Expression of apelin-13 and its negative correlation with TGF-β1 in patients with diabetic kidney disease

QI WANG[1], XUJING LIU[2], AIHUA ZHAI[1], HUA XU[3], SHIZHAN MA[4] and YULIN LIU[5,6]

Departments of [1]Pharmacy, [2]Clinical Laboratory and [3]Endocrinology, The Fifth People's Hospital of Jinan; [4]Department of Endocrinology, Shandong Provincial Hospital Affiliated to Shandong First Medical University, Jinan, Shandong 250022; [5]Shandong Analysis and Test Center, Qilu University of Technology (Shandong Academy of Sciences); [6]Department of General Surgery, The First Affiliated Hospital of Shandong First Medical University, Jinan, Shandong 250013, P.R. China

Received April 3, 2023; Accepted December 15, 2023

DOI: 10.3892/etm.2024.12398

Abstract. Diabetic kidney disease (DKD) is a severe microvascular complication of diabetes, one key feature of which includes renal fibrosis. As apelin is an adipokine closely related to diabetes, the present study aimed to evaluate apelin-13 expression levels and the relationship between apelin-13 and disease indicators in patients with diabetic kidney disease (DKD). The present case-control study enrolled 70 patients with diabetes, including 31 with diabetic kidney disease (DKD group), 39 without DKD (non-DKD group) and 30 healthy controls. The levels of serum apelin-13 and TGF-β1, the key driver of renal fibrosis, were determined by ELISA. Additionally, age, mean disease duration, weight, blood pressure, fasting blood glucose, triglyceride, low-density lipoprotein cholesterol, high-density lipoprotein, cholesterol, urea nitrogen, blood creatinine and 24-hour urinary total protein (24-h UTP) were recorded. The results demonstrated that apelin-13 and TGF-β1 expression levels, age, blood pressure, fasting blood glucose, cholesterol and blood urea nitrogen levels were significantly higher in patients with diabetes compared with the healthy controls (P<0.05). Moreover, apelin-13 and TGF-β1 expression levels, mean disease duration, systolic pressure, blood creatinine, blood urea nitrogen and 24-h UTP were significantly higher in the DKD group compared with the non-DKD group (P<0.05). The estimated glomerular filtration rate (eGFR) was significantly reduced in the DKD group compared with the non-DKD group (P<0.05). Correlation analysis demonstrated a negative correlation between apelin-13 and eGFR expression and a positive correlation between apelin-13 expression and 24-h UTP in both the DKD and non-DKD groups (P<0.05). A negative correlation was also demonstrated between apelin-13 and TGF-β1 expression levels in the DKD group and non-DKD groups (both P<0.05). In conclusion, apelin-13 and TGF-β1 expression levels were significantly higher in the DKD group compared with those in the non-DKD group. Additionally, apelin-13 expression was negatively correlated with TGF-β1 expression in the DKD and non-DKD groups. Therefore, apelin-13 could potentially be used in the future as an indicator of renal fibrosis or destruction in patients with DKD. The present trial was retrospectively registered in the Chinese Clinical Trial Registry (trial registration no. ChiCTR2200060945) on 14.06.2022.

Correspondence to: Dr Yulin Liu, Department of General Surgery, The First Affiliated Hospital of Shandong First Medical University, 16766 Jingshi Road, Jinan, Shandong 250013, P.R. China
E-mail: xiaohang880605@163.com

Key words: apelin-13, diabetes, diabetic kidney disease, fibrosis, transforming growth factor-β1

Introduction

Diabetic kidney disease (DKD) is a serious microvascular complication that can progress to severe end-stage renal failure (1). A previous epidemiological survey between 2015 and 2017 reported that there are a large number of individuals with diabetes, with a prevalence of 11.2% among individuals aged ≥18 years (2). Of the total number of patients with diabetes, ~33.6% develop DKD, which can seriously affect their quality of life (3). However, its pathogenesis is still unclear. Clinically, DKD can be quantitatively diagnosed based on urinary microalbumin levels. However, the severity and prognosis of the disease cannot be accurately assessed based on the degree of proteinuria (4). Therefore, attention should be paid to the importance of pathological changes observed in patients with DKD. Renal fibrosis is the main pathological feature closely associated with DKD progression (5). Therefore, determining the occurrence and progression of renal fibrosis is important.

Tubular epithelial-mesenchymal transition (EMT) is widely regarded as the underlying mechanism of renal fibrosis (6). One of the important profibrotic cytokines that induces EMT is TGF-β1 (7). In children with type 1 diabetes, the level of serum TGF-β1 was significantly higher compared with that in healthy children (8). Qiao *et al* (9) reported that serum and urinary TGF-β1 levels were significantly increased in patients with type 2 diabetes mellitus (T2DM) and DKD. A number of previous studies reported that TGF-β1 levels increased in streptozotocin-induced diabetic mouse models and inhibition

of TGF-β1 ameliorated renal fibrosis in DKD (10-12). The TGF-β1 pathway is also closely related to tubulointerstitial fibrosis in rats with DKD (13). The aforementioned reports suggest that TGF-β1 could be a potential target for the treatment of renal fibrosis in DKD.

Apelin, an adipokine secreted by adipocytes, is an endogenous peptide that was first isolated from the bovine stomach in 1998 (14). Apelin stimulates glucose use, enhances the sensitivity of target cells to insulin and serves a role in the pathogenesis and complications of diabetes (15). A previous study reported that circulating apelin levels increased in patients with T2DM (16), while another study reported that serum apelin levels decreased in patients with T2DM (17). The aforementioned study also reported that apelin might be a biomarker for early detection of diabetic nephropathy in patients with T2DM. However, serum apelin levels were not correlated with the urine protein to creatinine ratio (17). Therefore, the expression of apelin in DKD is yet to be fully elucidated. Fibrosis represents the final step of DKD and TGF-β1 has been established as the main mediator of renal fibrosis (18). However, the relationship between serum apelin levels and renal fibrosis in patients with DKD is not currently understood. Additionally, to the best of our knowledge, the relationship between serum apelin and TGF-β1 expression levels has not yet been reported.

