Connection between the Plasma Level of Apelin and Diabetic Nephropathy in Type 2 Diabetic Patients. A Case Control Study

Mona Yousry Helmy, Nehal Hamdy[1], Nagwa Abd El Ghaffar[2]

Lecturer of Internal Medicine, [1]Professor of Internal Medicine, Faculty of Medicine, Cairo University, Cairo, [2]Professor of Clinical and Chemical Pathology, National Research Center, Giza, Egypt

Abstract

Background: Apelin is an adipokine that may have an advantageous role in the prediction of early diabetic nephropathy. A few studies on apelin in diabetes have been performed and this research was performed to establish the connection between the apelinergic system and diabetic nephropathy. **Materials and Methods:** The research included 60 patients with type 2 diabetes mellitus (T2DM) who were equally divided into Group-I (diabetic nephropathy) and Group-II (non-diabetic nephropathy), and 30 healthy subjects in the control group (Group-III). Body mass index (BMI) and waist circumference were calculated. FBG, 2 h–PPG, HbA1c, fasting lipids, urea, creatinine, eGFR, urine analysis, A/C ratio, and apelin levels were assessed. **Results:** A statistically significant between-group difference in plasma apelin levels was found ($P < 0.001$). Apelin was the highest in Group-I than in Group-II relative to Group-III (325.79 ± 59.42 pg/mL, 162.83 ± 29.88 pg/mL, and 77.43 ± 8.44 pg/mL, respectively). Among diabetic patients, plasma apelin had a significantly positive correlation with disease duration ($r = 0.612$), SBP ($r = 0.427$), DBP ($r = 0.466$), weight ($r = 0.372$), and height ($r = 0.372$), FBG ($r = 0.684$), 2 h-PPG ($r = 0.744$), HbA1C ($r = 0.890$), total (T)-cholesterol ($r = 0.316$), low density lipoprotein (LDL)-C ($r = 0.397$), urea ($r = 0.575$), and creatinine ($r = 0.591$). A significantly negative correlation was observed between plasma apelin and HDL-C ($r = –0.303$), and eGFR ($r = –0.566$). **Conclusion:** Apelin levels in diabetics were elevated in the case of nephropathy, impaired glucose tolerance, and dyslipidemia. This supports the relationship between the apelinergic system and diabetic nephropathy.

Keywords: Apelin level, diabetic nephropathy, type 2 diabetes mellitus

INTRODUCTION

Diabetic nephropathy (DN) is demarcated by chronic albuminuria and a gradual decrease in renal function. The clinical course of kidney disease in diabetics became diverse and the diabetic kidney disease (DKD) term currently refers to diabetics who have either albuminuria or a decrease in renal function.[1]

Apelin is an adipokine that suppresses insulin secretion and glucose metabolism.[2] Apelin in diabetics mediates glomerular permeability and glomerular endothelial cell proliferation, influencing DN pathogenesis.[3,4]

The authors in this work aimed to assess the connection between plasma apelin levels and DN in Egyptian type 2 diabetes mellitus (T2DM) patients as this connection was not well established previously.

MATERIALS AND METHODS

Ninety participants were involved in this case–control study, aged 20 to 65 years. They were divided into 30 control participants and 60 patients with T2DM. Patients were gathered from the endocrinology outpatient clinic, Kasr El Ainy, Cairo University. The study took place between November 2019 and January 2021. The protocol of this work was accepted by the Research Ethics Committee of the Faculty of Medicine, Cairo University with ID. N-4-2020 and was conducted

Address for correspondence: Dr. Mona Yousry Helmy,
Lecturer of internal medicine, Faculty of Medicine,
Cairo University, Cairo,Egypt.
E-mail: Mona55508@yahoo.com

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in accordance with the principles outlined in the Helsinki declaration. It was registered at clinicaltrial.gov with an ID. NCT04380584. A signed informed consent was secured from the participants.

The participants were assigned to three groups: (Group I) 30 patients with T2DM and nephropathy, (Group II) 30 patients with T2DM without nephropathy and (Group III) 30 non-T2DM patients as the control group.

