

# Correlation of serum apelin level with carotid intima–media thickness and insulin resistance in a sample of Egyptian patients with type 2 diabetes mellitus

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**Background:** Type 2 diabetes mellitus (T2DM) is a growing health problem in Egypt, with a significant impact on morbidity and mortality. Measurement of the carotid Intima-media thickness (CIMT) allows early detection of atherosclerotic blood vessel diseases. Apelin is an adipose tissue-derived hormone that may be associated with insulin resistance (IR). This study aimed to assess the level of serum apelin in patients with T2DM and its relation to IR and CIMT. **Materials and Methods:** A case-control study was conducted on 60 patients with T2DM and 30 healthy controls. T2DM was diagnosed based on American Diabetes Association criteria. The study was carried out at Al-Zahraa University Hospital, Cairo, Egypt, through the period from June to December 2019. The laboratory investigations included serum apelin and blood glucose hemostasis markers. CIMT was assessed using B-mode ultrasonography. **Results:** Patients' group had a statistically significant higher apelin level than healthy controls ( $407.96 \pm 291.07$  versus  $83.32 \pm 10.55$  ng/dL,  $P < 0.001$ ). The correlation analysis showed that the serum apelin level correlated positively with glycemic indices, body weight, and waist circumference ( $P < 0.05$ ). At cutoff value of  $>96$  ng/dL, the serum apelin exhibited a sensitivity of 98.3% and specificity of 96.7%, positive predictive value of 98.1%, and negative predictive value of 96.5%, with a diagnostic accuracy of 95.1%. Serum apelin correlated positively with CIMT ( $r = 0.296$ ,  $P = 0.022$ ). Logistic regression analysis showed that systolic and diastolic blood pressures, Homeostasis Model Assessment of IR, and CIMT were independent predictors of serum apelin. **Conclusion:** Serum apelin may be correlated with the degree of carotid atherosclerosis and hence can be used as a prognostic biomarker.

**Key words:** Apelin, carotid intima–media thickness, insulin resistance, type 2 diabetes

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## INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a widespread chronic metabolic disorder characterized by hyperglycemia due to insulin resistance (IR) and is expected to grow to affect over 642 million adults in the next decades.<sup>[1,2]</sup> diabetes mellitus (DM) is the major cause of micro and macrovascular complications, which affect the quality of life associated with serious morbidity and

mortality.<sup>[3]</sup> Moreover, IR is observed to increase the risk of obesity-related morbidity and cardiovascular diseases (CVDs).<sup>[4]</sup> Given these complications, researchers strive to discover new targets that enable them to control DM. One of these targets is the apelin receptor, which was purified and extracted from the bovine stomach by Tatemoto *et al.* in 1998.<sup>[5]</sup>

Carotid intima–media thickness (CIMT) is a well-established tool to detect subclinical atherosclerosis

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and has been shown to be a reliable predictor of major cardiovascular events. Among T2DM patients, CIMT cut-off level of >0.8 mm has been demonstrated to be associated with a higher prevalence of ischemic heart disease.<sup>[6]</sup>

Apelin is an adipocytokine that is commonly distributed in organs such as the renal, heart, lungs, adipose, liver, endothelium, different brain regions, and human plasma.<sup>[7]</sup> The main active forms of apelin are apelin-13 and-17, which are characterized by higher resistance to degradation.<sup>[8]</sup> In mice, apelin showed a protective effect against CVDs by inhibiting the oxidative stress, which has an important role in the development of diabetic nephropathy.<sup>[9]</sup> Further, short- and long-term apelin treatment was associated with a significant improvement in terms of insulin sensitivity in obese mice.<sup>[10]</sup>

Moreover, it is efficient in enhancing altered glucose metabolism by increasing the glucose uptake in skeletal muscle.<sup>[11]</sup> The expression of apelin helps to regulate blood pressure, cardiac contractility, fluid balance, and to activate pituitary release of adrenocorticotrophic hormone.<sup>[12]</sup> Moreover, it increases cardiac output and lowers blood pressure.<sup>[13]</sup> In patients with greater CIMT, left ventricular hypertrophy, and systolic left ventricular dysfunction disease, the level of apelin is also altered, which indicates that apelin peptides could be successfully used as a biomarker of CVDs.<sup>[14]</sup>

Nevertheless, the current published literature shows scarcity regarding the association between serum apelin, IR, and CIMT in diabetic patients.<sup>[15,16]</sup> Thus, we performed the present case-control study to assess the level of serum apelin in T2DM patients and its relation to IR and CIMT.