The apelin gene encodes a 77 amino acid prepropeptide and can be cleaved into different active forms including apelin-12, -13, -15, -16, -17, -19, -28, -31 and -36 (19). All isoforms of apelin can bind to the G protein-coupled receptor, APJ (14). Apelin-13, the shorter isomer of apelin, exhibits the strongest biological activity of all the apelin isoforms (14). Apelin-13 is widely expressed in many tissues and organs including the cardiovascular, respiratory, digestive endocrine and neurological systems and it is particularly abundant in blood (20). Therefore, in the present study, serum apelin-13 and TGF-β1 levels were measured and their relationship in diabetic patients with and without DKD was analyzed.

Materials and methods

Participants. PASS 2021 software (NCSS LLC) was used to calculate the number of patients included in the present study. Based on the pre-experimental data, the sample size required for each group was ≥25 cases by taking 1-β=0.9 and α=0.05. In the present study, 70 patients with T2DM with or without DKD, who were admitted to The Jinan Fifth People's Hospital (Jinan, China) between February 18, 2021 and April 30, 2022, were enrolled. Additionally, 30 age- and sex-matched healthy controls from the examination center of The Jinan Fifth People's Hospital between March 1, 2021 and July 31, 2021 were selected for the study.

Ethics approval and patient consent. The present study was designed according to The Strengthening the Reporting of Observational Studies in Epidemiology guidelines (21). The present study was registered in the Chinese Clinical Trial Registry (https://www.chictr.org.cn/bin/userProject; trial registration no. ChiCTR2200060945) and was approved by the Ethics Committee of The Fifth People's Hospital of Jinan (Jinan, China; approval no. 20-ke-01). A total of 100 subjects including patients in the DKD, non-DKD and healthy control groups were included in the present study. Written informed consent was obtained from all participants.

Inclusion and exclusion criteria. The clinical diagnostic criteria for DKD were as follows: A 24-h urinary total protein level ≥30 mg/24 h and/or a prolonged decrease in eGFR <60 ml/min (1.73 m^2)$^{-1}$ without other types of kidney disease, based on The Kidney Disease Improving Global Outcomes 2020 Clinical Practice Guideline (22). The inclusion criteria of patients were as follows: i) Female or male patients who were hyperglycemic and being treated with glucose-lowering drugs; ii) aged 28-75 years; iii) majority ~55 years old; iv) a history of diabetes for ≥6 months; v) negative insulin autoantibodies, islet cell antibodies and glutamate decarboxylase antibodies; vi) fasting blood glucose (FBG) levels after treatment <7.0 mmol/l; and vii) postprandial 2-hour plasma glucose levels after treatment <10.0 mmol/l. Patients with type 1 diabetes, gestational diabetes, serious cardiovascular diseases, cerebrovascular diseases, respiratory disease, digestive disease, inflammatory diseases, malignant tumors, urinary tract infections, other kidney diseases, recent nephrotoxic drug exposure and proteinuria caused by other factors were excluded. The inclusion criteria of the controls were as follows: i) Healthy males and females; ii) aged 30-65 years; and iii) no history of diabetes. Exclusion criteria of the controls were any history of hypertension, tumors and a variety of serious acute and chronic diseases including brain, heart, lung, liver and kidney disease.

Control of bias. The methods to control confounding factors in the present study were as follows: i) Limiting the inclusion and exclusion conditions of subjects during the study design; and ii) taking some characteristics that interfere with the results as matching factors, which was a limiting method to maintain the same matching factors between the cases and controls. The confounding factors, such as BMI, FBG, cholesterol, triglyceride (TG), low-density lipoprotein cholesterol (LDL-c) and high-density lipoprotein cholesterol (HDL-c) levels were matched in the DKD group and the non-DKD group, which may potentially have resulted in selection bias due to the necessity to discard patients that did not have matching confounding factors. In order to reduce selection bias, random sampling was performed. By the strict selection of representative subjects, the cases and controls were balanced. Moreover, the criteria for inclusion and exclusion were strictly limited. When analyzing the final results, the blind method and objective statistical indicators such as FBG, blood pressure and serum lipids were applied. Good patient compliance and preventing loss in follow-up of patients also served a role in bias reduction.

Measurements of indicators. Sex, age, systolic blood pressure, diastolic blood pressure, mean disease duration, weight and BMI were recorded at the time of patient enrollment. The levels of FBG, serum TG, LDL-c, HDL-c, total cholesterol, blood urea nitrogen (BUN) and creatinine were detected using the TBA-FX8 automatic biochemical analyzer (Shenzhen Mindray Bio-Medical Electronics Co., Ltd.). The 24 h urinary total protein (24-h UTP) was measured using the BS-2000M

Table I. Characteristics of patients with diabetes compared with healthy controls.

