mg/dl)	0.190	0.058
LDL cholesterol (mg/dl)	0.020	0.286
HDL cholesterol (mg/dl)	–0.129	0.201
Fasting plasma glucose (mg/dl)	0.429	<0.001*
Postprandial glucose (mg/dl)	0.386	<0.001*
HbA1c	0.483	<0.001*
Fasting insulin (μIU/ml)	0.850	<0.001*
HOMA-IR	0.744	<0.001*
hs-CRP (μg/ml)	0.744	<0.001*
CIMT (mm)	0.433	<0.001*

CIMT, carotid intima-media thickness; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessments of insulin resistance; hs-CRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein. *P_1<0.001.

Comparison of serum copeptin in the studied population

Patients with T2DM had significantly higher values of serum copeptin (7.64±1.98) compared with control (4.64±1.11; Fig. 1a). Among patients with T2DM, patients with CAD had significantly higher values of serum copeptin (8.64±2.55) compared with control (6.36±0.86; Fig. 1b).

The Pearson correlation between serum copeptin with other parameters is shown in Table 3.

The accuracy of serum copeptin for discriminating patients with diabetes from the control group

Using the receiver operating characteristic curve, at the cutoff value of 5.7 and area under the curve of 0.768 [95% confidence interval (CI)=0.673–0.863], the sensitivity and the specificity of serum copeptin for discriminating patients with diabetes from the control group were 87.2 and 60.7%, respectively (Fig. 2).

The accuracy of serum copeptin for discriminating patients with coronary artery disease from those without coronary artery disease

At the cutoff values of 4.5 and area under the curve of 0.818 (95% CI=0.721–0.914), the sensitivity and the specificity of serum copeptin for discriminating patients with CAD from those without CAD were 90 and 63.3%, respectively (Fig. 3).

Logistic regression analysis evaluating the association of serum copeptin with type 2 diabetes mellitus among the studied patients

After adjusting for the traditional risk factors, logistic regression analysis test was done to evaluate the predictor of cardiovascular risk. Copeptin was a statistically significant predictor of cardiovascular risk among the patients (odds ratio=0.001, 95% CI=0.000–0.407, P<0.05; Table 4).

Multiple stepwise linear regression analysis to test the influence of the main independent variables against copeptin (pmol/l) (dependent variable)

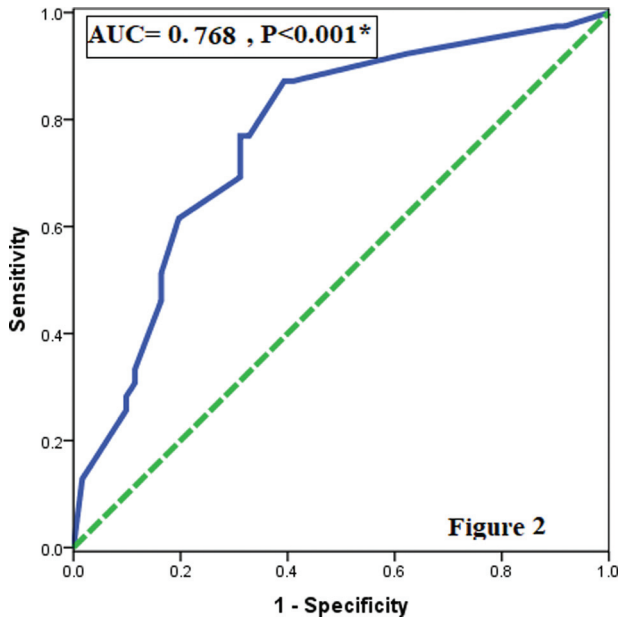
The stepwise linear regression analysis test revealed that CIMT and postprandial plasma glucose were independently correlated with copeptin (P<0.001; Table 5).

Discussion

There has been great evidence that the prevalence of T2DM is increasing since decades. Interestingly, CAD represents a major burden on public health. Although considerable research effort has been focused on CAD, the pathophysiology of the disease remains incompletely understood [10].

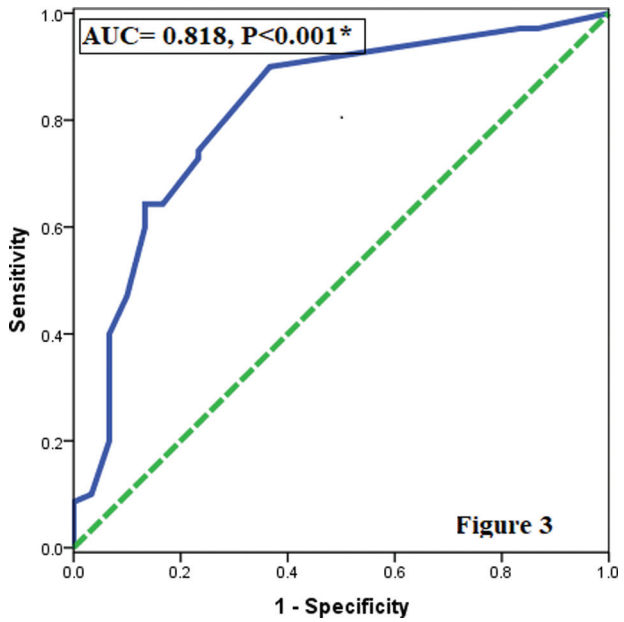
Regarding the importance and efficacy of prevention strategies by identifying the susceptible populations, this study was designed to investigate the association of serum copeptin with susceptibility of CAD. To address

Figure 2



Receiver operating characteristic (ROC) for serum copeptin (pmol/l) in discriminating patients with type 2 diabetes mellitus from control group.