DN is clinically diagnosed by a persistently high urinary albumin-to-creatinine (A/C) ratio ≥30 mg/g, which ranges from microalbuminuria (A/C ratio of 30 to 300 mg/g) to macroalbuminuria (A/C ratio >300 mg/g), and/or a prolonged decrease in eGFR <60 mL/min per 1.73 m^2.[5]

Criteria of exclusion involved nephropathic patients due to factors other than diabetes. Moreover, patients with hepatic, intrinsic renal or coronary artery disease, DN, and retinopathy and hypertensive patients on angiotensin receptor blockers (ARBS) or angiotensin converting enzyme inhibitor (ACEI) medications are excluded from the research.

A complete history of the medical condition was obtained from every participant, emphasizing the age, gender, T2DM duration, and the presence of DN. A thorough examination comprising measuring the blood pressure, weight, height, body mass index (BMI; kg/m^2), and waist circumference was assessed.

The investigated laboratory parameters comprised the fasting glucose in the blood (FBG), 2 h-postprandial blood glucose (2 h-PPG), glycosylated hemoglobin (HbA1c), fasting lipids (triglycerides [TAG], total cholesterol [TC], high-density lipoprotein cholesterol [HDL-C], low-density lipoprotein cholesterol [LDL-C]), urea, creatinine, urine analysis, A/C, eGFR, and apelin levels.

The clinical pathologist that analyzed the samples was blinded to the source of the sample by specific code.

Sampling

Five milliliters of blood was withdrawn by a venipuncture and divided into three parts: The first 2 mL of blood poured into an EDTA containing tube for evaluation of HbA1C by a cation exchange resin. The second 3 mL of blood was kept in plastic serum tubes and the specimens were left for clotting at room temperature. The serum was separated from the cells by centrifugation at 3000 ×g for 5 min. The separated serum was stored at –20°C until analysis. The third part was collected in a tube having EDTA and centrifuged for 15 min at 1000 ×g at 2 to 8°C within 30 min of the sampling. The supernatant was gathered and stored at –80°C for apelin determination.

Serum urea, creatinine, TC, TAG, LDL, and HDL were determined on a Dimension RxL Max analyzer (Siemens Healthcare GmbH - Henkestr; 127, 91052 Erlangen, Germany) by colorimetry.

Plasma apelin was evaluated using a competitive ELISA kit delivered from Elabscience Biotechnology Inc. (1 Shizishan Street, Hongshan District, Wuhan, Hubei, China).[6]

The eGFR was estimated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation: eGFR = 141 × min $(S_{cr}/\kappa, 1)^\alpha$ × max $(S_{cr}/\kappa, 1)^{-1.209}$ × 0.993^{Age} × 1.018 [if female] × 1.159 [if African American],

Where

S_{cr} is serum creatinine in mg/dL,

κ is 0.7 for females and 0.9 for males,

α is –0.329 for females and -0.411 for males,

min indicates the minimum of S_{cr}/κ or 1, and

max indicates the maximum of S_{cr}/κ or 1.

The analysis of urine was conducted to identify the presence of active urinary sediments (proteinuria, pyuria, red blood cells [RBCs] or RBCs casts, granular cast).

The concentration of albumin in urine was assessed with a Minineph micro-albumin kit using nephlometry (Minineph-nephelometer [AD200]; The Binding Site, Birmingham, UK).[7]

Creatinine in urine was measured by colorimetry using the Dimension RxL Max analyzer and the urinary albumin/creatinine ratio was utilized to describe microalbuminuria.

Outcomes

This work investigated the association of plasma apelin levels to DN in T2DM patients as the primary outcome. Secondary outcomes investigated the relationship between plasma apelin, glycemic status, and metabolic factors in T2DM patients.

Sample size

The G*power software 3.1.9.4. was used to calculate the sample size. The analysis was conducted on the plasma apelin levels in T2DM patients with and without nephropathy and controls as the primary outcome. The previous studies reported an apelin level of 433 ± 221.031 pg/dL, 665.967 ± 110.991 pg/dL, and 502.95 ± 201 pg/dL for T2DM patients with nephropathic and non-nephropathic patients and control, respectively.[8] Taking a study power of 95% and an α error of 0.05, 30 patients were computed for every group.

