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Serum Irisin Level as a Marker of Erectile Dysfunction

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ABSTRACT

Background: Erectile dysfunction (ED) is characterised by a persistent or recurring inability to achieve and/or sustain an erection adequate for sexual pleasure.

Objectives: This study aimed to measure serum irisin levels in ED individuals comparing with those in healthy controls, and to identify whether it can be correlated with the ED severity.

Methods: This prospective case-control work had been performed on 50 male participants with ED, aged from 20 to 50 years old, with ED, and BMI between 18.5 – 24.9 kg/m^2 (ED group) and 25 healthy individuals as control (Control group). Each subject was exposed to pharmacopenile colour Doppler ultrasound, biochemical blood tests, lipid profile, and hormonal analysis. Assessing serum irisin by ELISA and being contrasted with the control group.

Results: There was a significantly decreased level of serum irisin in ED group contrasted to the control group. There was a significant negative association among the level of irisin in serum and age, BMI, duration of ED, glycated haemoglobin (HbA1c) and fasting blood glucose (FBG). While, there was a significant positive association among the level of irisin in serum with mean peak systolic velocity and international index of erectile function (IIEF-5) scores. Irisin level demonstrated an excellent validity for discrimination between ED group and control group with higher sensitivity and specificity. **Conclusions:** Low serum irisin level could be deemed as a risk factor for developing ED especially in type 2 DM (T2DM) making screening of irisin level in diabetic patient to be an investigation that can be added to the work up for patients with T2DM.

Keywords: Serum irisin, Erectile dysfunction, Diabetes mellitus.

INTRODUCTION

ED represents a global public health issue, impacting as many as 52% of males between the ages of 30 and 80 years. The vascular aspect is the predominant factor influencing the occurrence of ED [(1)]. Traditional risk factors for ED usually include age, obesity, dyslipidemia, metabolic syndrome, diabetes mellitus (DM), hypertension, and aspects of an unhealthy lifestyle such as smoking [(2)].

Irisin is among the most regarding hormones. The molecular weight is about 22 kDa, comprising around 163 amino acid residues [(3)]. Prior studies have demonstrated that irisin is a broke down and secreted fragment of fibronectin type III domain containing 5 (FNDC5), functioning as a hormone-like polypeptide that mediates various functions [(4-5)].

Irisin is a myokine, which is expelled from skeletal muscle as an exercise responses. Research indicates that irisin could be useful as a therapeutic agent for metabolic disorders that might be ameliorated through exercise [(6)]. This myokine enhances the uncoupling protein-1 (UCP1) expression in adipocytes, facilitating the "browning" of white adipose tissue, that results in higher energy expenditure, thermogenesis, and enhanced sensitivity to insulin [(3)].

The correlation between level of irisin and ED in clinical contexts has not been extensively studied. **Kumsar *et al.*** [(7)] investigated the level of irisin in serum in diabetic individuals with ED, and found that irisin was significantly low among individuals with ED, and hence considered as a microvascular complication of type 2 DM. We hypothesised that irisin serum level may be considered as an efficient biomarker for anticipating the occurrence and ED severity.

The aim of this work was to measure serum irisin levels in ED individuals as contrasted to healthy controls and to identify whether it can be correlated with the severity of ED.

PATIENTS AND METHODS

This prospective case-control work had been performed on 50 male participants aged from 20 to 50 years old with ED who experienced challenges in achieving or sustaining an erection for a minimum of six months, were -active within a stable heterosexual relationship, cohabitated with their partner for a minimum of one year, and had only one sexual partner. Also, who attempted sexual intercourse at an average rate of one or more times per week? Type 2 DM diagnosis was done depending on the European Association for the Study of Diabetes (EASD) guidelines criteria [(8)]. BMI between 18.5 – 24.9 kg/m^2. 25 healthy individuals were involved as control.