## MATERIALS AND METHODS

This study was approved by the institutional review board of Faculty of Medicine for Girls, Al-Azhar University, Cairo (AFMG IRB, reference number: 202001091) and was obliged to the standards of the Declaration of Helsinki. Sixty patients (aged 40–60 years old) with T2DM, who were on oral hypoglycemic agents, were included. The diagnosis of T2DM was based on American Diabetes Association 2019 criteria (fasting plasma glucose  $\geq 126$  mg/dL, or 2-h plasma glucose  $\geq 200$  mg/dL during a 75-g oral glucose tolerance test, or random plasma glucose  $\geq 200$  mg/dL in a patient with classic symptoms of hyperglycemia or hyperglycemic crisis, or hemoglobin A1c  $\geq 6.5\%$ ).<sup>[17]</sup>

In addition, 30 age and sex-matched healthy volunteers were recruited as control group. Written informed consents were signed by all recruited participants. Patients with morbid obesity, familial dyslipidemia, organ failure, malignancies, thyroid disorder, and/or associated immunological

disorders were excluded. All eligible patients were recruited from the outpatient's endocrinology clinic of Al-Zahraa University Hospital through the period from June to December 2019.

The following data were collected from participants: demographic characteristics, anthropometric measures, lipid profile, blood glucose profile, HbA1c, fasting serum insulin, serum apelin, assessment of IR index using Homeostatic Model Assessment (HOMA-IR), and CIMT (done for patients only).

### Biochemical analysis

For biochemical investigations, 10 ml of venous blood was collected from every eligible patient after at least 8 h of fasting. The sample was divided into two ethylenediaminetetraacetic acid tubes; 7 ml for routine investigations and the rest of the sample was centrifuged at 1000 rpm for 15 min and stored at  $-20^{\circ}\text{C}$  until assayed. The enzymatic colorimetric methods were used for the measurement of serum lipids and blood glucose using a Hitachi Autoanalyzer 704 (Roche Diagnostics, Switzerland). The HbA1c was measured using Automated Glycohemoglobin Analyzer (Tosoh Bioscience's HLC-723GX<sup>®</sup>, Tosoh, India), while the serum insulin (INS) was measured by chemiluminescent immunoassay (Immulite2000, Siemens, Germany). The HOMA-IR was calculated as the following:  $\text{HOMA-IR} = \text{fasting insulin (IU/mL)} \times \text{plasma glucose (mg/dL)} / 405$ .<sup>[18]</sup>

The enzyme linked immunoassay (ELISA) technique was used to measure serum apelin (APLN) using the Human Apelin ELISA Kit (Cat No. E2014Hu, Bioassay Technology Laboratory Inc., Shanghai, China). The detection range of the kit is 7–1500 ng/dL. The kit is designed to target the C-terminus of the 77-aa apelin peptide; thus, the active forms of apelin, including apelin-36 and apelin-13, can be detected.

### Assessment of carotid intima-media thickness

High-resolution B-mode ultrasound (Philips infinity 70) with a high frequency 7.5–12 MHz linear probe of both carotids was done for all patients. An ultrasound consultant performed all ultrasound examinations and measurements. The CIMT was measured by the ultrasound physician. Intima Media Thickness (IMT) is defined by measuring two parallel echogenic lines, which refer to the lumen intima and the media adventitia interfaces. B-mode images of the distal right and left common carotid artery (CCA) were traced in real time at longitudinal axis. Middle segment posterior wall of each CCA was obtained as a source of IMT measurement. Mean of the left and right CIMT was calculated (MCIMT). The focal thickening was considered as a discrete plaque and locally thickened IMT of 1.5 mm was defined as carotid atherosclerosis.