Statistical analysis

The information was coded and submitted utilizing version 26 of the Social Sciences Statistical Package (SPSS) (IBM Corp., Armonk, NY, USA). The means and standard deviations expressed the quantitative variables. The categorical data are expressed as numbers and percentages. The analysis of inter-group comparisons used the analysis of variance (ANOVA) test. For analyzing multiple comparisons of normally distributed quantitative statistics, the post hoc test was utilized. The quantitative statistics that were not normally

distributed were analyzed by the nonparametric Kruskal–Wallis and Mann–Whitney tests.[9] The Chi-square test was utilized to distinguish categorical statistics. Instead, the exact test was applied if the expected frequency was less than 5.[10] Spearman correlation coefficient was utilized for studying the correlations between quantitative statistics.[11] The receiver operating characteristics (ROC) curve was created with the area under the curve (AUC) to identify the favored cutoff value of apelin for the determination of DN. Linear regression analysis was performed to find the independent forecasters of apelin.[12] A P value below 0.05 was needed to assume the statistical significance.

Data availability

The data associated with the study are not publicly available but are available from the corresponding author on reasonable request.

RESULTS

One hundred ten participants were involved in this research, whereas only 90 completed the analysis [Figure 1]. The demographic data showed a statistically significant between-group difference regarding the gender ($P = 0.016$), age, and disease duration ($P < 0.001$) [Table 1].

Patients with DN included 24 (80%) patients were on insulin treatment, 2 (6.7%) of them were receiving insulin + metformin and 4 (13.3%) were on oral hypoglycemics mainly metformin + gliclazide. Patients with T2DM patients without nephropathy included seven (23.3%) patients receiving insulin, five (16.7%) receiving insulin + metformin, and 19 (60%) receiving oral hypoglycemics mainly metformin + glimepride + gliclazide + glibeclamide.

Diabetic nephropathy cases included 18 (60%) hypertensive patients and 12 (40%) non-hypertensive patients. Diabetic patients without nephropathy included 6 (20%) hypertensive patients and 24 (80%) non-hypertensive patients. A highly significant inter-group difference existed as regard to SBP and DBP ($P < 0.001$) [Table 1]. However, the correlation between apelin level and blood pressure in different groups was weak, suggesting that apelin can predict DN independent of blood pressure.

The contrast between the studied groups found significant difference regarding weight, height, BMI, and WC ($P < 0.05$) [Table 1]. The laboratory findings showed a significant difference between the three groups according to their FBG, 2h-PPG, HbA1c, lipid profile, creatinine, urea, and eGFR ($P < 0.05$) [Table 1].

The mean level of plasma apelin in the DN group was significantly higher compared with T2DM without nephropathy and control groups ($P < 0.001$) [Table 1].
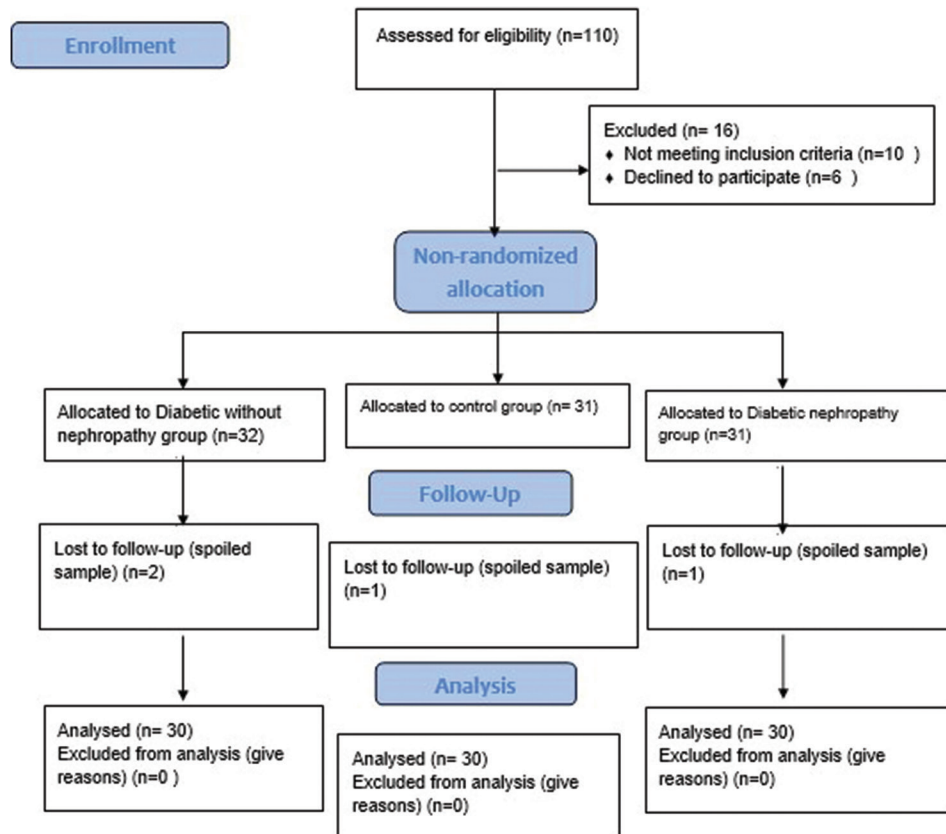