Exclusion criteria: Patients diagnosed with an additional sexual disorder, significant relationship issues, tobacco use, substance abuse, Peyronie's disease, penile anatomical abnormalities, prior genitourinary surgeries, medical conditions, hypertension, or symptomatic coronary artery disease within the last six months, respiratory disease, hyperlipidemia, infection, endocrinal disorders, renal, hepatic or neurological diseases, malignancy, professional athletes participated in intense physical

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exercise during the final week of sampling, and major psychological or psychiatric disorders.

Each subject had been exposed to complete history taking, physical examinations, laboratory tests [fasting blood sugar (FBS), hemoglobin (Hb) A1c, lipid profile, total serum testosterone, serum prolactin and thyroid stimulating hormone (TSH)] and radiological investigations [pharmaco-penile colour Doppler ultrasound (PPDU)].

Individuals with a BMI ≥ 30 kg/m² had been categorized as obese depending on the WHO classification [9]. The validated Arabic version of IIEF-5 was used for diagnosing the existence of ED and to evaluate its severity. Items of the IIEF-5 are phrased regarding the last 6-month period. The ED severity was categorised as mild, moderate, or severe, with scores of 5–7 which suggests severe ED, 8–11 moderate ED, 12–16 mild-moderate ED, 17–21 mild ED, and 22–25 suggesting no ED [10].

Sample size calculation: Using Stata Corp. 2021. Stata statistical software: Release 17. College Station, TX: STATA CORP LLC., and published work by **Kumsar *et al.*** [7], concerning serum irisin level on ED in diabetic men compared to healthy control groups. The null hypothesis was considered as the absence of difference between studied groups regarding the level of irisin in serum. The expected mean serum irisin level in DM with ED group is 5.4 ng/mL, in DM without ED group is 8 ng/mL, while in control group, mean was 16.7 ng/mL as reported by **Mai *et al.*** [11]. Number of groups was 3 (DM with ED, DM without ED and control groups). Expected effect size was 0.4; utilising estimated sample size for one-way ANOVA F test for group effect. Minimal required sample size was 22 subjects per group (total 66 subjects) using α error 5% and a power of 80%.

International index of erectile function (IIEF): The most frequently employed sexual adjustment questionnaire (SAQ), a 5-item questionnaire addressing and quantifying erectile function. Four of the five variables in the IIEF-5 are derived from the erectile functioning domain of the IIEF-15. The final item pertains to sexual intercourse satisfaction, selected to represent a key component of the NIH definition of ED that correlates ED to satisfaction by emphasising the necessity to preserve an erection with adequate rigidity and duration for satisfactory sexual performance. ED severity is categorised into five levels according to the IIEF: severe (one to seven), moderate (eight to eleven), mild to moderate (twelve to sixteen), mild (seventeen to twenty one), and no ED (twenty two to twenty five) [12]. The validated Arabic version of IIEF-5 was used for Egyptian subjects [13].

Pharmaco-penile color Doppler ultrasonography (PPDU): Pharmacological stimuli were conducted employing 10 micrograms of PGE1 administered intracavernuosly. Subsequently, a high-resolution colour Doppler US (Mindray DC-N2, Shenzhen Mindray Biomedical Electronics Co., Ltd., Shenzhen, China) equipped with a 5–10 MHz linear probe (75L38EA) had been utilised. Prior to injection, a penile assessment was conducted to rule out corporeal fibrosis and Peyronie's plaques. Both cavernous arteries diameters have been determined at the proximal penile shaft. The assessment of intracavernous blood flow was conducted at the peno-scrotal junction over a 20-minute period, throughout which end diastolic velocity (EDV), peak systolic velocity (PSV), and resistance index have been assessed for each cavernous artery at the point of maximal clinical action. In the interpretation of PPCDU studies, the following values are regarded as normal: A rise in cavernous artery diameter exceeding 75%, PSV of 35 cm/sec or greater, EDV less than 5 cm/sec, and RI greater than 0.9. The patient is classified as having arterial insufficiency if the PSV is less than 25 cm/sec; veno-occlusive dysfunction if the EDV exceeds 5 cm/sec; and a nonvascular disorder if the PSV is > 30 cm/sec, the EDV is less than or equal to 5 cm/sec, and the RI is greater than 0.75 [14]. Each participant adhered to the study protocol with no complications.

