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Association between irisin and vascular complications of type 2 diabetic patients: a prospective case–control study

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Abstract

Background Diabetes vascular complications are classified as either macrovascular (cardiovascular disease) or microvascular (nephropathy). These complications considerably raise the risk of morbidity and death. Irisin is a myokine that has been linked to metabolic disorders and cardiovascular disease. The purpose of this study was to look at the relationship between irisin and vascular complications among type 2 diabetic (T2DM) individuals. In this case–control study, the patients were put into four groups based on the occurrence of a diabetic cardiovascular complications and the presence of diabetic nephropathy into group 1: twenty T2DM cases without complications, group 2: twenty T2DM cases with diabetic nephropathy, group 3: twenty T2DM cases with cardiovascular complications, and group 4: thirty controls. History was taken, and clinical examination was done. Laboratory investigations (fasting blood glucose, 2-h postprandial blood glucose, HbA1C, cholesterol, triglycerides, HDL-C and LDL-C, serum urea and creatinine, albumin/creatinine ratio, eGFR, serum irisin) were analyzed.

Results Serum level of irisin was significantly lower in T2DM patients than in control. Also, irisin level was significantly lower in diabetic cases with vascular complications versus those without complications. Irisin level had a negative correlation to BMI and lipid profile in diabetic cases and had a positive correlation to eGFR in diabetic patients with cardiovascular complications.

Conclusions Irisin level was significantly lower in T2DM patients than control and in diabetic patients with vascular complications than patients without complications. So, irisin may have a role as a marker of vascular complications in T2DM.

Keywords Type 2 diabetes, Diabetic nephropathy, Cardiovascular complications, Irisin

Background

Diabetes complications include retinopathy, nephropathy, and neuropathy, as well as macrovascular complications such as coronary and peripheral artery disease, cardiomyopathy, and cerebrovascular disease [1]. In affected people, these consequences are the principal cause of morbidity and death [2].

Irisin, a novel myokine secreted from the skeletal muscles after exercising, reduces insulin resistance and type 2 diabetes (T2DM) by enhancing the insulin receptor's sensitivity in the skeletal muscle and the translocation of the glut4 protein, which increases glucose uptake and improves glucose utilization by inhibiting gluconeogenesis [3, 4].

Previous research found a significantly decreased serum irisin concentration in T2DM patients with macrovascular complications, an association between irisin and diabetic microvascular complications, and a

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significant decrease in serum irisin concentration in patients with chronic kidney disease compared to healthy controls. This demonstrates the potential use of irisin as a biomarker in T2DM patients who have a higher probability of developing CVD [5–8].

The availability of novel biomarkers related to diabetic nephropathy and cardiovascular risk may constitute a major advance in prevention of the vascular complications of diabetes. As few studies had investigated serum irisin as a predictive agent, so the target of this study was to evaluate the diagnostic utility of serum irisin as a predictor of DN and CVD complications in T2DM patients.

Materials and methods

This case–control study was done in the period between January 2022 and January 2023 on patients visiting the outpatient clinics and the inpatient department of the internal medicine at Faculty of Medicine of Cairo University. Approval to start the research was ensured from the Research Ethics committee of the Faculty of Medicine of Cairo University (MS-232–2022). An informed signed consent was taken from all participants.

The study involved ninety participants, aged 20 to 65, divided into 4 groups: group I, which involved 20 T2DM patients without complications; group II, which involved 20 T2DM patients with diabetic nephropathy; group III, which involved 20 T2DM patients with cardiovascular complications; and group VI, which involved 30 non-DM participants. The study also included 60 patients with T2DM. Included were diabetic individuals on insulin or oral hypoglycemic medications.

Patients were not included in the study if they were with type 1 diabetes, severe infection, patients on regular dialysis, patients using pioglitazones, hormonal preparations, or immune inhibitors such as hydrocortisone, prednisone, and cyclosporine.

All individuals provided a complete medical history, with particular emphasis on their age, gender, duration of their diabetes mellitus, how it was being treated, any vascular complications from type 2 diabetes, and other comorbidities. Blood pressure, waist circumference, weight, height, and body mass index (BMI) measurements were all part of the clinical evaluation.

Laboratory investigations

Fasting blood glucose (FBG), 2-h postprandial blood glucose (2-h PPG), fasting lipids {total cholesterol (TC), triglycerides (TAG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C)}, glycosylated hemoglobin (Hb A1c), urea, creatinine, albumin/creatinine ratio, eGFR, and irisin levels were measured.

Measurement of eGFR

GFR (ml/min/1.73 m^2) = 175 × (Scr)-1.154 × (age)-0.203 × (0.742 if female) × (1.212 if African American) [9].

