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Circulating myokines and apelin-13 levels as predictive biomarkers for newly diagnosed type 2 diabetes mellitus

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Type 2 diabetes mellitus (T2DM) is mostly caused by insulin resistance, while insulin sensitivity can be affected by myokines such as myonectin and irisin. In order to manage metabolic disorders, including T2DM, lipid and glucose metabolism must be regulated by apelin-13. The present study was designed to identify the levels of myonectin, irisin, and apelin-13 in prediabetes and recently developed T2DM that may help in the early diagnosis of the disease. The 180 individuals participated in this cross-sectional study. Four millilitres of venous blood were drawn in the morning after fasting for the whole night. Tests were carried out for each participant involving glucose using the glucose oxidase methods, HbA1c using the ion exchange high-performance liquid chromatography (HPLC) technique, insulin using the sandwich-based electrochemiluminescence immunoassay (ECLIA) technique, myonectin, irisin, and apelin-13 using sandwich-based enzyme-linked immunosorbent (ELISA) technique. Notably, the levels of myonectin and apelin-13 were increased gradually in T2DM more than in prediabetes and healthy controls. Conversely, the irisin level was lowered progressively in T2DM than in prediabetes and healthy subjects with statistical variations ($P< 0.001$) among study groups. This study concluded that the increased levels of myonectin and apelin-13 as well as decreased irisin levels may be considered predictive biomarkers for the early diagnosis of T2DM.

Keywords: Blood, Diagnosis, Hyperglycemia, Hyperinsulinemia, Insulin resistance, Obesity, Prediabetes

T2DM is typified by hyperglycemia triggered by insufficient insulin synthesis, action, or both, systemic inflammation, and beta cell dysfunction in the pancreas[1].T2DM accounts for 90-95% of instances of diabetes, which impacts 422 million individuals globally[2]. T2DM reduces the triggering of signals from proteins that are not insulin-dependent in a number of tissues, including skeletal muscle, due to insulin binding to its receptor impairing signaling[3].Hyperglycemia and hyperinsulinemia develop from insulin resistance (IR), which happens when the pancreatic beta cells that produce insulin to maintain blood glucose levels continue to do so without obtaining any response from the cells that are sensitive to it[4,5].

Myonectin is a member of the C1q/tumor necrosis factor-related protein isoform 15 (CTRP) family, which is made by skeletal muscle to control the metabolism of lipid and glucose throughout the body[6]. During exercise, myonectin is released into the bloodstream through the elevated calcium levels brought on by the contraction of muscles[7].By controlling glucose and lipid metabolism through increased AMPK activity, triggering glucose transporters (GLUTs) in the skeletal muscle, and enhancing glucose absorption in the myocytes, myonectin may help avoid the development of IR[8].Therefore, myonectin is a myokine that senses nutrients and may play a significant function in diabetes and associated conditions. Furthermore, myonectin may be a diagnostic biomarker for anticipating the onset of T2DM and prediabetes[9].Another myokine that is produced in skeletal muscle is irisin[10]. The disintegrin and metallopeptidase proteins cleave fibronectin type III domain containing 5 proteins (FNDC5) in order to generate 112 amino acid peptide irisin[11]. Moreover, this myokine enhances skeletal muscle and heart insulin receptor sensitization, hepatic glucose and lipid metabolism, pancreatic β cell functions, and the transformation of white adipose tissue to energy-burning brown adipose tissue, all of which diminish obesity, insulin resistance, and T2DM[12,13]. The G-protein-coupled APJ receptor has an endogenous ligand termed apelin[14]. The 77-amino acid peptide apelin can split into subtypes of varying fractions, such as apelin-36, apelin-17, apelin-13, and apelin-12 in its pyroglutamate form[15].