Assessment of serum irisin by ELISA method: Irisin had been assessed in serum utilising human irisin ELISA kit (Bio Vision, Milpitas, CA). This test kit supplied one original standard reagent. It was diluted depending on the instruction. Samples and irisin-antibody labelled wasn't introduced with biotin and streptavidin-HRP. Only chromogen solutions A and B, as well as the stop solution, are permitted and all other procedures remained unchanged. Standard 50 µl; streptavidin-HRP 50 µl. Forty microlitres of sample was added, followed by the addition of ten microlitres of irisin-antibody and fifty microlitres of streptavidin-HRP. The sealing membrane was then applied, and the mixture was gently shaken and incubated for sixty minutes at 37°C. The membrane was meticulously removed, and the plate had been rinsed five times with wash buffer. Wells had been treated with 300 µl of wash buffer for duration of 30 seconds to 1 minute per wash. Fifty microlitres of chromogen solution A were added, followed by fifty microlitres of chromogen solution B to each well. Gently mixed and incubated for ten minutes at 37°C in the absence of light. Fifty microlitres of stop solution was introduced to each well to terminate the reactions, resulting in an immediate colour change from blue to yellow. The blank well was designated as zero. The OD was detected at a wavelength of 450 nm, conducted within 10 minutes after the introduction of the stop solution employing a microplate reader. The linear regression equation for the standard curve was derived from the concentration of standards and their corresponding OD values. Subsequently, the values of the OD of the sample were applied to this regression equation to determine the sample's concentration.

Ethical approval: The work had been performed following approval from The Ethics Committee Mansoura University Hospitals, Mansoura, Egypt (approval code: MS.22.09.2127). Each subject signed an informed consent. The study adhered to the Helsinki Declaration throughout its execution.

Statistical analysis

Statistical analysis was conducted employing SPSS version 26.0. Shapiro-Wilks test and histograms were employed to evaluate the data distribution normality. Quantitative parametric variables were displayed as mean ± SD and contrasted among both groups employing unpaired Student's t- test. Quantitative non-parametric data were displayed as median and IQR and were analyzed by Mann Whitney-test. Qualitative parameters were displayed as frequencies and percentages (%) and were analysed employing the X^2-test or Fisher's exact test when appropriate. A two tailed correlation between various variables was done using Pearson moment correlation equation. Roc curve was used for evaluation of diagnostic performance specificity, sensitivity, negative predictive value (NPV) and positive predictive value (PPV). Univariate regression was utilised to measure the relationship among dependent parameter and one independent parameter. Multivariate regression was also utilised to measure the relationship among dependent parameter and more independent parameters. P value ≤ 0.05 was considered statistically significant.

RESULTS

An insignificant variation existed regarding age, BMI, creatinine and TGs between both groups. The FBG, HbA1c, s. prolactin level and cholesterol were significantly greater in the ED group contrasted to the control group (P < 0.05). Serum testosterone, s. irisin levels and IIEF-5 had been significantly decreased in the ED group contrasted to the control group (P <0.05) (Table 1).