Patient characteristic	Healthy control group (n=30)	Patients with diabetes (n=70)	P-value
Sex, n (%)			0.335
Male	14 (25.9)	40 (74.1)	
Female	16 (34.8)	30 (65.2)	
Proteinuria, n (%)			<0.001
Yes	0 (0.0)	25 (100.0)	
No	30 (40.0)	45 (60.0)	
Age, years	48.20±4.59	57.91±8.42	<0.001
Weight, kg	67.19±11.96	68.34±12.26	0.666
BMI, kg/m2	24.31±3.83	24.85±3.84	0.517
Systolic blood pressure, mmHg	122.60±13.68	141.39±21.26	<0.001
Diastolic blood pressure, mmHg	77.00±12.89	84.31±10.82	0.004
Fasting blood glucose, mmol/l	5.06±0.53	8.46±2.56	<0.001
Serum low-density lipoprotein cholesterol, mmol/l	2.86±0.67	3.01±1.13	0.406
Serum high-density lipoprotein cholesterol, mmol/l	1.23 (1.06/1.48)	1.17 (0.99/1.31)	0.112
Serum triglyceride, mmol/l	1.13 (0.79/1.49)	1.34 (0.93/2.06)	0.093
Cholesterol, mmol/l	4.70±0.74	5.14±1.43	0.046
Creatinine, μmol/l	63.00 (52.75/69.25)	62.50 (54.00/109.50)	0.250
Blood urea nitrogen, mmol/l	4.55 (3.90/5.40)	6.05 (5.08/8.75)	<0.001
Estimated glomerular filtration rate, ml/min (1.73 m^2)$^{-1}$	122.02 (104.55/131.94)	116.31 (58.18/146.43)	0.339
Apelin-13, pg/ml	14,415.16 (10,285.52/18,011.01)	29,716.03 (17,278.46/49,354.44)	<0.001
TGF-β1, ng/ml	79.32 (48.20/103.00)	103.02 (64.45/150.92)	0.003

Data were presented as percentages of frequency for qualitative variables and the mean ± standard deviation or median (25th/75th percentiles) for continuous variables.

automatic biochemical analyzer (Shenzhen Mindray Bio-Medical Electronics Co., Ltd.). Glycosylated hemoglobin A1c (HbA1c) levels were analyzed using the Tosoh Automated Glycohemoglobin Analyzer HLC-723G11 (Tosoh Bioscience Co., Ltd.). Fasting C-peptide (FCP) levels were measured using a MAGLUMI X8 automatic chemiluminescence immunoanalyzer (Shenzhen New Industry Biomedical Engineering Co., Ltd.).

Serum samples were collected, centrifuged at 1,000 x g at room temperature for 20 min and stored at -80˚C until analysis. Serum apelin-13 concentration was measured using an ELISA kit (cat. no. E-EL-H0458c; Wuhan Elabscience Biotechnology Co., Ltd.). Serum TGF-β1 concentration was measured using an ELISA kit (cat. no. CSB-E04725h; Wuhan Huamei Biological Engineering Co., Ltd.). The standards and samples were added to the coated assay plates. After incubation at 37˚C for 1 h (apelin ELISA kit) or 2 h (TGF-β1 ELISA kit), horseradish peroxidase-avidin was added to biotin-labeled antibodies (1:100). Then, the assay plate containing apelin-13 was incubated at 37˚C for 30 min and the assay plate containing TGF-β1 was incubated at 37˚C for 1 h . After 5'-tetramethylbenzidine substrate incubation for 15 min at 37˚C, stop solution was injected into each well and the absorbance of each sample was measured at 450 nm.

Statistical analysis. Statistical analyses were performed using SPSS (version 22.0; IBM Corp.). The Shapiro-Wilk test was used to determine the normal distribution of the quantitative parametric data. An unpaired t-test was used to analyze the quantitative parametric data which were presented as the mean ± standard deviation. Variables with non-normal distributions were evaluated using the Mann-Whitney U test and were presented as the median with the 25th and 75th percentiles. The χ^2 test was used to analyze qualitative variables and the data were presented as percentages of frequency. Pearson's correlation was used to calculate the correlation between continuous data. Spearman's correlation analysis was used to evaluate potential correlations between rank variables. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Comparison of indicators between the diabetic groups and the controls. The groups of patients with diabetes consisted of 40 males and 30 females, with an average age of 57.91±8.42 years. The control group consisted of 14 males and 16 females, with an average age of 48.20±4.59 years. There were no significant differences observed in sex, weight,

Table II. Characteristics of patients in the DKD and non-DKD groups.

Patient characteristic	DKD group (n=31)	Non-DKD group (n=39)	P-value
Sex, n (%)			0.728
Male	17 (42.5)	23 (57.5)	
Female	14 (46.7)	16 (53.3)	
Proteinuria, n (%)			<0.001
Yes	25 (100.00)	0 (0.00)	
No	6 (13.33)	39 (86.67)	
Age, years	59.74±10.08	56.46±6.60	0.106
Duration of disease, years	15.00 (10.00/20.00)	4.00 (1.00/10.00)	<0.001
Weight, kg	68.27±12.43	68.38±12.29	0.970
BMI, kg/m^2	25.00±4.03	24.73±3.73	0.769
Systolic blood pressure, mmHg	151.52±24.46	133.33±14.08	0.001
Diastolic blood pressure, mmHg	86.74±12.26	82.38±9.24	0.106
Fasting blood glucose, mmol/l	8.32±2.74	8.58±2.44	0.670
Glycosylated hemoglobin A1c, %	9.00±1.88	8.87±2.25	0.800
Fasting C peptide, ng/ml	2.13 (1.31/3.59)	1.85 (1.40/2.50)	0.226
Serum low-density lipoprotein cholesterol, mmol/l	3.09±1.38	2.95±0.90	0.650
Serum high-density lipoprotein cholesterol, mmol/l	1.15 (0.97/1.28)	1.18 (0.99/1.37)	0.603
Serum triglyceride, mmol/l	1.62 (1.05/2.39)	1.27 (0.85/1.84)	0.170
Cholesterol, mmol/l	5.22±1.78	5.08±1.09	0.695
Creatinine, μmol/l	111.00 (67.00/175.00)	56.00 (48.00/64.00)	<0.001
Blood urea nitrogen, mmol/l	8.30 (5.80/15.40)	5.30 (4.40/6.80)	<0.001
24 h urinary total protein, mg/24 h	681.90 (332.00-3,603.60)	58.88 (36.45-100.20)	<0.001
Estimated glomerular filtration rate, ml/min $(1.73\ m^2)^{-1}$	66.91±42.82	138.57±39.62	<0.001
Apelin-13, pg/ml	50,720.36 (26,954.76/78,880.05)	20,490.58 (16,008.67/33,997.97)	<0.001
TGF-β1, ng/ml	141.37±71.93	96.47±43.81	0.004

Data were presented as percentages of frequency for qualitative variables and the mean ± standard deviation or median (25th/75th percentiles) for continuous variables. DKD, diabetic kidney disease.