GLUTs are responsible for transferring glucose across the cell membrane[16]. The movement of GLUT4

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from the cytoplasm to the plasma membrane can be facilitated by apelin-13, which is related to glucose uptake and IR[17]. Patients with T2DMmay have lower levels of GLUT4, resulting in less glucose being absorbed and used[18]. By activating the AMPK and PI3K/Akt signalling pathways and phosphorylating Akt, Apelin-13 can improve the metabolism of lipids and glucose[19,20]. Early diagnosis of prediabetes and T2DM is crucial in the prevention of T2DM complications and treatment of it. Therefore, this study was designed to detect circulating myonectin, irisin, and apelin-13 in prediabetes and recently developed T2DM to help in the diagnosis and treatment of the disease.

Materials and Methods

Study design

The 180 individuals who participated in this study were separated into three categories: those with T2DM, prediabetes, and healthy controls. Newly identified groups with prediabetes and type 2 diabetes visited specialist clinics at Basra city's Al-Fayhaa Teaching Hospital. They were between thirty and seventy-five years old. WHO criteria were applied to identify participants with prediabetes and T2DM using fasting serum glucose and HbA1c[21]. Healthy controls did not have diabetic mellitus (DM).Participants in the current study were excluded if they had kidney, cardiovascular, liver, or thyroid gland diseases, were pregnant, had T1DM, or were taking medications that influence glucose metabolism. Each participant had four millilitres of venous blood drawn in the morning after fasting for the whole night, and the blood was split into two tubes using a disposable syringe.

Initially, two millilitres of blood were moved into a vacutainer tube that contained EDTA K3 in order to estimate the HbA1c right away.Next, two millilitres of blood were put into an evacuated tube with a gel and clot activator. The serum was separated, and the insulin and glucose levels were immediately determined by centrifuging the blood for 15 min at 3500 revolutions per minute (RPM) after it had stood for half an hour. The remaining serum was placed in an Eppendorf tube and was stored in a deep freeze at -80°C for later tests of myonectin, irisin, and apelin-13.

The collection of blood samples occurred between the start of April 2024 and the last day of August 2024. Name, age, sex, height, weight, and waist circumference were among the details gathered from each participant.

Methods

The application of a balance for evaluating weight and a stadiometer to determine standing height, this formula was utilized to determine the body mass index (BMI): This is the BMI: weight (kg) divided by height (m^2)[22].

Serum glucose has been determined by the enzymatic (glucose oxidase) method in a fully automated biochemistry analyzer[23] (Spin 200 analyzer, Spinreact Company, Spain). Whole blood was tested for glycosylated haemoglobin A1c (HbA1c) by the ion exchange high-performance liquid chromatography (HPLC) technique in a fully automated analyzer (D-10 analyzer, Bio-Rad Company, USA)[24].

The sandwich-based electrochemiluminescence immunoassay (ECLIA) technique was applied in a fully automated immunoassay instrument (Cobas e 411 analyzer, Roche business, Germany) to determine the serum insulin level[25]. Insulin resistance was assessed using the homeostatic model assessment of insulin resistance (HOMA-IR) formula[26]: HOMA-IR is equivalent to fasting insulin (mIU/L) × fasting glucose (mg/dl)/405.After thawing the frozen serum for thirty min at 22°C, the serum was centrifuged for five min at 3000 RPM to determine the amounts of myonectin, irisin, and apelin-13. Quantitative sandwich-based enzyme-linked immunosorbent test (ELISA) kits (LOT No. 202406, Shanghai Ideal Medical Technology Company, China) were used to estimate them. With the Elisys Uno (ELISA) instrument (Human company, Germany), a fully automated system was employed.

Statistical analysis

All data analysis was done using version 26.0of SPSS. A one-way ANOVA (analysis of variance) Tukey's HSD in post hoc analysis was used for group comparison. Pearson's correlation coefficients were utilized for evaluating the association between numerical variables. Compared to healthy controls, the data's mean, standard deviations (SD), and F-value were shown. A significance value below 0.05 was regarded as statistically significant.

Results

Three equal-number categories were formed from the 180 participants in the current study and matched in terms of anthropometric characteristics, such as age, height, weight, BMI, and sex, with non-significant differences ($P > 0.05$). However, newly identified T2DM had statistically significant increases in waist

circumference, glucose, HbA1c, insulin, and HOMA-IR compared to prediabetes and healthy controls ($P< 0.001$), as present in clinical baseline data in (Table 1).