Table (1): Comparison between participants with ED and the control group regarding demographic data and laboratory parameters

parameter		ED (n=50)	Control (n=25)	Test	P
Age (years)		49.0(28.0–59.0)	56.0(26.0–59.0)	U =521	0.241
BMI (kg/m^2)		23.90(20.0–24.90)	23.80(21.0–24.90)	U 642.0	0.848
Blood glucose level	**FBG (mg/dL)**	105.0(70.0–243.0)	88.0(70.0–105.0)	U=293.5*	**<0.001***
	HbA1c (%)	5.80(4.50–10.10)	5.20(4.20–6.20)	U=378.0*	**0.005***
Hormonal analysis	**S. testosterone (ng/mL)**	5.75(3.10–21.70)	8.20(3.80–12.30)	U=889.5*	**0.003***
	S. prolactin (ng/mL)	9.74±2.36	8.06±2.00	t=2.256*	**0.027***
Creatinine (mg/dL)		0.90(0.70–1.40)	0.80(0.70–1.20)	U =490.0	0.121
Cholesterol (mg/dL)		160.0(97.0–195.0)	123.0(89.0–189.0)	U=329.5*	**0.001***
TGs (mg/dL)		102.0(58.0–149.0)	94.0(59.0–145.0)	U=520.5	0.240
IIEF-5		8.0(5.0–17.0)	23.0(21.0–25.0)	U=0.001	**<0.001***
S. Irisin Level (ng/ml)		3.50(0.01–8.0)	9.0(3.40–41.90)	U=1165.5*	**<0.001***

Median and IQR: Non-parametric test, *Significant <0.05. BMI: Body mass index, FBG: fasting blood glucose, HbA1c: Hemoglobin, ED: Erectile dysfunction, TGs: Triglycerides, IIEF-5: International Index of Erectile Function, U: Mann Whitney test, S: Serum.

Comparison between ED with DM and ED without DM groups showed that age, BMI, s. testosterone, prolactin, creatinine, cholesterol, triglycerides, pharmaco penile colour Doppler ultrasound and penile blood flow showed non-significant variation among both groups. IIEF-5 scores and levels of serum irisin were significantly decreased in participants with DM than in those without DM (Table 2).

Table (2): Comparison between ED with DM and without DM regarding age, BMI, hormonal analysis, s. creatinine, cholesterol, TGs, IIEF-5 and s. irisin level

parameter		ED with DM (n = 25)	ED without DM (n =25)	Test (U)	P
Age (years)		52.0(30.0–59.0)	42.0(28.0–59.0)	230.0	0.109
BMI (kg/m^2)		24.0(21.60–24.90)	23.60(20.0–24.90)	224.0	0.086
Hormonal analysis	**S. testosterone(ng/mL)**	5.60(3.10–13.60)	5.80(3.34–21.70)	349.0	0.479
	S. prolactin(ng/mL)	10.02±2.48	9.46±2.34	t=0.587	0.560
Creatinine (mg/dL)		0.90(0.70–1.40)	0.90(0.70–1.30)	293.0	0.699
Cholesterol (mg/dL)		153.0(99.0–195.0)	162.0(97.0–190.0)	357.5	0.382
TGs (mg/dL)		97.0(69.0–147.0)	102.0(58.0–149.0)	329.0	0.749
IIEF-5		8.0(5.0–11.0)	11.0(5.0–17.0)	472.0*	**0.002***
S. irisin level (ng/ml)		3.20(0.01–8.0)	4.30(3.30–5.70)	578.0*	**<0.001***

Median and IQR: Non-parametric test, *significant <0.05. BMI: Body mass index, HbA1c: Hemoglobin, ED: Erectile dysfunction, TGs: Triglycerides, IIEF-5: International Index of Erectile Function, U: Mann Whitney test, DM: Diabetes Mellitus, S: Serum.

There were insignificant differences in the measured parameters related to penile blood flow between both groups (ED with DM and ED without DM) (**Table 3).**

Table (3): Comparison between ED with DM and without DM regarding Pharmaco penile color Doppler ultrasound