Diagnostic criteria for diabetes mellitus [10]

- HbA1c level ≥ 6.5% (≥ 48 mmol/mol Hb)
- Fasting plasma glucose value of ≥ 126 mg/dl (≥ 7.0 mmol/l)
- Two-hour oral glucose tolerance test (OGTT) value in venous plasma ≥ 200 mg/dl (≥ 11.1 mmol/l) (in the absence of unequivocal hyperglycemia, results should be confirmed by repeated testing).
- In a patient with classic symptoms of hyperglycemia or hyperglycemic crisis, a random plasma glucose ≥ 200 mg/dl (11.1 mmol/l) is diagnostic.

Diagnosis of diabetic nephropathy [11]

Before a patient is diagnosed with albuminuria, two of three urine albumin-creatinine ratio specimens collected during 3 to 6 months must be abnormal (> 30 mg/g).

Diagnosis of cardiovascular disease

Assessment of cardiovascular damage was done by echocardiography and angiography.

Sampling

Five milliliters of venous blood was taken following a 12- to 16-h fast and separated into two portions through venipuncture. In order to measure HbA1C using a cation exchange resin, 2 ml of blood was first added to an EDTA-containing tube. The second component, 3 ml of residual blood in plastic serum tubes, was maintained at − 80 °C for irisin analysis. Serum irisin levels were determined using ELISA kits. This kit comprises a microtiter plate that has been pre-coated with a specific antibody. After the samples were added, a biotin-conjugated-specific antibody was added to the corresponding microtiter plate wells.

The clinical examination and laboratory tests were performed by investigators who were blinded to the patient groups (diabetic or non-diabetic).

Outcomes

The ability of serum irisin to predict vascular complications in T2DM patients was the primary outcome of this work. The secondary outcomes were comparing the level of irisin in all groups, to explore the relation between irisin and body mass index (BMI), blood pressure, fasting blood glucose, 2-h postprandial blood glucose, HbA1C, cholesterol, triglycerides, HDL-C and LDL-C, serum urea and creatinine, albumin/creatinine ratio, and eGFR.

Statistical approaches

Version 26 of the Social Sciences Statistical Package (SPSS) (IBM Corp., Armonk, NY, USA) was used to code and submit the data. Quantitative variables were summed using the mean and standard deviation, while categorical components were summed using frequencies (number of instances) and relative frequencies (percentages), where comparing the groups, the unpaired *t*-test was used to compare differences between two groups, and analysis of variance (ANOVA) was used to compare differences between multiple groups where there were more than two [12]. Categorical data were separated using the chi-squared test. When the anticipated frequency was less than 5, the exact test was instead used [13]. The Pearson correlation coefficient was used to construct correlations between the quantitative variables [14]. To determine the preferred cut-off value of irisin for determining T2DM and associated vascular complication, an area under curve analysis was used to produce a ROC curve. In order to identify independent irisin forecasters, linear regression analysis was used [15]. Statistical significance was considered if *P*-values < 0.05.

Sample size

To determine the relationship between irisin and vascular complications in type 2 diabetes patients, an analytical case–control research was proposed. An earlier investigation found that irisin has a 0.98 accuracy as possible indicators for atherosclerosis in association to type 2 diabetes [1]. Therefore, the sample size to examine the findings of the current investigation with a significant *P* 0.05 and 80% study power is determined using the following formula: Z1-α/2 = 1.96, $AUC = 0.98$, and $d = 0.05$. Therefore, the minimum sample size for cases is $n = 30$. For the comparable degree of confidence, the standardized value is Z1-/2. At 95% CI, it is 1.96, and at 99% CI or 1% type I error, it is 2.58. d is the marginal error. Therefore, the study should enroll at least 30 diabetes participants and 30 healthy controls [16]. A total of ninety participants—thirty healthy controls, twenty type 2 diabetics without problems, twenty type 2 diabetics with diabetic nephropathy, and twenty type 2 diabetics with cardiovascular issues—will take part in our study.

$$n_{case} = n_{non-case} \geq \frac{Z^2_{1-a/2}\, V(AUC)}{d^2}$$
$$V(AUC) = \left(0.009 \times e^{-a^2/2}\right) \times \left(6a^2 + 16\right)$$
$$a = Z_{AUC} \times 1.414$$

Results

One-hundred-two patients were enrolled in the study, but only 90 completed the study. 30 apparently healthy individuals represented the control group and sixty T2DM represented patients' groups. Table 1 shows the demographics of the groups.

Regarding SBP and DBP, their values were higher in all 3 groups of patients compared with the control with *P*-value (< 0.0001), but there was insignificant difference between diabetic patients vs diabetic with DN, diabetic patients vs diabetic with CVD, and diabetic with DN vs diabetic with CVD with *P*-values 0.051, 0.388, and 0.268, respectively (Table 2).