The results in Table 2 displayed mean ± SD of myonectin and apelin-13 levels that were increased gradually in newly developed T2DMmore than in prediabetes and healthy controls. Conversely, the irisin level was lowered progressively in recently identified T2DMthan in prediabetes and healthy subjects with significant differences ($P<0.001$) among study groups.

Circulating myonectin, irisin, and apelin-13 among study groups based on age groups were summarized in (Table 3). As compared to subjects in the 30-45 age group, those in the 41-60 age group and those in the 61-75 age group had myonectin levels that increased steadily with age in normal persons. However, in prediabetes and newly diagnosed T2DM, myonectin levels decreased inversely with age over time. These differences across study groups and age groups were significant ($P< 0.001$).

Table 1 — Clinical baseline data of study groups

Variables	Control Mean ± SD (n=60)	Prediabetes Mean ± SD (n=60)	T2DM Mean ± SD (n=60)	Mean difference	95% Confidence Interval		F-value
					Lower Bound	Upper Bound	
Age (years)	51.8 ± 13.55	52.75 ± 12.90	52.48 ± 11.68	-0.95	-6.45	4.55	0.09
				-0.68	-6.18	4.81	
				0.27	-5.23	5.76	
BMI (kg/m^2)	27.80 ± 4.13	28.77 ± 5.01	28.63 ± 4.89	-0.97	-2.99	1.06	0.75
				-0.84	-2.86	1.19	
				0.13	-1.89	2.16	
Waist circumference (cm)	99.37 ± 4.38	107.27 ± 7.10[a]	111.43 ± 6.13[b, c]	-7.90[a]	-10.48	-5.32	63.12
				-12.07 [b]	-14.65	-9.49	
				-4.17 [c]	-6.75	-1.59	
Glucose (mg/dL)	85.5 ± 8.27	117.15 ± 4.27[a]	208.27 ± 73.70[b, c]	-31.65[a]	-50.16	-13.14	132.53
				-122.77 [b]	-141.27	-104.26	
				91.12 [c]	72.61	109.62	
HbA1c (%)	5.01 ± 0.29	6.10 ± 0.22[a]	9.09 ± 1.60[b, c]	-1.09[a]	-1.50	-.68	297.61
				-4.09 [b]	-4.49	-3.68	
				-2.99[c]	-3.40	-2.58	
Insulin (µlU/mL)	7.79 ± 2.22	21.09 ± 5.09[a]	28.32 ± 6.85[b, c]	-13.30[a]	-15.50	-11.11	251.10
				-20.53 [b]	-22.73	-18.33	
				-7.23 [c]	-9.42	-5.03	
HOMA-IR	1.64 ± 0.50	6.11 ± 1.53[a]	13.93 ± 4.41[b, c]	20.53 [a]	-5.63	-3.30	316.10
				7.23 [b]	-13.45	-11.12	
				-12.29 [c]	-8.99	-6.65	

[a]comparison between prediabetes and control groups was significant at p<0.001,[b] comparison between T2DM and control groups was significant at p<0.001, and[c] comparison between T2DM and prediabetes groups was significant at p<0.001, as determined by one-way ANOVA Tukey HSD.

Table 2 — Comparison of myonectin, irisin, and apelin-13 levels among study groups

Biomarkers	Control Mean ± SD (n=60)	Prediabetes Mean ± SD (n=60)	T2DM Mean ± SD (n=60)	Mean Difference	95% Confidence Interval		F-value
					Lower Bound	Upper Bound	
Myonectin (pmol/mL)	63.54 ± 4.35	83.62 ± 5.15[a]	103.20 ± 4.54[b, c]	-20.08[a]	-22.11	-18.06	1071.47
				-39.66[b]	-41.69	-37.64	
				-19.58[c]	-21.61	-17.56	
Irisin (ng/mL)	8.06 ± 0.29	7.08 ± 0.27[a]	5.07 ± 0.28[b, c]	0.98 [a]	0.86	1.10	1745.54
				2.99 [b]	2.87	3.11	
				-2.01 [c]	1.89	2.13	
Apelin-13 (ng/mL)	0.33 ± 0.18	0.63 ± 0.09[a]	0.97 ± 0.13[b, c]	-0.30[a]	-0.36	-0.24	313.77
				-0.64 [b]	-0.70	-0.58	
				-0.34 [c]	-0.40	-0.28	