		ED with DM (n = 25)	ED without DM (n =25)	Test (U)	P
Pharmaco penile color doppler ultrasound (PPDU)	**PSV right**	17.50(8.47–102.0)	19.22(8.10–116.2)	359.0	0.367
	PSV left	19.60(8.10–102.0)	22.70(10.35–114.8)	376.0	0.218
	PSV mean	18.80(8.65–102.0)	20.84(10.90–115.5)	366.0	0.299
	EDV right	2.40(0.0–33.02)	2.60(0.0–109.3)	324.0	0.823
	EDV left	2.77(0.0–30.0)	2.40(0.0–71.34)	326.5	0.785
	EDV mean	2.10(0.0–31.50)	2.31(0.0–90.30)	314.0	0.977
	RI right	0.85(0.19–1.84)	0.84(0.06–1.34)	288.5	0.640
	RI left	0.83(0.14–1.20)	0.89(0.17–1.23)	316.0	0.946
	RI mean	0.85(0.50–1.11)	0.85(0.13–1.12)	307.5	0.923

Median and IQR: Non-parametric test, *significant <0.05, U: Mann Whitney test, PSV: Peak systolic velocity, EDV: End-diastolic volume, RI: resistive index.

Regarding type of ED, an insignificant variation existed among arteriogenic and venogenic ED indicating that the type of ED was not associated with variations in s. irisin levels (**Table 4).**

Table (4): Association between S. irisin level and type of ED among patients with ED

		N	S. irisin level (ng/mL)	Test	P
Type of ED	**Arteriogenic**	**34**	3.50(0.01–5.70)	U=311.5	0.410
	Venogenic	**16**	3.70(0.02–8.00)		

Median and IQR: Non-parametric test, ED: Erectile dysfunction.

A significant negative association existed among levels of s. irisin and age, BMI, duration of ED, FBG, and HbA1c. A significant positive association existed among s. irisin levels with mean PSV and IIEF-5 scores, indicating that higher s. irisin levels were correlated with better erectile function and vice versa. Other parameters showed non-significant correlations (Table 5).

Table (5): Correlation between s. irisin level and different parameters among patients with ED

	S. irisin level (ng/ml)	
	rs	**P**
Age (years)	-0.472*	**0.001***
BMI (kg/m^2)	-0.726*	**<0.001***
Duration of ED (years)	-0.500*	**<0.001***
FBG (mg/dL)	-0.596*	**<0.001***
HbA1c (%)	-0.682*	**<0.001***
S. testosterone (ng/mL)	0.108	0.457
S. prolactin (ng/mL)	-0.033	0.820
S. creatinine (mg/dL)	-0.071	0.624
S. cholesterol (mg/dL)	-0.017	0.906
S. TGs (mg/dL)	-0.023	0.877
PSV Mean	0.326*	**0.021***
EDV Mean	0.088	0.543
RI Mean	-0.066	0.650
IIEF-5	0.608*	**<0.001***

*significant <0.05. rs: Spearman's rho , BMI: Body mass index, HbA1c: Hemoglobin, ED: Erectile dysfunction, TGs: Triglycerides, IIEF-5: International Index of Erectile Function, DM: Diabetes Mellitus, PSV :Peak systolic velocity, EDV :End-diastolic volume, RI: resistive index , S: Serum, FBG: fasting blood glucose.

S. irisin level demonstrated an excellent validity for discrimination between ED case group and the control group. And also between ED cases with DM and ED cases without DM, with an AUC of 0.932 and 0.925. Using a cutoff of ≤ 5 and 3.4 ng/mL, the sensitivity was 90.0% and 80.0%, specificity was 80.0% and 96.0%, PPV was 90.0% and 95.24% , NPV was 80.0% and 82.76% , and accuracy was 86.67% and 88.0% respectively (Figure 1).