Regarding FBG and 2HPP, their values were higher in all 3 groups of patients compared with control with

Table 1 Demographics of the studied patients

		Control group No. = 30	Patients group No. = 60	*p*-value	Control group No. = 30	DM only No. = 20	Diabetic nephropathy No. = 20	DM + cardiovascular No. = 20	*p*-value
Age (years)	**Mean ± SD**	**31.20 ± 7.77**	**55.00 ± 9.81**	**< 0.0001**	**31.20 ± 7.77**	**50.55 ± 13.28**	**55.40 ± 7.76**	**59.05 ± 4.91**	**< 0.0001**
Gender	**Female**	24 (80.0%)	29 (48.3%)	0.004	24 (80.0%)	10 (50.0%)	12 (60.0%)	7 (35.0%)	0.012
	Male	6 (20.0%)	31 (51.7%)		6 (20.0%)	10 (50.0%)	8 (40.0%)	13 (65.0%)	
BMI (kg/m^2)	**Mean ± SD**	23.60 ± 4.23	29.32 ± 4.80	< 0.0001	23.60 ± 4.23	31.39 ± 5.43	29.17 ± 4.00	27.40 ± 4.20	< 0.0001
Duration (years)	Median		10 (4 – 14)			4.5 (4–10)	7 (4–10)	13.5 (11–15)	< 0.0001
Medication	Insulin		22 (36.7%)			2 (10.0%)	4 (20.0%)	16 (80.0%)	< 0.0001
	OHG		36 (60.0%)			18 (90.0%)	14 (70.0%)	4 (20.0%)	
	Both		2 (3.3%)			0 (0.0%)	2 (10.0%)	0 (0.0%)	
Comorbidity	No		37 (61.7%)			15 (75.0%	10 (50.0%	12 (60.0%)	0.262
	HTN		23 (38.3%)			5 (25.0%	10 (50.0%	8 (40.0%)	

Data are expressed by mean ± standard deviation (SD), median (interquartile 1–3), numbers, percent (%), *P*-value < 0.05 is significant, *P*-value < 0.0001 is highly significant

Table 2 Comparison between control and patients' groups regarding clinical and laboratory data

		Control group No. 30	Patients group No. 60	*p*-value	DM only No. 20	Diabetic nephropathy No. 20	DM + cardiovascular No. 20	*p*-value
Systolic BP (mmHg)	**Mean ± SD**	**109.00 ± 9.14**	**128.83 ± 14.39**	**< 0.0001**	**125.00 ± 11.47**	**133.00 ± 17.20**	**128.50 ± 13.48**	**< 0.0001**
Diastolic BP (mmHg)	Mean ± SD	70.00 ± 6.43	81.17 ± 8.65	< 0.0001	81.50 ± 6.71	82.00 ± 11.96	80.00 ± 6.49	< 0.0001
FBG (mg/dl)	Mean ± SD	86.00 ± 10.98	137.20 ± 46.35	< 0.0001	116.95 ± 31.29	156.60 ± 39.42	138.05 ± 57.54	< 0.0001
2hPP (mg/dl)	Mean ± SD	122.00 ± 8.73	241.52 ± 82.38	0.0001	202.75 ± 71.12	274.10 ± 55.56	247.70 ± 100.95	< 0.0001
HbA1C%	Mean ± SD	5.35 ± 0.14	8.15 ± 1.71	< 0.0001	7.43 ± 1.49	8.93 ± 1.16	8.11 ± 2.08	< 0.0001
Urea (mg /dl)	Mean ± SD	21.73 ± 4.39	39.87 ± 15.04	< 0.0001	28.30 ± 4.62	54.30 ± 14.03	37.00 ± 10.74	< 0.0001
Creatinine (mg/dl)	Mean ± SD	0.67 ± 0.10	1.04 ± 0.24	< 0.0001	0.95 ± 0.21	1.13 ± 0.30	1.05 ± 0.17	< 0.0001
A/C (mg)	Median	7 (4 – 9)	16 (9.3 – 168)	< 0.0001	12.5 (8–19)	201.5 (168–411)	11.5 (8–13)	< 0.0001
e-GFR (ml/min/1.73 m^2)	Mean ± SD	121.13 ± 17.70	73.32 ± 19.06	< 0.0001	81.50 ± 19.64	67.50 ± 21.86	70.95 ± 12.34	< 0.0001
LDL/cholesterol (mg/dl)	Mean ± SD	74.87 ± 11.38	118.15 ± 32.95	< 0.0001	114.45 ± 20.08	115.00 ± 37.79	125.00 ± 38.28	< 0.0001
TG (mg/dl)	Mean ± SD	84.00 ± 29.17	122.78 ± 36.33	< 0.0001	129.15 ± 36.70	133.50 ± 25.25	105.70 ± 40.55	< 0.0001
Total cholesterol (mg/dl)	Mean ± SD	123.73 ± 40.66	185.98 ± 47.38	< 0.0001	173.95 ± 44.73	196.30 ± 37.57	187.70 ± 57.36	< 0.0001
HDL (mg/dl)	Mean ± SD	69.6 ± 6.1	52.72 ± 11.65	< 0.0001	56.00 ± 11.08	48.30 ± 12.34	53.85 ± 10.98	< 0.0001