Figure 1: Patients' flow chart

Table 1: Comparison of the parameters among the three studied groups

Variable	Group-I		Group-II		Group-III		P
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	
Age (years)	53.10	9.08	52.10	10.07	36.20	8.30	<0.001*
Disease duration (years)	12.47	4.58	5.77	4.35	.	.	<0.001*
SBP (mmHg)	141.67	21.19	124.50	9.59	120.33	1.83	<0.001*
DBP (mmHg)	87.67	7.28	80.67	3.88	80.33	1.83	<0.001*
Weight (kg/m²)	88.20	17.16	78.67	15.70	74.63	15.39	0.005*
Height (cm)	165.47	6.02	157.67	8.42	162.27	8.20	0.001*
BMI (kg/m²)	32.41	7.04	31.55	6.24	28.52	6.42	0.061*
WC (cm)	107.20	14.17	104.80	14.42	95.83	11.51	0.004*
FBG (mg/dL)	191.50	68.22	135.77	37.48	95.53	7.77	<0.001*
2 h-PPG (mg/dl)	239.93	65.26	169.43	39.83	161.80	12.54	<0.001*
HbA1c (%)	7.81	1.16	6.49	0.56	5.01	0.27	<0.001*
T-cholesterol (mg/dL)	215.80	31.24	193.63	42.13	192.37	23.60	0.011*
Triglycerides (mg/dL)	213.33	60.39	201.83	55.50	160.47	37.93	<0.001*
HDL-C (mg/dL)	40.30	10.98	45.10	7.49	51.50	9.28	<0.001*
LDL-C (mg/dL)	123.60	15.05	115.60	12.27	106.50	9.19	<0.001*
Creatinine (mg/dL)	4.45	3.09	0.79	0.15	0.76	0.14	<0.001*
Urea (mg/dL)	100.53	59.07	33.10	6.10	31.37	5.68	<0.001*
eGFR (1.73 mL/min/m²)	44.34	50.39	104.84	10.37	122.68	14.34	<0.001*
Apelin (pg/mL)	325.79	59.42	162.83	29.88	77.43	8.44	<0.001*
	Count	**%**	**Count**	**%**	**Count**	**%**	**P**
Sex							
M	12	40.0%	4	13.3%	4	13.3%	0.016
F	18	60.0%	26	86.7%	26	86.7%	
Retinopathy	10	33.3%	3	10%	0	0%	<0.001
Cardiovascular disease	9	30%	5	16.6%	0	0%	0.01

Values are expressed as numbers, percentages (%), means±standard deviations (SD), *P<0.05 is significant, P<0.001 is highly significant

The incidence of DN was significantly higher in diabetic nephropathy patients than patients in the other two groups ($P < 0.001$). A significant difference existed between the control and the two diabetic groups as regard the incidence of cardiovascular disease [Table 1].

The mean albumin/creatinine ratio in DN cases was 1357.67 ± 938.54 mg. Urine analysis of these patients found 7 (23.3%) had albumin +4, 8 (26.7%) had albumin +3, 8 (26.7%) had albumin +2, and 7 (23.3%) had albumin +3.

Post-hoc pair-wise comparisons between DN patients and diabetic patients without nephropathy found a significant difference regarding height, SBP, DBP, FBG, 2h-PPG, HbA1C, total cholesterol, LDL-C, creatinine, urea, e-GFR, and apelin [Tables 2 and 3].

A significant difference was found regarding age, SBP, DBP, waist circumference, FBG, 2h-PPG, HbA1C, total cholesterol, TAG, HDL-C, LDL-C, creatinine, urea, e-GFR, and apelin between diabetic patients without nephropathy and controls [Tables 2 and 3].