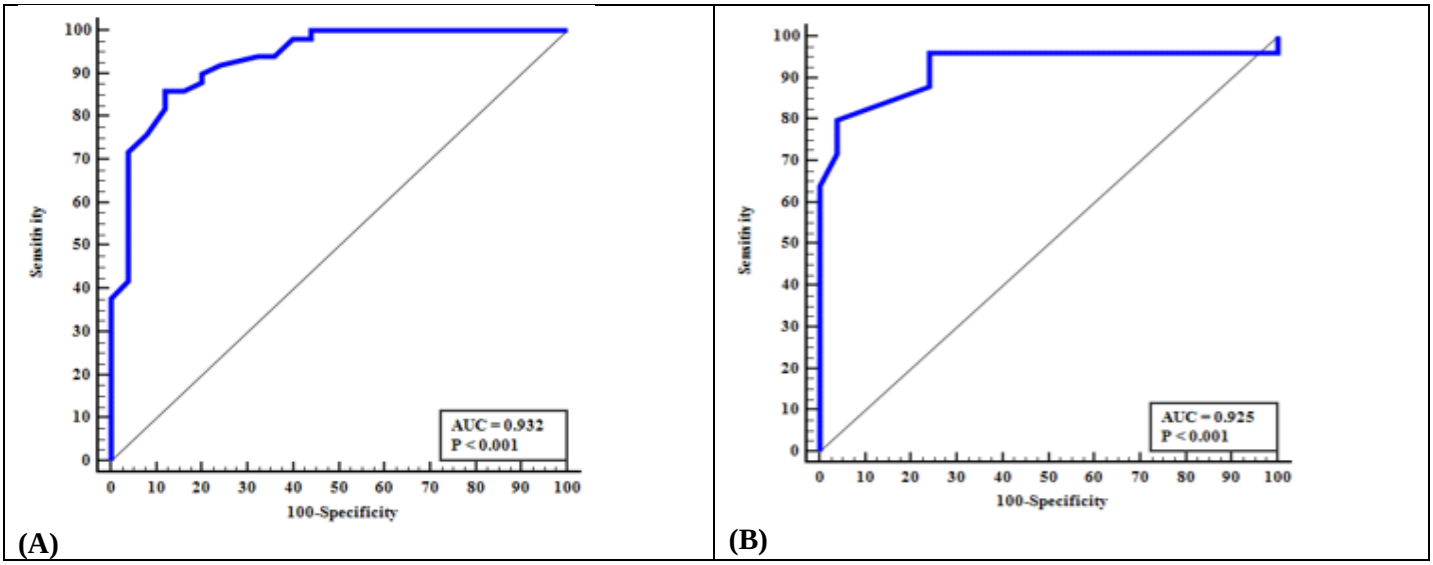

Figure (1): ROC curve for serum irisin level for discrimination between: (A) erectile dysfunction susceptibility and the control group and (B) erectile dysfunction with diabetes mellitus and erectile dysfunction without diabetes mellitus.

Logistic regression analysis was performed to predict of ED susceptibility. In univariable analysis, high FBG, HbA1c, prolactin, cholesterol, low testosterone and low irisin were correlated with risk of ED. However, in multivariable analysis, only high cholesterol and low irisin were considered independent risk predictors for ED susceptibility (Table 6).

Table (6): Logistic regression analysis for prediction of ED susceptibility

	Univariate			Multivariate		
	P	**OR**	**95% C.I**	**P**	**OR**	**95% C.I**
Age (years)	0.477	0.991	0.965-1.017	--	--	--
BMI (kg/m^2)	0.736	0.952	0.717–1.265	--	--	--
FBG (mg/dL)	**0.001***	1.035	1.013–1.057	0.274	1.032	0.975-1.091
HbA1c (%)	**0.002***	1.771	1.226–2.559	0.573	1.716	0.225-2.282
Testosterone (ng/mL)	**0.040***	0.902	0.818–0.996	0.116	0.869	0.729-1.035
Prolactin (ng/mL)	**0.026***	1.124	1.014–1.245	0.058	1.273	0.992-1.635
Cholesterol (mg/dL)	**0.001***	1.018	1.008–1.029	**0.005***	1.039	1.012-1.066
TGs (mg/dL)	0.177	1.008	0.996–1.020	--	--	--
Irisin (ng/mL)	**<0.001***	0.543	0.393–0.750	**0.009***	0.395	0.197-0.794

*significant <0.05. BMI: Body mass index, HbA1c: Hemoglobin, ED: Erectile dysfunction, TGs: Triglycerides, FBG: fasting blood glucose, OR: Odd Ratio, CI: Confidence interval.