[a]comparison between prediabetes and control groups was significant at p<0.001, [b] comparison between T2DM and control groups was significant at p<0.001, and[c] comparison between T2DM and prediabetes groups was significant at p<0.001, as determined by one-way ANOVA Tukey HSD.

Age-related changes in irisin levels were not observed in normal persons, prediabetes, or newly developed T2DM, but there were notable variations within the study groups ($P< 0.001$). When comparing respondents in the 30-45 age range to those in the 41-60 age group and the 61-75 age group, the apelin-13 level increased gradually and directly with age in normal persons. Additionally, patients in the age groups of 30-45 years, 46-60 years, and 61-75 years had lower apelin-13 levels in prediabetes and recently diagnosed T2DM. These differences across study categories were significant ($P< 0.001$).

Circulating levels of myonectin, irisin, and apelin-13 among study groups based on sex were summarized in (Table 4). Men had significantly higher levels of myonectin in normal subjects, prediabetes, and recently identified T2DM than in women in healthy individuals, prediabetes, and newly diagnosed T2DM.Whereas there was a non-significant difference ($P \geq 0.05$) in irisin and apelin-13 level between men of normal subjects, prediabetes, and recently developed T2DM compared to women of healthy controls, prediabetes, and newly developed T2DM.

Table 5 presented the levels of serum myonectin, irisin, and apelin-13 in all groups in the study depending on BMI. Myonectin levels gradually elevated with body weight in healthy subjects; obese subjects had higher levels than overweight subjects and normal subjects. Myonectin levels in prediabetes were similarly higher in obese people than in overweight people and people of normal weight.

Table 3 — Comparison of circulating myonectin, irisin, and apelin-13 among study groups based on age groups

Biomarkers	Groups	Group 1 (Mean ± SD)	Group 2 (Mean ± SD)	Group 3 (Mean ± SD)	Significance level
Myonectin (pmol/mL)	Control (n=60)	62.24 ± 3.78	63.1 ± 4.35	65.42 ± 4.21	0.000
	Prediabetes (n=60)	84.67 ± 4.71	83.77 ± 4.72	82.63 ± 6.03	0.000
	T2DM (n=60)	104.64 ± 4.76	103.04 ± 4.35	101.8 ± 4.34	0.000
Irisin (ng/mL)	Control (n=60)	8.11 ± 0.31	8.09 ± 0.29	7.99 ± 0.29	0.000
	Prediabetes (n=60)	7.05 ± 0.32	7.08 ± 0.25	7.08 ± 0.24	0.000
	T2DM (n=60)	5.02 ± 0.28	5.1 ± 0.31	5.1 ± 0.25	0.000
Apelin-13 (ng/mL)	Control (n=60)	0.26 ± 0.14	0.28 ± 0.17	0.44 ± 0.16	0.000
	Prediabetes (n=60)	0.6 ± 0.09	0.61 ± 0.07	0.70 ± 0.09	0.000
	T2DM (n=60)	0.96 ± 0.14	0.92 ± 0.14	1.03 ± 0.11	0.000

*Group 1 (30-45 years), Group 2 (46-60 years), Groups 3 (61-75 years)

Table 4 — Comparison of myonectin, irisin, and apelin-13 levels among study groups according to sex