BMI, LDL-c, HDL-c, TG, blood creatinine and the estimated glomerular filtration rate (eGFR) of patients with diabetes and the control group (P>0.05). Serum apelin and TGF-β1 levels, age, systolic and diastolic pressure, FBG, total cholesterol and BUN were significantly higher in the diabetic group compared with the controls (P<0.05; Table I).

Comparison of indicators between the DKD group and the non-DKD groups of patients. Subgroup analysis was performed for diabetic groups with and without DKD. Age, sex, weight, BMI, diastolic pressure, FBG, HbA1c, FCP, LDL-c, HDL-c, TG and total cholesterol levels were evaluated and no significant differences were demonstrated between the DKD and non-DKD groups of patients. However, the mean disease duration was significantly longer in the DKD group compared with the non-DKD group (P<0.05). Additionally, a statistically significant increase was demonstrated in serum apelin-13 and TGF-β1 levels, systolic pressure, blood creatinine, BUN and 24-h UTP in the DKD group compared with the non-DKD group (P<0.05). By contrast, eGFR was significantly decreased in the DKD group compared with the non-DKD group of patients (P<0.05; Table II).

Correlation analysis of apelin-13 and parameters in the DKD and non-DKD groups. The Pearson and Spearman's correlation analyses demonstrated a significant negative correlation between serum apelin-13 and TGF-β1 levels in the DKD and non-DKD groups (P<0.05); however, no significant correlation was demonstrated in the control group (Fig. 1). Serum apelin-13 levels were associated with FCP, blood creatinine, BUN and 24-h UTP in the DKD group (Table III; P<0.05). A significant positive correlation was demonstrated between apelkin-13 and 24-h UTP levels in both the DKD and non-DKD groups (P<0.05; Fig. 2). In addition, apelin-13 levels were significantly negatively correlated with eGFR in the DKD group (P<0.05; Fig. 3) and a marked negative correlation was demonstrated between eGFR and apelin-13 levels in the non-DKD group (P>0.05; Fig. 3). No significant correlation was observed between serum apelin-13 levels and age, disease duration, weight, BMI, blood pressure, FBG, HbA1c,

Table III. Correlation between apelin-13 expression levels and characteristics of patients in the DKD and non-DKD groups.

	DKD group		Non-DKD group	
Patient characteristic	r	P-value	r	P-value
Age, years	-0.149	0.423	0.032	0.847
Disease duration, years	0.082	0.663	-0.076	0.645
Weight, kg	-0.122	0.513	-0.004	0.980
BMI, kg/m^2	-0.108	0.565	0.029	0.862
Systolic blood pressure, mmHg	0.088	0.636	0.176	0.283
Diastolic blood pressure, mmHg	0.195	0.293	0.246	0.131
Fasting blood glucose, mmol/l	-0.284	0.122	-0.108	0.513
Glycosylated hemoglobin A1c, %	-0.201	0.279	-0.008	0.960
Fasting C peptide, ng/ml	0.405	0.024	0.225	0.168
Serum low-density lipoprotein cholesterol, mmol/l	-0.029	0.876	0.094	0.568
High-density lipoprotein cholesterol, mmol/l	-0.029	0.878	-0.038	0.817
Serum triglyceride, mmol/l	-0.005	0.980	-0.023	0.889
Cholesterol, mmol/l	-0.017	0.927	0.081	0.622
Creatinine, μmol/l	0.848	<0.001	0.152	0.354
Blood urea nitrogen, mmol/l	0.810	<0.001	0.023	0.891

DKD, diabetic kidney disease.