DISCUSSION

ED is a significant health issue, which prevalence is progressively increasing with age and strongly associated with the existence of additional co-morbid conditions, including hypogonadism, diabetes, and cardiovascular conditions [(15)]. Serum testosterone level was significantly decreased in the ED group contrasted to the control group, which is in line with **Blute *et al.*** [(16)] who reported an inverse correlation between ED severity and serum testosterone level.

On the contrary, serum prolactin levels had been significantly greater in ED group contrasted to the control group. This finding is in accordance with the results of **Xu *et al.*** [(17)] who demonstrated the negative effect of prolactin on erectile function.

Regarding lipid profile, ED patients showed significantly higher level of serum total cholesterol than control group, but serum TGs did not show significant correlation with ED. These findings agree with the outcomes of **Nikoobakht *et al.*** [(18)] who stated that ED was positively correlated with serum total cholesterol level however, serum TGs level did not show a significant correlation.

In the current study, level of irisin in serum revealed a highly decrease in the ED group versus the control group. These findings agree with the outcomes of **Kumsar *et al.*** [(7)] who demonstrated that level of irisin in serum was significantly lower in ED with type 2 DM, however they didn't notice a relationship between irisin level and severity of ED, which might be due to the different features of the population of the work by including individuals with BMI > 25 and the use of a control group of subjects with type 2 DM, which could be considered as confounding factors. Also, in accordance with our results, **Liu *et al.*** [(19)] showed that serum irisin level was significantly low in T2DM and even lower in existence of T2DM

complication including macrovascular and microvascular complications.

The current work showed a significant negative association among serum irisin level and duration of ED, although **Kumsar *et al.*** [7] did not find such significant relationship between irisin level and duration of ED, which could be attributed, at least partly, to the difference in the inclusion criteria of the control group subjects by including type 2 DM patients. Our results also showed significant negative correlations among level of irisin in serum and age, which is in agreement with results of other investigator who found that irisin level decreases with age [20].

In the current study, we found a significant negative relationship between serum irisin level and BMI. Although, several study results agree with ours regarding the negative association between irisin and BMI, **Choi *et al.*** [21] and **Park *et al.*** [22] revealed a positive relationship between irisin and BMI.

In the present study, fasting blood glucose, and HbA1c were significantly negatively associated with the level of irisin in serum, which agree with the results of **Choi *et al.*** [21]. However, **Liu *et al.*** [19] revealed a significant positive association among irisin and FBG and no significant correlation with HBA1c.

In the current work, logistic regression analysis for predicting risk factors of ED, revealed that high FBG, HbA1c, prolactin, cholesterol, low testosterone and irisin were correlated with risk of ED in univariate analysis. However, in multivariate analysis, only high cholesterol and low irisin were considered independent risk factors for ED, which agree with the findings of **kumsar *et al.*** [7]

In the current work, serum irisin level revealed high accuracy and a significant diagnostic value for ED. Serum irisin level demonstrated an excellent validity for discrimination between ED susceptibility and the control group, with an AUC of 0.932. Using a cutoff of $\leq$ 5 ng/mL, the sensitivity was 90.0%, specificity was 80.0%, PPV was 90.0%, NPV was 80.0%, and accuracy was 86.67%. These results align with the findings of **Kumsar *et al.*** [7] who reported serum irisin cutoff value of 14.7 ng/ml by performing the ROC curve analysis.

CONCLUSIONS

Irisin molecule could be considered as a protective biomarker against ED. Low serum irisin level could be deemed as a predisposing factor for ED development, especially in T2DM, making screening of irisin level in diabetic patient is an investigation that can be added to the work up of patients with T2DM.

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Conflict of Interest: Nil.

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