**Statistical analysis**

We performed the statistical analysis using the SPSS software, version 22.0 (SPSS Inc., Chicago, Illinois, USA).  $P < 0.05$  was significant. The distribution of continuous variables was tested using Kolmogorov–Smirnov test. The quantitative data were expressed with mean  $\pm$  standard deviation or median (interquartile range) according to data normality, while the frequency was used to present categorical data. The difference between continuous variables was tested using Student’s *t*-test or Mann–Whitney U-test. Categorical variables were examined using the Chi-square or Fisher’s exact tests. Spearman correlation test was used to examine the correlation for variables that did not follow the normal distribution, and Pearson correlation was used for the normally distributed variables. Linear regression analysis was used to predict the outcome of categorical variables based on one or more predictor variables. Receiver operating characteristic (ROC curve) analysis was used to find out the overall predictivity of the parameter and to find out the best cutoff value with detection of sensitivity and specificity at this cutoff value.

**RESULTS**

T2DM group included 60 patients (30 females and 30 males; mean age  $48.3 \pm 9.7$  years), and the control group consisted of 30 healthy subjects (21 females and 9 males; mean age  $44.9 \pm 7.6$  years). The baseline characters of the cohort are presented in Table 1 including age, diseases duration (DD), systolic and diastolic blood pressures (SBP and DBP), anthropometric measurements, glycemic indices, Homeostatic Model Assessment (HOMA-IR), lipid profile, apelin, and CIMT. No differences were detected in terms of age, height, SBP, and low-density lipoprotein cholesterol (LDL) ( $P \geq 0.05$  for all). On the other hand, the T2DM group had statistically significant higher body weight (BW) ( $P = 0.004$ ), body mass index (BMI) ( $P < 0.001$ ), waist circumference (WC) ( $P < 0.001$ ), DBP ( $P = 0.002$ ), total cholesterol (CHO) ( $P = 0.018$ ), triglyceride (TG) ( $P = 0.003$ ), and significant lower high-density lipoprotein cholesterol (HDL) ( $P < 0.001$ ). HOMA-IR was significantly higher in the patients’ group compared with healthy controls ( $P < 0.001$ ).

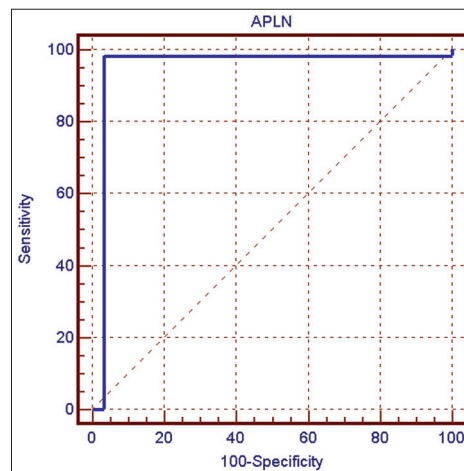
In terms of the primary outcome of the present study, we found that patients’ group had statistically significant higher serum apelin levels than healthy controls ( $407.96 \pm 291.07$  versus  $83.32 \pm 10.55$  ng/dL, respectively;  $P < 0.001$ ) [Table 1]. ROC curve was used to define the best cutoff value of apelin which was  $>96$  ng/dL, with sensitivity of 98.3%, specificity of 96.7%, positive predictive value of 98.1%, negative predictive value of 96.5%, and diagnostic accuracy of 95.1% [Figure 1].