Data are presented by mean ± standard deviation (SD), median (interquartile 1–3), numbers, percent (%), *P*-value < 0.05 is significant, *P*-value < 0.0001 is highly significant. P1, control vs DM; P2, control vs DN; P3, control vs DM + cardiovascular; P4, DM vs DN; P5, DM vs DM + cardiovascular; P6, DN vs DM + cardiovascular

P-value (< 0.0001); also, a highly significant difference between diabetic patients vs diabetic with DN was found, but there was insignificant difference between diabetic patients vs diabetic with CVD and diabetic with DN vs diabetic with CVD with *P*-values 0.071 and 0.112, respectively. HbA1C in different DM groups was of higher values denoting inefficient diabetes control in diabetic patients with significant difference between controls and patient groups (Table 2).

Regarding renal functions (urea, creatinine, A/C ratio, eGFR), there was significant difference between control group and all 3 groups of patients that have higher values compared to control group. According to creatinine level, there was insignificant difference between diabetic patient vs diabetic patient with DN and diabetic patient with DN vs diabetic with CVD with *P*-values 0.140 and 0.187, respectively (Table 2). A/C ratio shows significant difference between all groups except between diabetics vs diabetic patient with DN that show no significant difference with *P*-value 0.606. According to eGFR, there was no significant difference between diabetic patient vs diabetic patient with DN and diabetic patient with DN vs diabetic with CVD with *P*-values 0.070 and 0.550, respectively (Table 2).

All groups of T2D with and without vascular complications show hyperlipidemia in comparison to control group, but there were no significant difference in between different groups of patients.

As regarding LDL/cholesterol level, there was no significance between diabetic patients vs diabetic with DN, diabetic patients vs diabetic with CVD, and diabetic with DN vs diabetic with CVD with *P*-values 0.950, 0.233, and 0.258, respectively. Regarding triglyceride levels, there was no significant difference between diabetics vs diabetic with DN with *P*-value (0.678).

Cholesterol levels show no significance between diabetic patients vs diabetic with DN, diabetic patients vs diabetic with CVD, and diabetic with DN vs diabetic with CVD with *P*-values 0.121, 0.338, and 0.549, respectively. HDL levels show significant difference between diabetic patients vs diabetic with DN and diabetic with DN vs diabetic with CVD with *P*-values 0.018 and 0.086, respectively. Yet, it shows no significance between diabetic patients vs diabetic with CVD with *P*-value 0.503 (Table 2).

Serum irisin shows a significantly lower levels in all patients and patients with DN and diabetic patients with CVD than in control group with *P*-values 0.005, 0.009, and 0.041, respectively, but it shows insignificant difference between patients with T2DM without cardiovascular complications and control group with *P*-value 0.057 (Table 3).

Table 3 Comparison and post hoc analysis between control, DM, DN, and DM with cardiovascular disease regarding serum irisin

		Control group No. 30	All patients No. 60	DM only No. 20	Diabetic nephropathy No. 20	DM + cardiovascular No. 20	*p*-value
Serum irisin (ng/dl0)	**Mean ± SD**	**1.39 ± 0.90**	1.02 ± 0.28	**1.06 ± 0.27**	**0.94 ± 0.17**	**1.05 ± 0.36**	**0.039**
	Post hoc analysis						
	P1	**P2**	**P3**	**P4**	**P5**	**P6**	**P7**
Serum irisin	**0.005**	**0.057**	**0.009**	**0.041**	**0.532**	**0.942**	**0.570**

Data are presented by mean ± standard deviation (SD). *P*-value > 0.05, nonsignificant; *P*-value < 0.05, significant; *P*-value < 0.01, highly significant. P1, control vs all patients; P2, control vs DM only; P3, control vs DN; P4, control vs DM + cardiovascular; P5, DM vs DN; P6, DM vs DM + cardiovascular; P7, DN vs DM + cardiovascular

There was also insignificant difference between diabetic patients vs diabetic with DN, diabetic patients vs diabetic with CVD, and diabetic with DN vs diabetic with CVD with *P*-values 0.532, 0.942, and 0.570, respectively (Table 3).

Among all diabetic patients, type 2 diabetes patients with DN, and patients with type 2 diabetes with CVD, there was a significant lower level of serum irisin in female diabetic patients than diabetic males except for type 2 diabetes patients with CVD which there was no significant difference. There was no significant difference in serum irisin level among patients as regards treatment, the presence of hypertension, albuminuria, and different grades of eGFR except for patients with diabetic nephropathy where different grades of eGFR among diabetic patients with DN have significant relation with irisin serum levels (Table 4).