Biomarkers	Groups	Men (Mean ± SD)	Women (Mean ± SD)	Significance level
Myonectin (pmol/mL)	Control (n=60)	67.31 ± 2.09	59.76 ± 2.17	0.000
	Prediabetes (n=60)	88.25 ± 2.10	78.99 ± 2.30	0.000
	T2DM (n=60)	107.23 ± 2.09	99.17 ± 2.00	0.000
Irisin (ng/mL)	Control (n=60)	8.07 ± 0.31	8.04 ± 0.28	0.761
	Prediabetes (n=60)	7.12 ± 0.23	7.03 ± 0.30	0.219
	T2DM (n=60)	5.09 ± 0.21	5.04 ± 0.34	0.496
Apelin-13 (ng/mL)	Control (n=60)	0.33 ± 0.17	0.33 ± 0.18	0.942
	Prediabetes (n=60)	0.63 ± 0.10	0.63 ± 0.08	1.000
	T2DM (n=60)	0.97 ± 0.13	0.97 ± 0.14	0.924

Table 5 — Comparison of myonectin, irisin, and apelin-13 among study groups based on BMI

Biomarkers	Groups	Normal weight (Mean ± SD)	Overweight (Mean ± SD)	Obese (Mean ± SD)	Significance level
Myonectin (pmol/mL)	Control (n=60)	61.83 ± 4.59	63.56 ± 4.27	65.23 ± 3.67	0.000
	Prediabetes (n=60)	81.78 ± 5.01	83.44 ± 5.11	102.97 ± 4.44	0.000
	T2DM (n=60)	65.23 ± 3.67	86.81 ± 4.64	105.43 ± 4.39	0.000
Irisin (ng/mL)	Control (n=60)	8.35 ± 0.11	8.11 ± 0.09	7.71 ± 0.15	0.000
	Prediabetes (n=60)	7.35 ± 0.11	7.11 ± 0.08	6.84 ± 0.15	0.000
	T2DM (n=60)	5.35 ± 0.11	5.11 ± 0.08	4.78 ± 0.17	0.000
Apelin-13 (ng/mL)	Control (n=60)	0.13 ± 0.04	0.33 ± 0.05	0.54 ± 0.05	0.000
	Prediabetes (n=60)	0.54 ± 0.05	0.63 ± 0.04	0.74 ± 0.05	0.000
	T2DM (n=60)	0.83 ± 0.04	0.95 ± 0.05	1.13 ± 0.05	0.000

*Normal weight (18.5 – 24.9 kg/m2), Overweight (25.0 – 29.9 kg/m2), Obese (≥ 30 kg/m2)

Table 6 — Correlation between anthropometric parameters and predictive biomarkers in T2DM

Parameters	HOMA-IR	Age	Waist circumference	BMI	Glucose	HbA1c	Insulin	Myonectin	Irisin	Apelin-13
HOMA-IR	1	0.04	0.19	0.38	0.53	0.30	0.37	0.22	-0.38	0.42
Age	0.04	1	-0.03	-0.12	-0.01	-0.07	-0.06	-0.26	0.12	0.22
Waist circumference	0.19	-0.03	1	0.76	-0.09	-0.01	0.29	0.02	-0.76	0.66
BMI	0.38	-0.12	0.76	1	-0.02	-0.04	0.44	0.25	-0.98	0.82
Glucose	0.53	-0.01	-0.09	-0.02	1	0.51	-0.54	-0.01	0.04	-0.04
HbA1c	0.30	-0.07	-0.01	-0.04	0.51	1	-0.30	-0.04	0.07	-0.09
Insulin	0.37	-0.06	0.29	0.44	-0.54	-0.30	1	0.24	-0.45	0.51
Myonectin	0.22	-0.26	0.02	0.25	-0.01	-0.04	0.24	1	-0.25	0.22
Irisin	-0.38	0.12	-0.76	-0.98	0.04	0.07	-0.45	-0.25	1	-0.80
Apelin-13	0.42	0.22	0.66	0.82	-0.04	-0.09	0.51	0.22	-0.80	1

Obese people with newly diagnosed T2DM had higher myonectin levels than overweight people and normal weight people. There were also statistically significant differences between the BMI groups and study groups ($P< 0.001$). In contrast, irisin levels in normal controls were steadily diminished in obese individuals compared to normal-weight people and overweight persons. In cases of prediabetes, obese people had lower irisin levels than overweight people and people of normal weight. In newly diagnosed type 2 diabetes, the obese group's irisin level was lower than that of the overweight group and the normal weight group. It was found that there were high differences between the BMI groups and study groups. Lastly, in healthy individuals, Apelin-13 levels increased progressively with body weight; obese individuals exhibited greater levels than overweight individuals and subjects of normal weight. Similar to prediabetes, obese individuals had higher levels of apelin-13 than in overweight people and people of normal weight. Obese people with newly diagnosed T2DM had higher apelin-13 levels than overweight people and normal weight people, and statistically significant differences were found among BMI groups and study groups ($P< 0.001$).