Gender-based comparison for apelin level showed an insignificant difference between males and females within the groups and a highly significant difference between groups as regard apelin levels in males and females of each group ($P < 0.001$) [Table 4].

By generating a ROC curve, an AUC of 1.000 and cut-off value of 230 was calculated. The sensitivity and specificity would be 100% with ($P < 0.001$). These findings emphasize the discriminated ability of plasma apelin as a biomarker for DN in T2DM [Table 5].

Among DN patients, a highly significantly positive correlation was reported between plasma apelin and FBG, 2 h-PPBG, HbA1C, and e-GFR ($P < 0.001$). Although a significantly negative correlation was reported between plasma apelin and creatinine ($P < 0.001$), urea, and age ($P = 0.014$, $P = 0.027$, respectively) [Table 6].

Among diabetic patients without nephropathy, a significantly positive correlation was reported between plasma apelin and disease duration, BMI, FBG, 2 h-PPBG, HbA1C, total cholesterol, LDL-C, urea, and creatinine, and a significantly negative correlation between plasma apelin and HDL-C [Table 6].

Among diabetic cases, a significantly positive correlation was reported between plasma apelin and disease duration, SBP, DBP, weight, height, FBG, 2 h-PPBG, HbA1C, LDL-C, T cholesterol, urea, and creatinine. A highly significant positive correlation was found between apelin level and proteinuria in DN patients ($P < 0.001$), and a significantly negative correlation was found between plasma apelin and HDL-C and eGFR [Table 7].

Table 2: *Post hoc* pair-wise comparisons of clinical data (between each two groups)

Variable	Group I	Group II	Group III
Age (years)			
Diabetic nephropathy		1.000	<0.001
DM without nephropathy	1.000		<0.001
control	<0.001	<0.001	
SBP (mmHg)			
Diabetic nephropathy		<0.001	<0.001
DM without nephropathy	<0.001		0.702
control	<0.001	0.702	
DBP (mmHg)			
Diabetic nephropathy		<0.001	<0.001
DM without nephropathy	<0.001		1.000
control	<0.001	1.000	
Weight (kg/m^2)			
Diabetic nephropathy		0.073	0.005
DM without nephropathy	0.073		1.000
control	0.005	1.000	
Height (cm)			
Diabetic nephropathy		<0.001	0.323
DM without nephropathy	<0.001		0.065
control	0.323	0.065	
WC (cm)			
Diabetic nephropathy		1.000	0.005
DM without nephropathy	1.000		0.034
control	0.005	0.034	

*$P<0.05$ is significant, $P<0.001$ is highly significant

Table 3: *Post hoc* pair-wise comparisons of laboratory data

Variable	Group I	Group II	Group III
FBG (mg/dL)			
Diabetic nephropathy		0.031	
DM without nephropathy			<0.001
Control	<0.001		
2 h-PPG (mg/dL)			
Diabetic nephropathy		<0.001	<0.001
DM without nephropathy	<0.001		1.000
Control	<0.001	1.000	
HbA1c (%)			
Diabetic nephropathy		<0.001	<0.001
DM without nephropathy	<0.001		<0.001
Control	<0.001	<0.001	
T-cholesterol (mg/dL)			
Diabetic nephropathy		0.034	0.023
DM without nephropathy	0.034		1.000
Control	0.023	1.000	
Triglycerides (mg/dL)			
Diabetic nephropathy		1.000	0.001
DM without nephropathy	1.000		0.009
Control	0.001	0.009	
HDL-C (mg/dL)			
Diabetic nephropathy		0.150	<0.001
DM without nephropathy	0.150		0.029
Control	<0.001	0.029	
LDL-C (mg/dL)			
Diabetic nephropathy		0.043	<0.001
DM without nephropathy	0.043		0.017
Control	<0.001	0.017	
Creatinine (mg/dL)			
Diabetic nephropathy			<0.001
DM without nephropathy	<0.001		
Control		1.000	
Urea (mg/dL)			
Diabetic nephropathy			<0.001
DM without nephropathy	<0.001		
Control		1.000	
GFR (1.73 ml/min/m^2)			
Diabetic nephropathy		<0.001	
DM without nephropathy			0.008
Control	<0.001		
Apelin (pg/mL)			
Diabetic nephropathy		<0.001	<0.001
DM without nephropathy	<0.001		1.000
Control	<0.001	1.000	

*$P<0.05$ is significant, $P<0.001$ is highly significant

Multivariate regression analysis showed that HbA1C, creatinine, height, total cholesterol, and HDL-C are independent predictors of plasma apelin levels [Table 8].