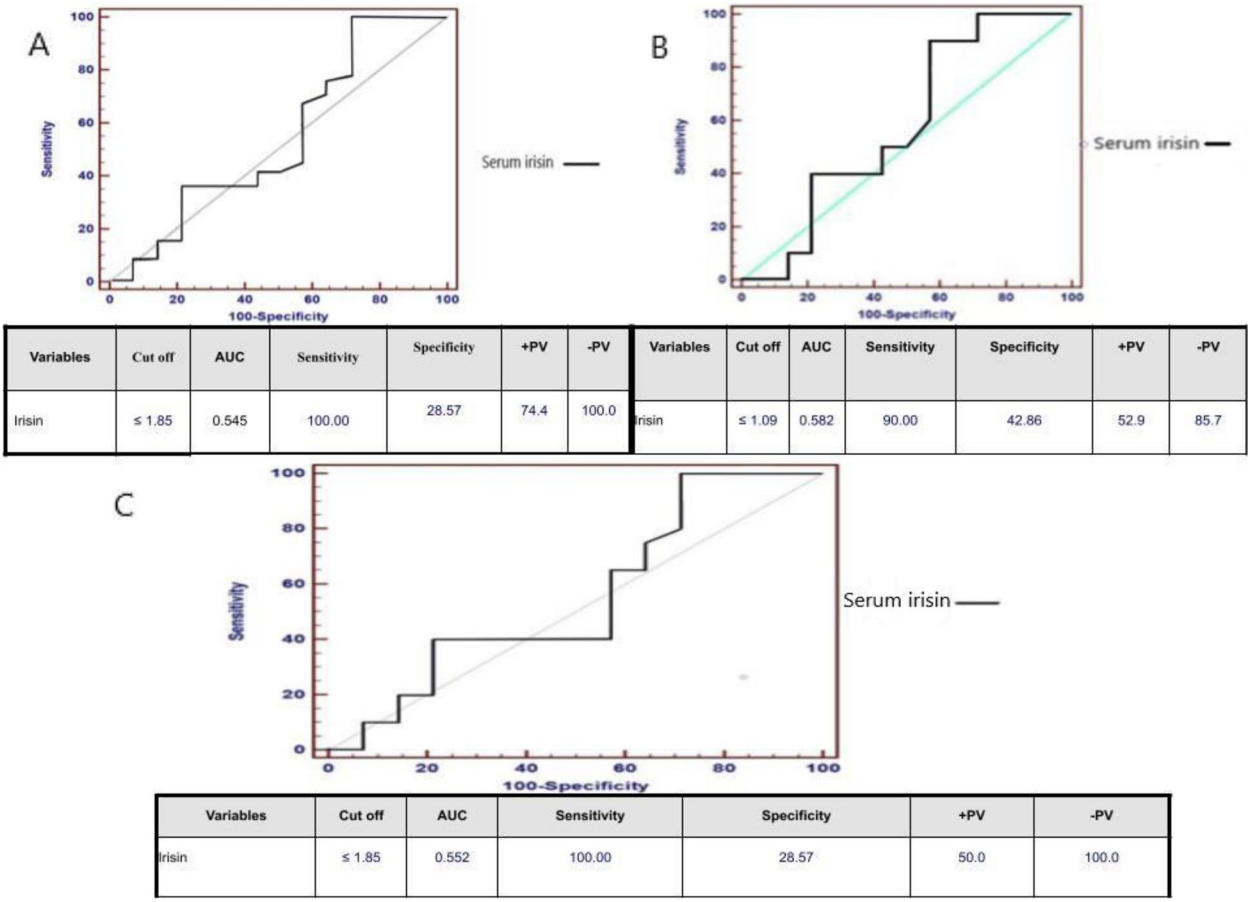
ROC curve was constructed to assess the best cut-off values of serum irisin between controls and type 2 diabetic patients. It revealed an AUC of 0.545, and at a cut-off level of ≤ 1.85, the sensitivity is 100%, and specificity is 28.57%. ROC curve was constructed to assess the best cut-off values of serum irisin between control group versus DN patients. It revealed an AUC of 0.582, and at a cut-off level of ≤ 1.09, the sensitivity is 90%, and specificity is 42.86%. Another ROC curve was constructed to assess the best cut-off values of serum irisin between control group versus diabetic patients with CVD. It revealed an AUC of 0.552, and at a cut-off level of ≤ 1.85, the sensitivity is 100%, and specificity is 28.57% (Fig. 1).