Table 6 shows the relationships between body measurements and biomarkers that can predict T2DM. The results show that there were no significant links between HOMA-IR and age. While insulin resistance had significant positive correlations with waist circumference, BMI, glucose, HbA1c, insulin, myonectin, and apelin-13. Whereas there was a significant negativeassociation between insulin resistance and irisin.There was no significant association between age and waist circumference, glucose, HbA1c, andinsulin.However, age had significant, weak direct associations with irisin and apelin-13. On the contrary, age had a significant inverse relationship withBMI and myonectin.

No significant correlations were found between waist circumference and serum glucose, HbA1c, and myonectin. While there was a significant direct relationship between waist circumference and BMI, insulin, and apelin-13. In contrast, there was a significant inverserelationship between waist circumference and irisin.BMI had no significant relationship with glucose and HbA1c. Inversely, there was a significantdirectassociation of BMI with insulin, myonectin, and apelin-13. whereas BMI had a significantly strong negative association with irisin.

Serum glucose level had a significant moderate positive association with HbA1c. Whereas serum glucose level had a significant moderate negative correlation with insulin. Conversely, serum glucose level had no significant association with myonectin, irisin, and apelin-13. There was a significant weak indirectrelationship between HbA1c and insulin. While HbA1c had a non-significant relationship with myonectin, irisin, and apelin-13. Circulating insulin had significant direct associations with myonectin and apelin-13. Inversely, there was a significant weakindirectassociation between insulin and irisin.Myonectin had a significant weak indirect correlation with irisin,althoughit had a significant weak direct association with apelin-13. Finally, there was a significantly strong negative correlation between irisin and apelin-13.

Discussion

This study revealed a higher mean waist circumference in T2DM cases and prediabetic people than in healthy subjects with significant differences ($P<0.001$). The results of this study were consistent with a Chinese study that indicated that individuals with T2DM and prediabetes had larger waist circumferences than people in good health[27]. Therefore, high waist circumference is considered a risk factor for developing prediabetes and T2DM.

Early diagnosis of prediabetes and T2DMiscrucial in the prevention of T2DM complications and treatment of it. Therefore, our study was designed to detect circulating myonectin, irisin, and apelin-13 in prediabetes and recently developed T2DM to help in the diagnosis and treatment of the disease. This study revealed that gradually increased serum myonectin and apelin-13 levels in recently developed T2DM were more than in prediabetes and healthy individuals, in contrast to progressively decreased serum irisin levels in recently developed T2DM lower than in prediabetes and healthy individuals.

Gamas *et al*.[28] reported that myonectin and irisin may prevent the development of IR as they are included in the metabolism of fats and glucose. On the other hand, the emergence of muscle insulin resistance could potentially have an impact on their activity. The adipose tissue is where myonectin and irisin function; therefore, their disturbance may affect cross-tissue communication, worsen insulin resistance, and disrupt the metabolism of fats and glucose.

The markedly diminished activity and expression of peroxisome proliferator-activated receptor gamma coactivator 1-alpha in the skeletal muscle of cases with T2DM and prediabetes could be responsible for lower blood irisin levels. This protein is crucial for insulin sensitivity, muscle and liver insulin secretion, and glucose metabolism. The gamma coactivator-1-α pathway of the peroxisome proliferator-activated receptor stimulates the irisin, which is produced during exercise[29].

Onalan *et al*.[30] reported that apelin may affect insulin resistance and glucose metabolism; it may also be a helpful marker for preventing complications with early detection in those with prediabetes, and it may play an essential role in treatment.