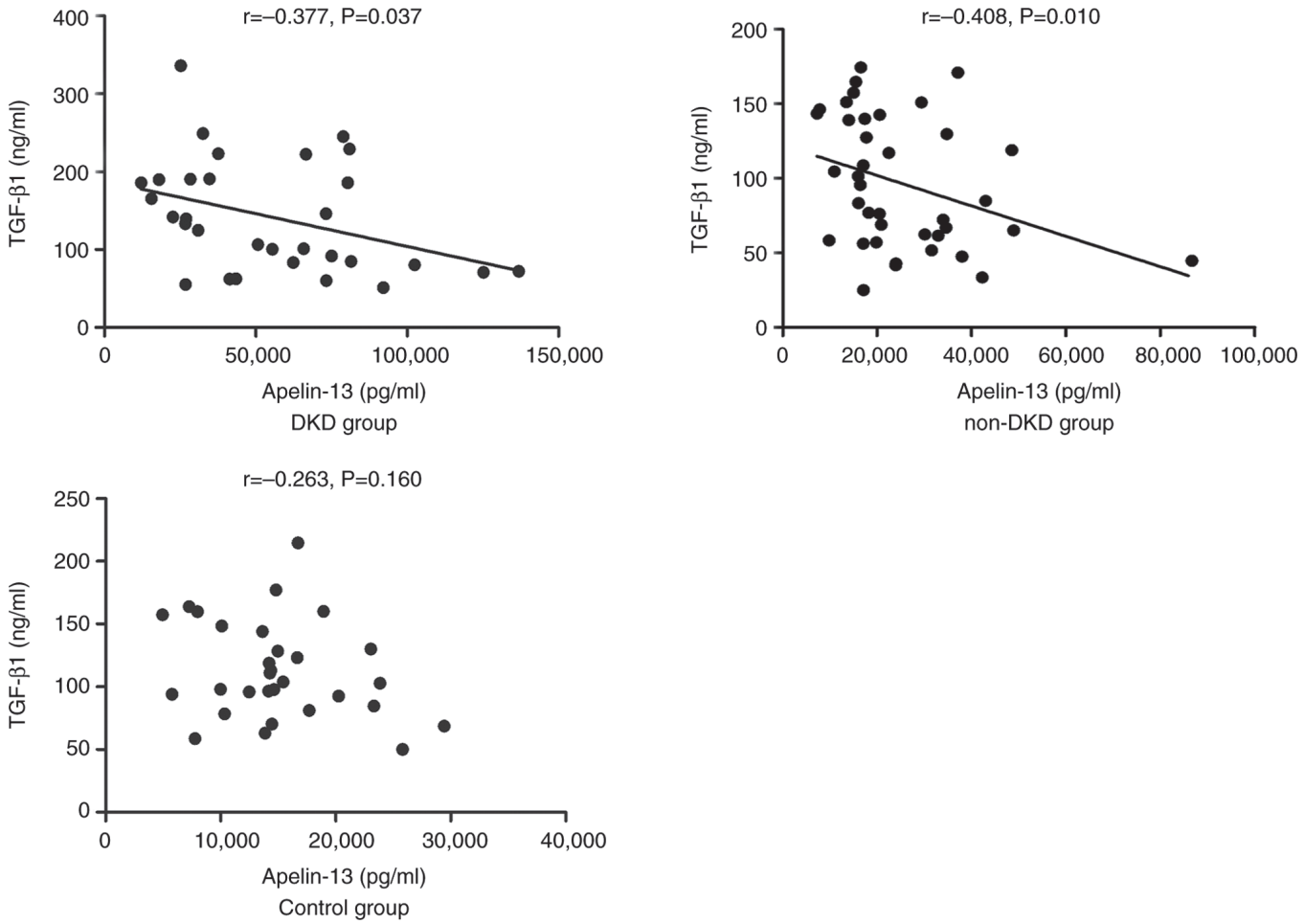

Figure 1. A significant negative correlation was demonstrated between serum apelin-13 and TGF-β1 expression levels in the DKD and non-DKD groups, whereas no correlation was observed in the control group. DKD, diabetic kidney disease.

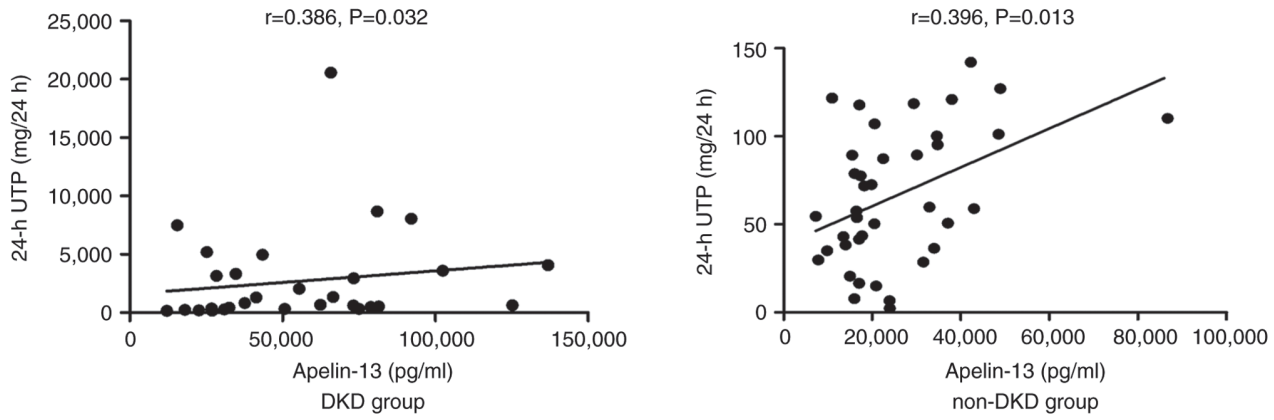

Figure 2. A significant positive correlation was demonstrated between serum apelin-13 levels and 24-h UTP in the DKD and non-DKD groups. DKD, diabetic kidney disease; 24-h UTP, 24 h urinary total protein.

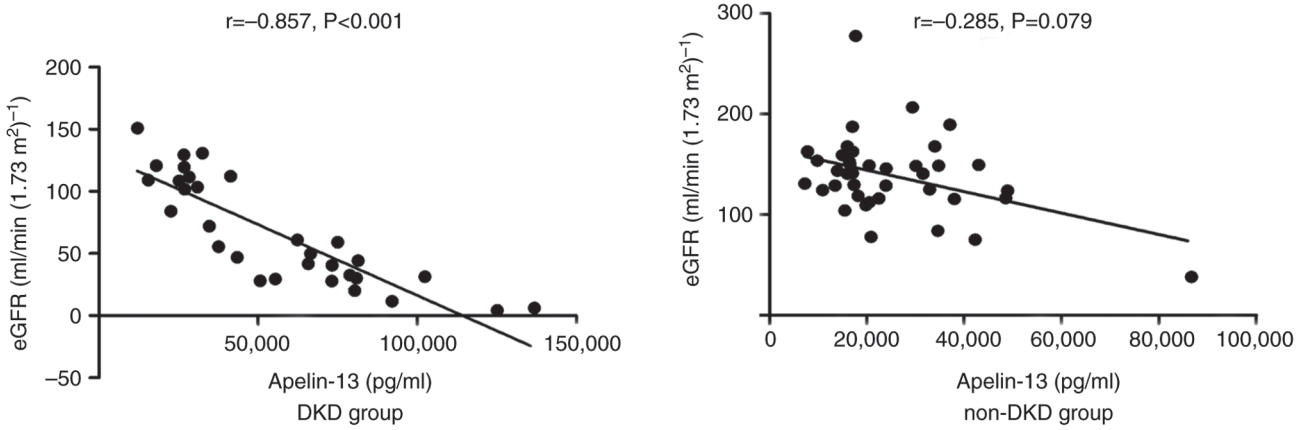

Figure 3. A significant negative correlation was demonstrated between serum apelin-13 levels and eGFR in the DKD group, whereas a marked negative correlation was observed in the non-DKD group. eGFR, estimated glomerular filtration rate; DKD, diabetic kidney disease.