The correlation between irisin and BMI, LDL/cholesterol, TAG, and T. cholesterol among diabetic patients group showed negative significant correlation with *P*-values 0.048, 0.026, 0.006, and 0.000, respectively, with no significant correlation regarding HDL/cholesterol with *P*-value 0.407. On the other hand, the correlations between serum irisin levels with other anthropometric and biochemical variables as age, duration of diabetes,

Table 4 Relation of serum irisin with other parameters among the whole patients, DM only, T2DM with DN, and T2DM with CVD groups

		All T2DM Serum irisin Mean ± SD	*p*-value	DM only Serum irisin Mean ± SD	*p*-value	Diabetic nephropathy Serum irisin Mean ± SD	*p*-value	DM only Serum irisin Mean ± SD	*p*-value
Gender	Female	0.92 ± 0.22	**0.007**	0.91 ± 0.29	**0.007**	0.88 ± 0.12	**0.033**	0.99 ± 0.25	0.620
	Male	1.11 ± 0.3		1.24 ± 0.07		1.04 ± 0.19		1.08 ± 0.41	
Insulin	No	1 ± 0.23	0.569	1.08 ± 0.27	0.518	0.95 ± 0.17	0.781	0.85 ± 0.17	0.227
	Yes	1.04 ± 0.34		0.94 ± 0.41		0.93 ± 0.19		1.1 ± 0.38	
OHG	No	1.04 ± 0.35	0.662	0.94 ± 0.41	0.518	0.85 ± 0.19	0.208	1.1 ± 0.38	0.227
	Yes	1 ± 0.22		1.08 ± 0.27		0.97 ± 0.16		0.85 ± 0.17	
Comorbidity	No	1.01 ± 0.32	0.846	1.05 ± 0.29	0.762	0.91 ± 0.09	0.433	1.05 ± 0.45	0.972
	HTN	1.02 ± 0.21		1.09 ± 0.26		0.98 ± 0.22		1.04 ± 0.17	
A/C groups	Normal	1.05 ± 0.32	0.217			1 ± 0.18	0.090		
	Microalbuminuria	1 ± 0.18				0.87 ± 0.13			
	Macroalbuminuria	0.87 ± 0.13				0.92 ± 0.02			
eGFR groups	G1	1.03 ± 0.14	0.207	1.15 ± 0.15	0.251	1.06 ± 0.19	**0.021**	0.99 ± 0	0.161
	G2	1.07 ± 0.32		0.98 ± 0.31		0.76 ± 0.08		1.15 ± 0.37	
	G3a	0.87 ± 0.27		1.29 ± 0		0.93 ± 0.09		0.79 ± 0.26	

Data are presented by mean ± standard deviation (SD), numbers, *P*-value < 0.05 is significant, *P*-value < 0.0001 is highly significant



Variables	Cut off	AUC	Sensitivity	Specificity	+PV	-PV
Irisin	≤ 1.85	0.545	100.00	28.57	74.4	100.0

Variables	Cut off	AUC	Sensitivity	Specificity	+PV	-PV
Irisin	≤ 1.09	0.582	90.00	42.86	52.9	85.7

Variables	Cut off	AUC	Sensitivity	Specificity	+PV	-PV
Irisin	≤ 1.85	0.552	100.00	28.57	50.0	100.0

Fig. 1 **A** ROC curve for serum irisin to detect DM type II patients. **B** ROC curve for serum irisin to differentiate between control and DM + cardiovascular groups. **C** ROC curve for serum irisin to differentiate between control and DN groups

SBP, DBP, FBG, 2HPP, HbA1c, urea, creatinine, A/C ratio, and eGFR were evaluated in diabetic patients group showed no significant correlations between irisin serum levels and those variables (Table 4).

The multivariate linear regression found that BMI, LDL, and triglycerides were independent predictors of serum irisin levels, while total cholesterol shows significant negative correlation with serum irisin among all diabetic patients with *P*-value 0.008. In diabetic patients without CVD, the multivariate linear regression found that BMI show significant negative correlation (*P*-value < 0.0001), and HbA1C show significant positive correlation (*P*-value < 0.0001) with serum irisin. In diabetic patients with DN, it showed that BMI and triglycerides were independent predictors of serum irisin levels, while A/C ratio show significant negative correlation with serum irisin (*P*-value 0.003). The univariate linear regression also found that T. cholesterol showed significant negative correlation with serum irisin among diabetic patients with CVD complications (*P*-value 0.012) (Table 4).

The association between irisin serum levels and BMI and HbA1C in type 2 diabetes patients without vascular problems ($n = 20$) revealed a negative significant correlation with *P*-values 0.016 and 0.034, respectively. In type 2 diabetes patients with diabetic nephropathy, the correlation between irisin serum levels and age showed a positive significant correlation with *P*-value 0.003 and a negative significant correlation with BMI, A/C ratio, and triglycerides with *P*-values 0.024, 0.024, and 0.037. The link between irisin serum levels and eGFR, LDL/cholesterol, triglycerides, and cholesterol in individuals with type 2 diabetes and CVD indicated a negative significant correlation with *P*-values 0.030, 0.004, 0.002, and 0.001, respectively (Table 5).