DISCUSSION

This case–control research was planned to assess the diagnostic value of plasma apelin as a DN predictor in patients with T2DM. Although this targeted connection allows early management and slows down the loss of renal function in diabetics, only a few previous studies attempted to investigate this relationship. We hypothesized that as the disease progresses to DN, plasma apelin levels would increase. Our results supported this hypothesis that plasma apelin levels were greater in patients with DN than in the controls and apelin levels were significantly higher in DN patients than in those with diabetics without nephropathy. A positive significant correlation was detected between plasma apelin and disease duration, SBP, DBP, weight, and height, FBG, 2 h-PPBG, HbA1C, LDL-C, T-cholesterol, urea, and creatinine. A positive significant correlation was found between apelin level and proteinuria in DN patients. A negative significant correlation was reported between plasma apelin and HDL-C and eGFR in studied patients. Also, the ROC curve emphasized the utility of the discriminated ability of plasm apelin as a predictor for DN in T2DM patients with an AUC of 1.000 and a cut-off value of 230 with a sensitivity of 100%, specificity of 100%, and a

P value <0.001. The multivariate linear regression found that HbA1C, creatinine, height, T-cholesterol, and HDL-C were independent predictors of plasma apelin levels.

This investigation has shown elevated apelin levels in patients with T2DM than in control patients. Apelin levels were significantly higher in the diabetics with nephropathy group than in the group with no nephropathy.

Concurring with our findings, prior studies done by El-Hassan, *et al.*[13] and Zhang *et al.*[4] reported that the elevated apelin levels in DN subjects were associated with increased glomerular permeability in the earlier DN phases. This can be assigned to the association between apelin, insulin resistance pathogenesis, and T2DM. Also, apelin has an impact on the advancement of DN through facilitating abnormal vessel proliferation in diabetic glomeruli. Therefore, apelin may be an important risk factor for glomerular angiogenesis that mediates DN pathogenesis. On the contrary, Yavuz *et al.*[14] stated that in terms of the apelin levels, there were no significant variations between diabetics and non-diabetics. However, it was conducted only on patients with chronic kidney disease (CKD) involving 60 DN patients.

Our findings showed that the plasma apelin, correlated significantly and positively with urea and creatinine in studied diabetic cases. This was agreed by Dawood *et al.*'s study.[15] This is supposed to be that apelin in animals increased the angiogenesis, permeability of glomeruli and albuminuria, resulting in DN.[4] However, when the diabetics were subdivided, there was a negative correlation between plasma apelin and urea and creatinine in DN patients, and a positive correlation between plasma apelin and urea and creatinine in diabetics without nephropathy. In addition, a non-significant correlation was found between apelin and urine A/C ratio in DN patients. This may be due to the small sample size. Also, these data implied that under physiological or pathological circumstances, there may be an inverse relationship between apelin and DN.[16] On the contrary, Zhang *et al.*[4] and Yonem *et al.*[17] stated that apelin levels and urinary albumin/creatinine ratio had a positive correlation in DN.

In the present investigation, a significantly positive correlation was observed between apelin level and FBG, 2 h-PPBG, and HbA1C in diabetics with and with no nephropathy. El-Hassan *et al.*,[13] agreed with this correlation in their study that stated a significantly positive correlation between apelin level and

Table 4: Gender-based comparison for apelin level

	Mean±SD			P (between groups)
	Group I	Group II	Group III	
Males	330.7±36.6	160.6±28.6	77.9±6.4	<0.001
Females	322.5±58.2	163.2±30.6	77.4±8.8	
P (within group)	0.8	0.9	0.99	

*P<0.05 is significant, P<0.001 is highly significant

Table 5: ROC curve for detection of diabetic nephropathy using plasma apelin

Area under the curve	P	95% Confidence interval				
		Lower bound	Upper bound	Cut-off	Sensitivity %	Specificity %
1.000	<0.001	1.000	1.000	230	100	100