Two endoplasmic reticulum (ER) stress receptors in pancreatic tissues are expressed by inositol-requiring protein 1a (IRE1a) and phosphorylated by protein kinase R (PKR)-like endoplasmic reticulum kinase (PERK), two signalling pathways that apelin-13 may regulate in order to reduce the pathological alterations brought on by T2DM. The synthesis of the pro-apoptotic transcription factor C/EBP homologous protein (CHOP) and the regulation of c-Jun N-terminal kinase (JNK) phosphorylation demonstrate that apelin-13 may prevent ER stress-dependent death of cells in the pancreas of Akita mice. Apelin-13 enhances the phosphorylation levels of AMPK, protein kinase B (Akt), and extracellular regulatory protein kinases (ERK) while also regulating ER stress[31].

The present study demonstrated thatmen have higher levels of myonectin compared to women in normal subjects, prediabetes, and recently diagnosed T2DM. Whereas men have no significant difference in irisin and apelin-13 levels compared to women of normal subjects, prediabetes, and recently developed T2DM.According to a recent study, obese people had considerably higher serum levels of myonectin and apelin-13, which may indicate that these proteins serve as circulating biomarkers of adiposity and metabolic disorders linked to obesity. Whereas serum irisin levels in the obese people with T2DM were significantly lower than in obese prediabetic and healthy individuals. These findings are identical to those of Kejia *et al*.[32], who discovered that obese T2DM patients had greater myonectin levels than overweight and normal weight T2DM patients. In addition, these results are similar to those of Chinmaya *et al*.[33], who concluded that there is a significant positive correlation between BMI and apelin levels. Insulin induces the generation of apelin in adipocytes, and IR, hyperinsulinemia, and diabetes mellitus have all been related to elevated apelin plasma levels[34]. Alkhader *et al*.[35] reported that the serum irisin levels of T2DM women were significantly less than those of non-diabetic obese women.

This study revealed that circulating insulin had significant direct associations with myonectin and apelin-13 but an inverse relationship between insulin and irisin. Myonectin had a significant indirect correlation with irisin, although it had a significant direct association with apelin-13. In addition, there was a significantly negative correlation between irisin and apelin-13. Myonectin and apelin-13 have a beneficial impact on insulin sensitivity because they are both involved in the metabolism of fat and glucose. Exercise has been demonstrated to raise apelin-13 levels, which could amplify myonectin's positive impacts on insulin sensitivity[31]. On the other hand, despite apelin-13 and myonectinbeing typically thought to be advantageous in metabolic settings, their dysregulation can play an essential part in the pathogenesis of T2DM and its related consequences, underscoring the need for more investigation into these proteins' functions in metabolic disorders. Serum irisin levels in this study were inversely associated with insulin, HOMA-IR, and BMI, indicating that irisin may contribute to the IR brought on by obesity that eventually developed prediabetes and T2DM. These findings have been confirmed by prior study[36]. By improving skeletal muscle, heart, and

liver insulin receptor sensitivity, supporting hepatic glucose metabolism, stimulating pancreatic cell activity, and transforming white to brown adipose tissue, irisin lowers IR [37,38].The study's primary limitations include its limited sample size, its exclusive emphasis on the Basra people, and its identification of apelin-13 and no other apelin isoforms.

Conclusion

This study revealed that levels of the hormones myonectin and apelin-13 in the blood gradually increased from prediabetes to T2DM, while irisin progressively decreased from prediabetes to T2DM. Myonectin and apelin-13 were directly associated with insulin resistance, waist circumference, and BMI. Whereas they were inversely associated with irisin. Irisin was directly correlated with age while indirectly correlated with insulin resistance, BMI, and waist circumference.Accordingly, myonectin, irisin, and apelin-13 may be helpful predictive biomarkers in the diagnosis of prediabetes and T2DM. However, further studies are necessary to elucidate the biochemical mechanism by which myonectin, irisin, and apelin-13 contribute to the pathogenesis of T2DM.

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Conflict of interest

All authors declare no conflict of interest.

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