Discussion

The aim of this case–control research was to evaluate the diagnostic ability of serum irisin as a DN and CVDs complication predictors in patients with T2DM. Previous investigations targeted this relationship as irisin plays an important role in slowing down diabetic

Table 5 Correlation of serum irisin with other studied parameters among cases with DM only, T2DM with DN, and T2DM with CVD groups

	DM only		T2DM with DN		T2DM with CVD	
	Serum irisin					
	r	*p*-value	*r*	*p*-value	*r*	*p*-value
Serum irisin (ng/dl)	–	–	–	–	–	–
Age years	0.255	0.308	0.634	0.003	0.067	0.779
BMI (kg/m^2)	−0.560	0.016	−0.502	0.024	−0.243	0.301
Duration (years)	0.068	0.789	−0.227	0.336	−0.402	0.079
Systolic BP (mmHg)	−0.113	0.656	0.147	0.536	0.302	0.196
Diastolic BP (mmHg)	−0.442	0.066	0.152	0.524	0.028	0.908
FBG (mg/dl)	0.222	0.377	−0.018	0.939	0.107	0.653
2hPP (mg/dl)	0.089	0.724	0.200	0.398	0.227	0.337
HbA1C%	0.501	0.034	0.085	0.721	0.097	0.685
Urea (mg/dl)	0.333	0.177	−0.049	0.839	−0.103	0.664
Creatinine (mg/dl)	0.219	0.383	−0.115	0.629	−0.034	0.886
A/C (mg)	−0.103	0.684	−0.503	0.024	−0.181	0.446
e-GFR (ml/min/1.73 m^2)	−0.137	0.587	0.236	0.316	0.485	0.030
LDL/cholesterol (mg/dl)	0.082	0.746	0.006	0.980	−0.612	0.004
TG (mg/dl)	0.222	0.376	−0.468	0.037	−0.658	0.002
Total cholesterol (mg/dl)	−0.096	0.706	−0.418	0.067	−0.693	0.001
HDL (mg/dl)	−0.092	0.717	−0.055	0.819	0.283	0.226

P-value > 0.05, nonsignificant; *P*-value < 0.05, significant; *P*-value < 0.01, highly significant Spearman correlation coefficient

nephropathy and has role in cardiovascular protection [5–8]. We hypothesized that as type 2 diabetes progresses to vascular complications, serum irisin level would decrease. Our findings confirmed that serum irisin levels were lower in cases with DN and cardiovascular complications than in controls, and that irisin levels were considerably lower in DN and CVD patients than in diabetics with no vascular complications. Serum irisin levels were shown to be inversely associated to BMI, LDL, triglyceride, and cholesterol levels. Serum irisin and eGFR were reported to have a significant positive correlation in diabetics with cardiovascular complications.

Furthermore, the ROC curve showed the efficacy of serum irisin's discriminating ability as a predictor of DN in T2DM patients, with an AUC of 0.582, and at a cut-off level of 1.09, the sensitivity is 90%, and the specificity is 42.86%. Another ROC curve highlighted the value of serum irisin's discriminating ability as a predictor of CVD in T2DM patients, with an AUC of 0.552, and at a cut-off level of 1.85, the sensitivity is 100%, and the specificity is 28.57%.

Obesity has raised the prevalence of T2DM in the majority of the developed countries as well as in developing ones. Sedentism is well recognized as one of the key causes of the expanding pandemic of T2DM; exercise affects glycemic parameters by improving blood glucose management, lipid profile, and lowering cardiovascular risk factors [17].

Myokines are cytokines generated by muscles that perform favorable metabolic tasks throughout the body in an autocrine, paracrine, or endocrine way. By attaching to receptors in many organs such as fatty tissue, liver, kidney, pancreas, and other organs such as the bone and brain, myokines operate as signal molecules to interact with other organs and participate in the development of metabolic and cardiovascular complications [18]. Myokines have a role in metabolic control and can help with a variety of diseases as insulin resistance, type 2 diabetes, and cardiovascular disease [19].

Irisin is a novel myokine released into the circulation by skeletal muscle contraction after its proteolytic breakdown from its precursor fibronectin type III domain-containing protein 5 (FNDC5), which is controlled by peroxisome proliferator-activated receptor gamma coactivator (PGC 1 alpha), a key controller of genes involved in metabolism, thermogenesis, and antioxidation [20]. According to a recent study, exercise causes favorable changes in T2DM, including a rise in irisin levels [21].