Figure 3



Receiver operating characteristic (ROC) for serum copeptin (pmol/l) in discriminating patients with coronary artery disease (CAD) from patient without CAD.

this need, we have focused on evaluating the value of serum copeptin as a predictive biomarker of CAD, and to assess the correlation between copeptin and cardiometabolic risk factors in patients with T2DM.

The results presented herein are innovative, as this study performs a robust evaluation of copeptin as a diagnostic marker of CAD. Our results confirmed that copeptin was significantly higher in patients with T2DM than the control group, in particular patients with CAD compared with those without CAD. Interestingly, copeptin was positively correlated with FPG, postprandial plasma glucose, FSI, HOMAR-IR, hs-CRP, and HbA1c.

Similar to our results, the study by Enhörning *et al.* [7] showed copeptin independently predicts T2DM and abdominal obesity but not the cluster of metabolic syndromes.

In agreement with our results, Schink *et al.* [11] observed higher levels of copeptin in the obese group compared with the lean one.

In accordance with our finding, the study by Enhörning suggested that elevated copeptin level predicts increased risk for diabetes mellitus independent of the established clinical risk factors, including fasting glucose and insulin. In addition, they observed that copeptin increased concomitantly with T2DM. However, the copeptin levels were not correlated with the parameters of insulin resistance [12].

An interesting experimental study reported that AVP infusion leads to increased blood glucose levels [13].

There is compelling evidence suggesting an important association of copeptin with impaired glucose tolerance, as in the study conducted by Aoyagi *et al.* [14], which found that mice lacking V1aR display impaired glucose tolerance, insulin resistance, and elevated AVP levels.

In another experimental study conducted by Fujiwara *et al.* [15], it was observed that mice that lack V1bR have the opposite phenotype of lower fasting plasma glucose and increased insulin sensitivity. These findings suggest a model in which impaired signaling through V1aR leads to elevated levels of AVP, which in turn stimulates V1bR and contributes to insulin resistance and the development of diabetes mellitus [16].

We in this study attempted to examine the association between serum copeptin and cardiometabolic risk factors. After adjusting for the traditional risk factors, logistic regression analysis test detected that serum copeptin level was a statistically significant predictor of cardiovascular risk among diabetic patients.

In agreement with our results, Tasevska *et al.* [17] observed that copeptin could predict CAD

Table 4 Logistic regression analysis evaluating the association of carotid intima-media thickness (mm) and serum copeptin with type 2 diabetes mellitus among the studied patients

Variables	*B*	SE	Wald	*P*	Odds ratio	95% CI	
						Lower	Upper
Copeptin	−7.008	3.116	5.057	<0.05*	0.001	0.000	0.407
CIMT	2.076	1.873	1.228	0.268	7.970	0.203	313.404
Constant	−13.620	6.675	4.163	<0.05*	0.000		

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

Table 5 Multiple stepwise linear regression analysis to test the influence of the main independent variables against copeptin (pmol/l) (dependent variable)

Models	Unstandardized coefficients		Standardized coefficients	*t*	*P*	95% CI	
	B	SE	β			Lower bound	Upper bound
Constant	3.977	0.545		7.298	<0.001*	2.895	5.058
CIMT	1.881	0.396	0.433	4.749	<0.001*	1.095	2.667
Constant	2.939	0.652		4.506	<0.001*	1.645	4.234
CIMT	1.360	0.429	0.313	3.170	<0.001*	0.509	2.212
Postprandial plasma glucose	0.009	0.003	0.267	2.709	<0.001*	0.003	0.016

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

development and cardiovascular mortality in both diabetics and nondiabetics. In addition, Boeckel *et al.* [18] found a significant increase of copeptin in patients having acute myocardial infarction.

Even more importantly, for further evaluation, we analyzed our results using stepwise linear regression analysis and found that CIMT and postprandial plasma glucose were independently correlated with copeptin.

Our findings are in concordance with the study by Enhörning *et al.* [12], in which they found that FPG was the strongest risk factor for new-onset diabetes mellitus, but after adjusting for FPG and all other available diabetes risk factors, copeptin had a 2–3-fold excess risk of developing diabetes mellitus.

The interesting study by Wannamethee *et al.* [19] found that copeptin was independently associated with an increased risk of incident stroke and CVD mortality in men with diabetes, but not in men without diabetes.

Recent evidence indicates that copeptin was elevated in acute coronary syndrome [20–22]. Interestingly, a study conducted by Khan *et al.* [23] suggested that the high level of serum copeptin measured immediately after Percutaneous coronary intervention (PCI) was associated with major adverse cardiac events (MACE) in patients with AMI during long-term follow-up.

Emerging evidence demonstrated that copeptin is a marker of insulin resistance and a possible surrogate measure of cardiovascular risk. Mounting evidence indicates that the AVP system contributes to the development of insulin resistance through a variety of mechanisms including the stimulation of glucagon, Adrenocorticotropic hormone (ACTH) secretion, glycogenolysis, etc. [24].

Conclusion

The high level of copeptin in obesity and T2DM is strongly correlated with CIMT and other cardiometabolic risk factors such as hypertension, dyslipidemia, insulin resistance, hyperglycemia, and obesity. Therefore, copeptin seems to be a marker of endothelial dysfunction and predictor of early atherosclerosis among patient with or without diabetes.

Author contribution

Nearmeen M. Rashad, Ayman E. Ali, and TME and MHS collected patients' samples and clinical data. Wesam M.R. Ashour and Reem M. Allam prepared the samples for laboratory investigations. Nearmeen M. Rashad wrote the paper. Statistical analysis, interpretation of data, and preparation of the paper for submitting internationally was done by Nearmeen M. Rashad. Critical revision of the manuscript was performed by all of the authors. All authors have read and approved the final manuscript.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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