The correlation analysis showed that the serum apelin level correlated positively with age, SBP, BW, WC, FBG, postprandial blood glucose (PPBG), HbA1c, serum insulin (INS), and HOMA-IR ( $P < 0.05$  for all) [Table 2]. Regarding the CIMT, serum apelin correlated positively with the MCIMT ( $r = 0.296, P = 0.022$ ) [Table 2]. The linear regression analysis showed that SBP, PPBG, HOMA-IR,

**Table 1: Comparison between patients and control according to baseline data**

| Variables                | Mean $\pm$ SD       |                    | P       |
|--------------------------|---------------------|--------------------|---------|
|                          | Patients (n=60)     | Control (n=30)     |         |
| Age (years)              | 48.30 $\pm$ 9.67    | 44.90 $\pm$ 7.59   | 0.096   |
| DD (years)               | 7.35 $\pm$ 4.65     |                    |         |
| SBP (mmHg)               | 124.83 $\pm$ 14.20  | 119.67 $\pm$ 7.65  | 0.066   |
| DBP (mmHg)               | 81.17 $\pm$ 7.39    | 76.33 $\pm$ 4.90   | 0.002*  |
| BW (kg)                  | 86.63 $\pm$ 13.37   | 78.03 $\pm$ 11.85  | 0.004*  |
| Height (cm)              | 163.60 $\pm$ 9.20   | 166.67 $\pm$ 8.13  | 0.125   |
| BMI (kg/m <sup>2</sup> ) | 32.36 $\pm$ 4.27    | 28.05 $\pm$ 3.70   | <0.001* |
| WC (cm)                  | 108.58 $\pm$ 9.68   | 98.97 $\pm$ 9.53   | <0.001* |
| FBG (mg/dL)              | 165.40 $\pm$ 54.78  | 85.20 $\pm$ 10.20  | <0.001* |
| PPBG (mg/dL)             | 218.63 $\pm$ 76.10  | 118.47 $\pm$ 16.33 | <0.001* |
| HbA1c (%)                | 8.35 $\pm$ 1.76     | 5.07 $\pm$ 0.37    | <0.001* |
| CHO (mg/dL)              | 195.27 $\pm$ 44.64  | 173.43 $\pm$ 30.72 | 0.018*  |
| TG (mg/dL)               | 167.70 $\pm$ 69.90  | 127.17 $\pm$ 21.61 | 0.003*  |
| HDL (mg/dL)              | 37.82 $\pm$ 6.40    | 43.30 $\pm$ 3.58   | <0.001* |
| LDL (mg/dL)              | 122.98 $\pm$ 39.99  | 107.80 $\pm$ 30.36 | 0.071   |
| INS (IU/mL)              | 17.88 $\pm$ 7.27    | 7.81 $\pm$ 1.73    | <0.001* |
| HOMA-IR                  | 7.50 $\pm$ 4.49     | 1.65 $\pm$ 0.45    | <0.001* |
| Apelin (ng/dl)           | 407.96 $\pm$ 291.07 | 83.32 $\pm$ 10.55  | <0.001* |
| Rt CIMT (mm)             | 0.87 $\pm$ 0.24     |                    |         |
| Lt CIMT (mm)             | 0.87 $\pm$ 0.24     |                    |         |
| MCIMT                    | 0.87 $\pm$ 0.22     |                    |         |

\*Significant ( $P < 0.05$ ). DD=Disease duration; SBP=Systolic blood pressure; DBP=Diastolic blood pressure; BW=Body weight; BMI=Body mass index; WC=Waist circumference; FBG=Fasting blood glucose; PPBG=Postprandial blood glucose; HbA1c=Glycosylated hemoglobin; CHO=Total cholesterol; TG=Triglycerides; HDL=High-density lipoprotein; LDL=Low-density lipoprotein; INS=Fasting serum insulin; HOMA-IR=Homeostasis Model Assessment of Insulin Resistance; Rt. CIMT=Right carotid intima-media thickness; Lt. CIMT=Left carotid intima-media thickness; MCIMT=Mean carotid intima-media thickness; SD=Standard deviation



**Figure 1:** The receiver operating characteristic curve for diagnostic accuracy of serum apelin

and MCIMT were independent predictors of serum apelin [Table 3].