Table 6: Correlation of apelin and each group parameters

Variable	Apelin					
	Group-I		Group-II		Group-III	
	Correlation coefficient	P	Correlation coefficient	P	Correlation coefficient	P
Age (years)	-0.403-	0.027	0.072	0.705	-0.015-	0.936
Disease duration (years)	-0.028-	0.885	0.440	0.015		
SBP (mmHg)	-0.141-	0.457	0.347	0.060	0.086	0.652
DBP (mmHg)	0.002	0.990	0.022	0.908	0.086	0.652
Weight (kg/m²)	0.143	0.451	0.355	0.054	-0.086-	0.650
Height (cm)	0.014	0.941	-0.045-	0.813	0.231	0.220
BMI (kg/m²)	0.113	0.552	0.390	0.033	-0.185-	0.328
WC (cm)	0.122	0.520	0.318	0.086	-0.189-	0.316
FBG (mg/dl)	0.753	< 0.001	0.679	< 0.001	-0.168-	0.376
2 h-PPG (mg/dL)	0.727	< 0.001	0.549	0.002	0.126	0.508
HbA1c (%)	0.911	< 0.001	0.994	< 0.001	-0.137-	0.472
T-cholesterol (mg/dL)	-0.215-	0.254	0.427	0.019	-0.074-	0.698
Triglycerides (mg/dL)	0.168	0.376	0.275	0.141	-0.109-	0.565
HDL-C (mg/dL)	-0.057-	0.763	-0.405-	0.026	0.112	0.554
LDL-C (mg/dL)	0.010	0.960	0.548	0.002	-0.267-	0.154
Creatinine (mg/dL)	-0.655-	< 0.001	0.369	0.045	-0.079-	0.680
Urea (mg/dl)	-0.443-	0.014	0.388	0.034	-0.102-	0.592
eGFR (1.73 mL/min/m²)	0.685	< 0.001	-0.201-	0.287	-0.037-	0.844
A/C ratio (mg)	-0.238-	0.206	.	.		

*P<0.05 is significant, P<0.001 is highly significant

HbA1c in diabetics with and without complications as DN and PN. This correlation confirms the association between apelin levels and the glycemic balance, representing, insulin resistance in T2DM patients. As insulin controls apelin secretion, the apelin level will rise in T2DM patients with insulin resistance. In addition, direct administration of apelin causes increased sensitivity of insulin, uptake of glucose peripherally, and decreased hyperinsulinemia. This supports the view of apelin's role in reducing insulin resistance and enhancing secretion of insulin.[18]

Although BMI mean in DN patients was higher than in DM patients without nephropathy. Apelin and BMI correlation, in this study, was significantly positive among T2DM patients without nephropathy but not with DN patients. This is supported by the trials of Xu *et al*.,[18] Soriguer *et al*.,[19] Daviaud *et al*.[20], and Cavallo *et al*.[21] However, these studies

had conflicting results concerning the assessment of relation between APLN and obesity.

Apelin was known, recent, to be an adipokine, which is not only formed but also secreted from the adipocytes. In addition, an evident association between apelin and insulin was observed, either *in vivo* or *in vitro*.[22] The adipocytes expressed apelin in increasing amounts in multiple rat models of hyperinsulin-related obesity. In these models, the form of apelin synthesis in adipocytes matched insulin levels in mice in the duration of fasting and after feeding, whereas apelin mRNAs are decreased in the absence of insulin in adipocytes.[23]

This investigation showed an elevated means of serum TG levels in the diabetics relative to the control. Besides, apelin and T-cholesterol, HDL-C, and LDL-C in diabetic cases were significantly correlated. This was supported by Jacobs *et al*.[22] who revealed a more prevalent dyslipidemia in T2DM relative to in controls. Also, Bonnet and Cooper[24] showed that DN was linked to lipid metabolism alteration, demonstrated by increased TG-rich lipoproteins in the earlier phases of kidney disorder. This is attributed to the fact that apelin expression and apelin receptors (APJ) increase in the fat tissues of obese subjects; besides, fatty tissue is an important source of apelin.[23]

Our findings showed that plasma apelin levels correlated significantly and positively with SBP and DBP in diabetic cases. This was supported by Zhu *et al*.,[25] who found a significant relationship between systolic BP, diastolic BP, MAP, and apelin levels ($P < 0.01$). So, apelin can be a therapeutic factor of hypertension. Apelin may be incorporated in improving the cardiac output (COP) and may provide cardiac protection against myocardial infarction (MI) and oxidative stress. Also, apelin can aid in regulating blood pressure as it acts as a sensor of pressure in response to myocardial hypertrophy.[26]

On the contrary, Rittig *et al*.[27] tested the cardiovascular risk effects of the levels of apelin, distribution of body fat, and insulin sensitivity to resistance ratio. Yet, they showed no influenced of serum apelin levels on blood pressure. However, the included cases were young patients prone to atherosclerosis and T2DM.