LDL-c, HDL-c, TG or total cholesterol levels in the DKD and non-DKD groups (P>0.05; Table III).

Discussion

With a worldwide increase in the incidence of DKD in recent decades, DKD has become one of the most serious complications of diabetes. Hyperglycemia is an important factor in the development of DKD (23). The potential mechanism by which this occurs may involve an increase in angiotensin II, growth factors and advanced glycation end products causing hyperfiltration, which leads to an increase in capillary pressure. Subsequently, the basement membrane thickens, the mesangium expands and the extracellular matrix thickens, which triggers fibrosis (24). In the present study, FPG was higher in patients with diabetes compared with the controls, which supported the previously published finding that hyperglycemia ultimately damages the kidney (24).

Reduced eGFR and increased albuminuria are indicators of DKD diagnosis and progression. In the present study, eGFR was lower and 24-h UTP was higher in the DKD group compared with the non-DKD patients. Moreover, serum eGFR and apelin-13 levels were negatively correlated and 24-h UTP was positively correlated with apelin-13 levels in the DKD group, which is in accordance with a previous study that reported that apelin-13 caused a significant increase in the ratio of microalbuminuria in diabetic mice (25). Another study reported that pyr-apelin-13 had the opposite effect on albuminuria, showing a tendency to reduce renal swelling and inflammation (26). These results indicate that the effects of apelin on albuminuria in the kidneys may be related to the morphology of apelin or its differential pathophysiological states.

Apelin is an endogenous peptide that may serve as an important biomarker for the detection of DKD (17). The results of the present study demonstrated that the serum levels of apelin-13 in patients in the DKD and non-DKD groups were significantly higher compared with those in the controls. This finding was consistent with a previous study that reported that circulating apelin concentration was significantly elevated in patients with diabetes compared to those in the control group (27). However, there are conflicting reports on decreased apelin levels in T2DM (17), possibly because different stages of DKD development may initiate different signaling pathways to regulate the expression of apelin, thus affecting serum apelin levels.

TGF-β1 is a recognized fibrogenic factor and may be considered a driver of renal fibrosis, which is an important pathological feature of DKD, as a previous study reported that TGF-β1 signaling pathways were key regulators of

renal fibrosis (28). In the present study, TGF-β1 expression levels in the serum of patients with DKD were increased, which was consistent with the previous study conducted by Sawires *et al* (8) that reported higher serum levels of TGF-β1 in children with DKD. The grouping was similar in the two studies, but the present study focused on the relationship between apelin-13 and disease indicators in T2DM nephropathy, while the previously published study reported the relationship between TGF-β1 and other related indicators in type 1 diabetic nephropathy in children.

Apelin has previously been reported to serve a crucial role in heart, liver and kidney fibrosis (29). Therefore, a relationship may exist between apelin and TGF-β1 expression levels under specific physiological and pathological conditions. In a previous study by Kocer *et al* (30), apelin was negatively correlated to TGF-β in patients with polycystic kidney disease. Animal and cell studies have also reported that apelin treatment served an inhibitory role on TGF-β1, prevented acute cell damage and improved the outcome of ischemia/reperfusion injury to the kidneys (31). Although it was observed that both apelin-13 and TGF-β1 expression levels increased in the DKD and non-DKD groups of patients, the increase was not the same in the present study as the values previously reported. The present study demonstrated that an increase in apelin-13 levels inhibited serum TGF-β1 levels. This phenomenon has also been reported by Lu *et al* (32), who suggested a negative correlation of axial length and peak distance in myopia, emmetropia, and hyperopic groups, although both axial length and peak distance increased in myopia group than emmetropia and hyperopia groups. Therefore, the results of the present study demonstrated that serum apelin-13 level were negatively associated with TGF-β1, which suggested there may be an inverse association between apelin-13 and renal fibrosis.

In the present study, apelin-13 was positively correlated with 24-h UTP and negatively correlated with eGFR, which served a detrimental role in DKD. However, apelin-13 was also negatively correlated with the fibrotic factor TGF-β1, which served a favorable role in DKD. This has been reported in several animal studies which showed that apelin-13 caused a significant increase in the ratio of microalbuminuria in diabetic KK-Ay mice (25) and inhibited autophagy in podocytes, which led to podocyte apoptosis and massive proteinuria in diabetic KK-Ay mice (33). Apelin-13 may be positively correlated with urinary protein and promote the progress of DKD by increasing fat mass, promoting angiogenesis in glomeruli to form abnormal vessels, increasing permeability or inducing podocyte apoptosis (18). However, it has also been reported that apelin inhibited the process of EMT in podocytes in diabetic KK-Ay mice (34), decreased the levels of TGF-β and suppressed kidney tissue fibrosis in Sprague Dawley rats with diabetic nephropathy (35). Therefore, apelin-13 may be relevant to increased fat mass, increased glomerular permeability and induced podocyte apoptosis during the early stages of DKD, while it may inhibit renal fibrosis in the advanced stage of DKD.