## DISCUSSION

The current published literature shows controversies regarding serum apelin, IR, and CIMT in patients with diabetes.<sup>[19]</sup> Thus, we performed the present case-control

study to assess the level of serum apelin in T2DM patients and its relation to IR and CIMT. Our analysis showed that patients with T2DM had statistically significant higher serum apelin concentration than healthy subjects. Moreover, in diabetic patients, serum apelin level correlated positively with IR and CIMT.

Apelin, a widely distributed adipocytokine in different body organs, plays a critical role in regulating various physiological processes such as energy metabolism, blood pressure control, cardiac functions, hemostasis, and hormonal-mediated stress response.<sup>[14]</sup> Alongside its physiological properties, a cumulative body of evidence suggests that serum apelin is significantly implicated in the pathogenesis of various disorders including components of metabolic syndromes;<sup>[20]</sup> previous reports suggested altered levels of serum apelin in patients with obesity<sup>[21]</sup> and diabetes.<sup>[22]</sup> In addition, high serum apelin level was linked to impaired insulin sensitivity and IR.<sup>[23]</sup> Thus, it was suggested that serum apelin can be used for the prediction of development of diabetes and its progression; nonetheless, the current literature is equivocal regarding the exact role of apelin in T2DM. In the present study, we found that patients with T2DM had statistically significant higher serum apelin concentration than the healthy control group. In addition, the serum apelin exhibited a strong positive correlation with IR. In concordance with our findings, Habchi *et al.*,<sup>[23]</sup> and Zheng *et al.*,<sup>[24]</sup> demonstrated a significant increase in the apelin-12 level in diabetic patients. Other reports showed similar findings.<sup>[25,26]</sup> Moreover, recent reports demonstrated that serum apelin correlated significantly with the degree of T2DM-related complications such as nephropathy and neuropathy.<sup>[27]</sup> In terms of IR, El Wakeel *et al.*,<sup>[20]</sup> reported a positive correlation between serum apelin and HOMA-IR in diabetic children. These findings were similar to other reports in Egyptian and Chinese population.<sup>[28,29]</sup> Owing to its potential role in diabetes development and IR, recent

**Table 2: Correlation between apelin with all parameters, using Pearson correlation coefficient in patients' group**

| Parameters               | Apelin (ng/dl) |          |
|--------------------------|----------------|----------|
|                          | r              | P        |
| Age (years)              | 0.285          | 0.027*   |
| DD (years)               | 0.115          | 0.310    |
| SBP (mmHg)               | -0.338         | 0.048*   |
| DBP (mmHg)               | -0.032         | 0.810    |
| BW (kg)                  | 0.254          | 0.049*   |
| Height (cm)              | 0.064          | 0.627    |
| BMI (kg/m <sup>2</sup> ) | 0.219          | 0.092    |
| WC (cm)                  | 0.276          | 0.033*   |
| FBG (mg/dL)              | 0.629          | <0.001** |
| PPBG (mg/dL)             | 0.613          | <0.001** |
| HbA1c (%)                | 0.543          | <0.001** |
| CHO (mg/dL)              | 0.028          | 0.829    |
| TG (mg/dL)               | -0.008         | 0.952    |
| HDL (mg/dL)              | 0.219          | 0.093    |
| LDL (mg/dL)              | -0.034         | 0.794    |
| INS (IU/mL)              | 0.786          | <0.001** |
| HOMA-IR                  | 0.908          | <0.001** |
| Rt. CIMT (mm)            | 0.175          | 0.181    |
| Lt. CIMT (mm)            | 0.296          | 0.022*   |
| MCIMT (mm)               | 0.257          | 0.048*   |

\*Significant (P<0.05). DD=Disease duration; SBP=Systolic blood pressure; DBP=Diastolic blood pressure; BW=Body weight; BMI=Body mass index; WC=Waist circumference; FBG=Fasting blood glucose; PPBG=Postprandial blood glucose; HbA1c=Glycosylated hemoglobin; CHO=Total cholesterol; TG=Triglycerides; HDL=High-density lipoprotein; LDL=Low-density lipoprotein; INS=Fasting serum insulin; HOMA-IR=Homeostasis Model Assessment of Insulin Resistance; Rt. CIMT=Right carotid intima-media thickness; Lt. CIMT=Left carotid intima-media thickness; MCIMT=Mean carotid intima-media thickness