Irisin has been demonstrated to enhance insulin resistance, browning of white adipose tissue, fatty acid oxidation, metabolic disorder and oxidative stress inhibition, and endoplasmic reticulum stress. Furthermore, irisin reduces cardiac remodeling, decreases ROS damage in

the myocardium in diabetic cardiomyopathy, increases cardiac fibrosis reduction, and improves myocardial function [22].

Our results are agreed with Carmona-Maurici et al. research [5] where irisin levels had negative correlation with atheroma parameters, and irisin also showed good predictability for plaque presence (*AUC* 0.81). However, this study did not target diabetic patients. Khorasani et al. [23] showed that serum irisin had a significantly higher levels in the diabetics without coronary artery disease (CAD) in comparison to the cases with CAD, and also, irisin levels were associated with the presence of CAD in diabetes ($P=0.038$) according to the logistic regression.

The relationship between serum HDL, cholesterol, and plasma irisin is noteworthy; earlier research has shown that exercise can enhance HDL/cholesterol ratios [24].

Further research indicates that greater HDL/cholesterol levels have been associated with higher amounts of irisin, which can be used for medicinal purposes. Through reverse cholesterol transport and anti-inflammatory action, HDL/cholesterol protects against atherosclerosis [25].

More research is needed to determine irisin's capacity to directly elevate HDL/cholesterol and alter the atherosclerotic process. Our findings are consistent with those of Liu et al., who discovered that lower levels of serum irisin were related with the occurrence of nephropathy in diabetic individuals [26].

Albuminuria is an early sign of diabetic nephropathy development in diabetes. In our investigation, serum irisin exhibited no significant link with albumin/creatinine ratio in all diabetes patients; however, we found a negative correlation between serum irisin and A/C ratio in diabetic nephropathy patients. Previous research in diabetic individuals indicated that low circulating irisin levels were related to the grade of albuminuria [7].

In a sample of diabetic individuals with cardiovascular complications, we found a negative relation between serum irisin and eGFR. In our investigation of diabetic individuals without complications and diabetic nephropathy, we found no significant association between irisin levels and eGFR or serum creatinine levels. This discovery contradicts prior research, which found that a decrease in irisin levels correlated with the advancement of chronic kidney disease (CKD) stage [27]. One of the proposed explanations for decreased irisin levels in CKD is that an increase in serum creatinine generates negative feedback inhibition on muscle irisin production [24].

The findings of this investigation revealed no significant relationships between serum irisin and glycemic indices in all T2DM patients. However, we found a link between irisin and HbA1C in individuals with type 2 diabetes who did not have vascular complications. This is consistent with prior research, since Stengel et al. discovered no link between irisin and blood glucose levels in diabetes patients and controls in the study [28].

These findings contradict prior study that found a link between serum irisin and fasting hyperglycemia and insulin resistance in people with metabolic syndrome [29]. In nondiabetic patients, Liu et al. observed a connection between irisin levels and blood glucose levels [26]. El-Haddad et al. discovered a link between serum irisin and glycemic indices [30].

In individuals with type 2 diabetes, we found a negative relationship between irisin levels and atherogenic lipids (LDL, triacylglycerol, and cholesterol). Oelmann et al. discovered a link between serum irisin and an acceptable lipid profile in the general population [31]. Data on the relationship between irisin levels and BMI are currently contradictory. Some scientists found a favorable relationship between circulating irisin levels and BMI, while others found the inverse [32]. However, we discovered a link between circulating irisin levels and BMI.

Limitations of the study

The small sample size limited the significance of the research, so a larger sample is needed to verify our results. The control group was only 30 patients that could affect the results, and it was better to increase this group to 60 patients for more reliable results. The study was done in single center, and it would be more favorable to be multicentric. The association with insulin resistance was not investigated in this study. However, the findings of this study can open new areas for future research.

Conclusions

Serum irisin levels were lower in cases with DN and cardiovascular complications than in the controls, and irisin levels were significantly lower in DN cases and CVD patients than in those with diabetics with no vascular complications. The ROC curve used to determine serum irisin's potential as a predictor of DN and CVD in T2DM cases revealed that serum irisin can predict the incidence of DN and CVD in T2DM cases.

Abbreviations

AUC	Area under the curve
BMI	Body mass index
CVD	Cardiovascular
DN	Diabetic nephropathy
eGFR	Estimated glomerular filtration rate
T2DM	Type 2 diabetic

Authors' contributions

HK, conceptualization of study; MH, collected the data; AH, analyzed and interpreted the patient data; NT, study design; and MY was a major contributor in writing the manuscript.

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

Declarations

Ethics approval and consent to participate
Approval to start the research was ensured from the research ethics committee of the Faculty of Medicine of Cairo University (MS-232–2022). An informed signed consent was taken from all participants.

Consent for publication
Informed signed consent was taken from all participants.

Competing interests
The authors declare that they have no competing interests.

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