Confounding factors may interfere with the results in the present study. A number of studies reported that apelin may be associated with BMI, FBG, cholesterol, TG, LDL and HDL. Zaki M *et al*. concluded that serum apelin levels were positively correlated with BMI (36). FBG, cholesterol, TG, LDL and HDL are indicators of glycolipid metabolism and Bertrand *et al* (37) reported that apelin improved hepatic lipid metabolism in obese and insulin-resistant mice. This is in accordance with a previous study that reported that apelin-13 treatment resulted in the significant decreases in the FBG levels and the serum levels of cholesterol, TG and LDL in mice with gestational diabetes mellitus (38). Therefore, case-control matching is helpful for known confounders. BMI, FBG, LDL-c, HDL-c, TG and cholesterol were not statistically significant between the DKD group and the non-DKD group in the present study. On this basis, the expression and correlation of apelin-13 in DKD were studied.

In conclusion, the present study demonstrated that a significant negative relationship between apelin-13 and TGF-β1 in both DKD and non-DKD groups was observed. This phenomenon has laid a foundation for the future study of the roles of apelin and fibrosis in DKD. Apelin-13 may potentially be a negative indicator of renal fibrosis in patients with DKD. More studies are required to assess the association and the mechanism between apelin-13 and the fibrosis in renal disease from both cellular and animal experiments. Future studies will use high-glucose-treated human renal tubular epithelial cells with different dosages of apelin-13 treatment to observe the morphological changes of the cells and detect the levels of fibrosis-related markers such as E-cadherin, vimentin and α-SMA. Furthermore, rat models of DKD may be constructed by streptozocin injection. Upon injecting apelin-13 into diabetic rats, the weight, blood glucose levels, urinary protein content, levels of serum apelin-13 and TGF-β1 could be detected. Staining of renal tissues and analysis of RNA and protein expression levels of E-cadherin, vimentin and α-SMA should be performed. Performing these studies would aid in the understanding of how apelin-13 may potentially serve as a useful marker for renal fibrosis.

Acknowledgements

Not applicable.

Funding

This study was supported by the Shandong Provincial Natural Science Foundation (grant no. ZR2021QH106), Jinan Municipal Science and Technology Project (grant nos. 202019083 and 202019091) and The Dean Foundation of the First Affiliated Hospital of Shandong First Medical University (grant no. QYPY2019NSFC0805).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