**Table 3: Multiple linear regression analysis using apelin as dependent variable in diabetic group to identify the most predictor parameter**

| Parameters               | Unstandardized coefficients |        | Standardized coefficients (β) | P      | 95% CI (lower-upper) |
|--------------------------|-----------------------------|--------|-------------------------------|--------|----------------------|
|                          | B                           | SE     |                               |        |                      |
| Age (years)              | 2.253                       | 1.948  | 0.075                         | 0.253  | -1.666-6.172         |
| SBP (mmHg)               | -1.177                      | 1.334  | -0.086                        | 0.019* | -2.860-2.506         |
| BW (kg)                  | -0.447                      | 2.372  | -0.021                        | 0.851  | -5.219-4.324         |
| BMI (kg/m <sup>2</sup> ) | 5.318                       | 5.880  | 0.078                         | 0.073  | -6.512-17.147        |
| WC (cm)                  | 0.235                       | 3.016  | 0.008                         | 0.938  | -5.832-6.302         |
| FBG (mg/dL)              | 1.034                       | 1.075  | 0.195                         | 0.341  | -1.129-3.196         |
| PPBG (mg/dL)             | 1.582                       | 0.513  | 0.152                         | 0.026* | -0.450-1.614         |
| HbA1c (%)                | 3.537                       | 14.936 | 0.021                         | 0.814  | -26.511-33.585       |
| INS (IU/mL)              | 19.462                      | 8.266  | 0.486                         | 0.023* | 2.834-36.091         |
| HOMA                     | 15.297                      | 18.120 | 0.236                         | 0.024* | -21.156-51.750       |
| MCIMT (mm)               | -6.354                      | 1.625  | -0.005                        | 0.013* | -91.854-39.146       |

\*Significant (P<0.05). SBP=Systolic blood pressure; BW=Body weight; BMI=Body mass index; WC=Waist circumference; FBG=Fasting blood glucose; PPBG=Postprandial blood glucose; HbA1c=Glycosylated hemoglobin; INS=Fasting serum insulin; HOMA-IR=Homeostasis Model Assessment of Insulin Resistance; MCIMT=Mean carotid intima-media thickness; SE=Standard error; CI=Confidence interval

reports have investigated the therapeutic potentials of apelin in the setting of T2DM.<sup>[30,31]</sup>

In patients with greater CIMT, left ventricular hypertrophy, and systolic left ventricular dysfunction disease, the level of apelin is also altered, which indicates that apelin peptides could be successfully used as a biomarker of CVDs.<sup>[14]</sup> In the present study, we found that the serum apelin level correlated positively with IR and CIMT. Likewise, a recent case-control study on T1DM patients demonstrated a positive correlation between serum apelin and CIMT.<sup>[32]</sup> Similar findings were reported in patients with primary hypertension.<sup>[33]</sup> On the other hand, Büyükbakkal *et al.*<sup>[34]</sup> described that there was no association between apelin and CIMT in patients on dialysis, while Yasar *et al.*<sup>[35]</sup> found that apelin level was negatively correlated with CIMT in patients with subclinical hypothyroidism.

We acknowledge that the present study has some limitations. The sample size of our cohort was relatively low and was from a single-center only; these factors may affect the generalizability of our findings. In addition, the lack of long-term follow-up did not allow us to correlate between serum apelin and long-term sequels of diabetes.

## CONCLUSION

Our results add an additional evidence that apelin probably plays a role in T2DM and IR pathophysiology. In addition, our results highlight that serum apelin may be correlated with the degree of carotid atherosclerosis and hence could be used as a prognostic biomarker for atherosclerotic cardiovascular disease.

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Nil.

## Conflicts of interest

There are no conflicts of interest.

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