Table 7: Correlation of apelin and the parameters of diabetic cases

Diabetic cases	Apelin		
	Correlation coefficient	*P*	*N*
Age (years)	-0.048	0.715	60
Disease duration (years)	0.612	<0.001	60
SBP (mmHg)	0.427	0.001	60
DBP (mmHg)	0.466	<0.001	60
Weight (kg/m²)	0.372	0.003	60
Height (cm)	0.418	0.001	60
BMI (kg/m²)	0.193	0.139	60
WC (cm)	0.196	0.133	60
FBG (mg/dL)	0.684	<0.001	60
2 h-PPG (mg/dL)	0.744	<0.001	60
HbA1c (%)	0.890	<0.001	60
T-cholesterol (mg/dL)	0.316	0.014	60
Triglycerides (mg/dL)	0.222	0.088	60
HDL-C (mg/dL)	-0.303-	0.019	60
LDL-C (mg/dL)	0.397	0.002	60
Creatinine (mg/dL)	0.591	<0.001	60
Urea (mg/dL)	0.575	<0.001	60
GFR (1.73 mL/min/m²)	-0.566-	<0.001	60
A/C ratio (mg)	-0.238-	0.206	30
Proteinuria (mg/24 h)	0.94	<0.001	30

*$P<0.05$ is significant, $P<0.001$ is highly significant

Table 8: Multivariate regression analysis of apelin and HbA1C, creatinine, height, T-cholesterol, and HDL-C

Models	Unstandardized coefficients		Standardized coefficients	*t*	*P*	95.0% Confidence interval for *B*	
	B	Std. Error	Beta			Lower bound	Upper bound
Apelin							
(Constant)	-687.377	117.442		-5.853	<0.001	-922.834	-451.920-
HbA1c (%)	64.199	4.357	0.762	14.734	<0.001	55.463	72.934
Creatinine (mg/dL)	4.904	1.984	0.148	2.472	0.017	0.927	8.881
Height (cm)	2.684	0.676	0.234	3.972	<0.001	1.329	4.038
T. cholesterol (mg/dL)	0.358	0.141	0.146	2.538	0.014	0.075	0.640
HDL-C (mg/dL)	-1.098	0.523	-0.112	-2.098-	0.041	-2.148-	-0.049-

*$P<0.05$ is significant, $P<0.001$ is highly significant

The multivariate regression analysis for T-cholesterol, HDL-C, HbA1C, creatinine, and height proved their independent predictability to apelin levels. This is in line with the conclusions of Li *et al.*,[28] showing that T-cholesterol, BMI, and Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) were predictors for increasing plasma apelin levels. Although the study of Cavallo *et al.*[21] proved that T2DM, the index of basal disposition, and fasting blood glucose (FBG) were predictors for serum apelin levels. This may clarify the possible connection between the level of apelin and insulin resistance and T2DM development.[29]

Limitations

The authors did not focus on all complications of diabetes. The authors did not assess the physical activity of the patients. However, this may expand the future research for the relation of apelin levels to other complications related to diabetes. Also, the authors recommend a larger sample size to confirm the contribution of apelin to T2DM.

Conclusion

The study concluded that the apelin levels in T2DM cases were elevated with a further marked elevation in the number of DN cases. A significantly positive correlation existed between plasma apelin and duration of disease, SBP, DBP, weight, and height, FBG, 2h-PPBG, HbA1C, LDL-C, T-cholesterol, urea, and creatinine in studied patients. A significantly negative correlation was found between plasma apelin levels and HDL-C and e-GFR in studied patients. Also, the ROC curve emphasized the utility of the discriminated ability of plasm apelin as a biomarker for DN in T2DM patients. The multivariate linear regression found that HbA1C, creatinine, height, T-cholesterol, and HDL-C are independent predictors of plasma apelin levels, increasing the concern to the potential relationship between the apelin levels and the pathogenesis of T2DM and its complications.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

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