QW contributed to the conception and design of the study and drafting of the manuscript. XL and SM acquired, analyzed and interpreted the data. HX was responsible for data measurement and curation. AZ and YL were responsible for the study concept, manuscript editing and supervision of the manuscript. QW and YL confirm the authenticity of all the raw data. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of the Fifth People's Hospital of Jinan (approval no. 20-ke-01; Jinan, China) and written informed consent was obtained from all participants.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Ahmad J: Management of diabetic nephropathy: Recent progress and future perspective. Diabetes Metab Syndr 9: 343-58, 2015.
2. Li Y, Teng D, Shi X, Qin G, Qin Y, Quan H, Shi B, Sun H, Ba J, Chen B, *et al*: Prevalence of diabetes recorded in mainland China using 2018 diagnostic criteria from the American diabetes association: National cross sectional study. BMJ 369: m997, 2020.
3. Weng JP and Bi Y: Epidemiological status of chronic diabetic complications in China. Chin Med J (Engl) 128: 3267-3269, 2015.
4. Radcliffe NJ, Seah JM, Clarke M, MacIsaac RJ, Jerums G and Ekinci EI: Clinical predictive factors in diabetic kidney disease progression. J Diabetes Investig 8: 6-18, 2017.
5. Zhang Y, Jin D, Kang X, Zhou R, Sun Y, Lian F and Tong X: Signaling pathways involved in diabetic renal fibrosis. Front Cell Dev Biol 9: 696542, 2021.
6. Carew RM, Wang B and Kantharidis P: The role of EMT in renal fibrosis. Cell Tissue Res 347: 103-116, 2012.
7. Sisto M, Lorusso L, Ingravallo G, Tamma R, Ribatti D and Lisi S: The TGF-β1 signaling pathway as an attractive target in the fibrosis pathogenesis of Sjögren's syndrome. Mediators Inflamm 2018: 1965935, 2018.
8. Sawires H, Botrous O, Aboulmagd A, Madani N and Abdelhaleem O: Transforming growth factor-β1 in children with diabetic nephropathy. Pediatr Nephrol 34: 81-85, 2019.
9. Qiao YC, Chen YL, Pan YH, Ling W, Tian F, Zhang XX and Zhao HL: Changes of transforming growth factor beta 1 in patients with type 2 diabetes and diabetic nephropathy. A PRISMA-compliant systematic review and meta-analysis. Medicine (Baltimore) 96: e6583, 2017.
10. Zheng ZC, Zhu W, Lei L, Liu XQ and Wu YG: Wogonin ameliorates renal inflammation and fibrosis by inhibiting NF-κB and TGF-β1/Smad3 signaling pathways in diabetic nephropathy. Drug Des Devel Ther 14: 4135-4148, 2020.
11. Sun Z, Ma Y, Chen F, Wang S, Chen B and Shi J: miR-133b and miR-199b knockdown attenuate TGF-β1-induced epithelial to mesenchymal transition and renal fibrosis by targeting SIRT1 in diabetic nephropathy. Eur J Pharmacol 837: 96-104, 2018.
12. Geng XQ, Ma A, He JZ, Wang L, Jia YL, Shao GY, Li M, Zhou H, Lin SQ and Ran JH: Ganoderic acid hinders renal fibrosis via suppressing the TGF-β/Smad and MAPK signaling pathways. Acta Pharmacol Sin 41: 670-677, 2020.
13. Miao XJ, Bi TT, Tang JM, Lv R, Gui DK and Yang XF: Regulatory mechanism of TGF-β1/SGK1 pathway in tubulointerstitial fibrosis of diabetic nephropathy. Eur Rev Med Pharmacol Sci 23: 10482-10488, 2019.
14. Tatemoto K, Hosoya M, Habata Y, Fujii R, Kakegawa T, Zou MX, Kawamata Y, Fukusumi S, Hinuma S, Kitada C, *et al*: Isolation and characterization of a novel endogenous peptide ligand for the human APJ receptor. Biochem Biophys Res Commun 251: 471-476, 1998.
15. Chapman FA, Nyimanu D, Maguire JJ, Davenport AP, Newby DE and Dhaun N: The therapeutic potential of apelin in kidney disease. Nat Rev Nephrol 17: 840-853, 2021.
16. Habchi M, Duvillard L, Cottet V, Brindisi MC, Bouillet B, Beacco M, Crevisy E, Buffier P, Baillot-Rudoni S, Verges B and Petit JM: Circulating apelin is increased in patients with type 1 or type 2 diabetes and is associated with better glycaemic control. Clin Endocrinol (Oxf) 81: 696-701, 2014.
17. Demirpence M, Yilmaz H, Colak A, Pamuk BO, Karakoyun I and Basok B: Apelin: A potential novel serum biomarker for early detection of diabetic nephropathy in patients with type 2 diabetes. North Clin Istanb 6: 151-155, 2118.
18. Iwano M: EMT and TGF-beta in renal fibrosis. Front Biosci (Schol Ed) 2: 229-238, 2010.
19. Lv SY, Yang YJ and Chen Q: Regulation of feeding behavior, gastrointestinal function and fluid homeostasis by apelin. Peptides 44: 87-92, 2013.
20. Zhang Y, Jiang W, Sun W, Guo W, Xia B, Shen X, et al. Neuroprotective Roles of Apelin-13 in Neurological Diseases. Neurochem Res 48: 1648-1662, 2023.
21. Von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP and STROBE Initiative: The strengthening the reporting of observational studies in epidemiology (STROBE) statement: Guidelines for reporting observational studies. Lancet 370: 1453-1457, 2007.
22. Kidney Disease: Improving Global Outcomes (KDIGO) Diabetes Work Group: KDIGO 2020 clinical practice guideline for diabetes management in chronic kidney disease. Kidney Int 98(4S): S1-S115, 2020.
23. Brownlee M: The pathobiology of diabetic complications: A unifying mechanism. Diabetes 54: 1615-1625, 2005.
24. Stevens PE and Levin A: Evaluation and management of chronic kidney disease: Synopsis of the kidney disease: Improving global outcomes 2012 clinical practice guideline. Ann Intern Med 158: 825-830, 2013.
25. Zhang BH, Wang W, Wang H, Yin J and Zeng XJ: Promoting effects of the adipokine, apelin, on diabetic nephropathy. PLoS One 8: e60457, 2013.
26. Day RT, Cavaglieri RC and Feliers D: Apelin retards the progression of diabetic nephropathy. Am J Physiol Renal Physiol 304: F788-F800, 2013.
27. Meral C, Tascilar E, Karademir F, Tanju IA, Cekmez F, Ipcioglu OM, Ercin CN, Gocmen I and Dogru T: Elevated plasma levels of apelin in children with type 1 diabetes mellitus. J Pediatric Endocrinol Metab 23: 497-502, 2010.
28. Meng XM, Nikolic-Paterson DJ and Lan HY: TGF-β: The master regulator of fibrosis. Nat Rev Nephrol 12: 325-338, 2016.
29. Wang LY, Diao ZL, Zhang DL, Zheng JF, Zhang QD, Ding JX and Liu WH: The regulatory peptide apelin: A novel inhibitor of renal interstitial fibrosis. Amino Acids 46: 2693-2704, 2014.
30. Kocer D, Karakukcu C, Ozturk F, Eroglu E and Kocyigit I: Evaluation of fibrosis markers: Apelin and transforming growth factor-β1 in autosomal dominant polycystic kidney disease patients. Ther Apher Dial 20: 517-522, 2016.
31. Chen H, Wan D, Wang L, Peng A, Xiao H, Petersen RB, Liu C, Zheng L and Huang K: Apelin protects against acute renal injury by inhibiting TGF-β1. Biochim Biophys Acta 1852: 1278-1287, 2015.
32. Lu LL, Hu XJ, Yang Y, Xu S, Yang SY, Zhang CY and Zhao QY: Correlation of myopia onset and progression with corneal biomechanical parameters in children. World J Clin Cases 10: 1548-1556, 2022.
33. Liu Y, Zhang J, Wang YJ and Zeng XJ: Apelin involved in progression of diabetic nephropathy by inhibiting autophagy in podocytes. Cell Death Dis 8: e3006, 2017.
34. Yin J, Wang Y, Chang J, Li B, Zhang J, Liu Y, Lai S, Jiang Y, Li H and Zeng X: Apelin inhibited epithelial-mesenchymal transition of podocytes in diabetic mice through downregulating immunoproteasome subunits β5i. Cell Death Dis 9: 1031, 2018.
35. Gao Z, Zhong X, Tan YX and Liu D: Apelin-13 alleviates diabetic nephropathy by enhancing nitric oxide production and suppressing kidney tissue fibrosis. Int J Mol Med 48: 175, 2021.
36. Zaki M, Kamal S, Ezzat W, Hassan N, Yousef W, Ryad H, Mohamed R, Youness E, Basha W and Elhosary Y: Serum apelin levels and metabolic risk markers in obese women. J Genet Eng Biotechnol 15: 423-429, 2017.
37. Bertrand C, Pradère JP, Geoffre N, Deleruyelle S, Masri B, Personnaz J, Le Gonidec S, Batut A, Louche K, Moro C, *et al*: Chronic apelin treatment improves hepatic lipid metabolism in obese and insulin-resistant mice by an indirect mechanism. Endocrine 60: 112-121, 2018.
38. Zheng XD, Huang Y and Li H: Regulatory role of Apelin-13-mediated PI3K/AKT signaling pathway in the glucose and lipid metabolism of mouse with gestational diabetes mellitus. Immunobiology 226: 152135, 2021.