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Evaluation of Serum Irisin Levels in Patients Developing Nephropathy Due to Type 2 Diabetes Mellitus

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ABSTRACT

Objective: Type 2 diabetes mellitus is a metabolic disease characterized by multi-organ involvement, mostly the renal system, as a result of impaired blood sugar regulation. Damage to the renal system can be serious and can be noticed late. Type 2 diabetes mellitus is associated with adipokine disorders, and irisin molecule, a myokine involved in glucose and lipid metabolism, has also been reported to play a role in the pathogenesis. In this study, it was aimed to compare serum irisin levels between healthy individuals and type 2 diabetes mellitus patients at different stages in terms of nephropathy and to demonstrate that irisin can be a guide in the diagnosis and treatment of the disease in earlier stages before kidney damage develops.

Material and Methods: The study included 101 samples of type 2 diabetes mellitus and 28 healthy volunteers who applied to Çukurova University Balcalı Hospital. The participants in the study were divided into five groups in total. The groups consisted of the first group including healthy volunteers without a history of diabetes, and four separate groups of diabetic nephropathy patients, which were formed by considering Mogensen's staging criteria. Serum irisin levels and routine biochemical parameters were also evaluated in the groups. Chi-square and One Way Anova tests were used for statistical analysis to compare group data.

Results: Serum irisin level was found to be significantly higher in group 1 ($p< 0.001$). Especially in group 2 (Mogensen stage 1 hyperfiltration and hypertrophy stage), serum irisin level was found to be significantly lower than the control group ($p< 0.001$). Hemoglobin A1c, micoralbumin/creatinine and HOMA-IR values were found to be significantly lower in group 1 ($p< 0.001$ for each). It was determined that irisin had a weak negative correlation between hemoglobin A1c, microalbumin/creatinine and glomerular filtration rate respectively ($p= 0.001$, $r= -0.286$), ($p= 0.199$, $r= -0.015$), ($p= 0.142$, $r= -0.158$), while it had a weak positive correlation between HOMA-IR ($p= 0.008$, $r= 0.308$).

Conclusion: According to the data obtained from the study, it was thought that serum irisin levels could be used as a guide in the early diagnosis of diabetic nephropathy before the symptoms related to nephropathy appear and the glomerular filtration rate decreases.

Keywords: Irisin, diabetic nephropathy, type 2 diabetes mellitus, microalbuminuria

ÖZ

Tip 2 Diyabetes Mellitusa Bağlı Nefropati Gelişen Hastalarda Serum İrisin Düzeylerinin Değerlendirilmesi

Giriş: Tip 2 diyabetes mellitus metabolik bir hastalık olup; kan şeker regülasyonunun bozulması sonucu, sıklıkla renal sistem olmak üzere, çoklu organ tutulumuyla karakterize bir hastalıktır. Renal sistemde meydana gelen hasar ciddi olabilmekte ve geç fark edilebilmektedir. Tip 2 diyabetes mellitus, adipokin bozukluklarla ilişkilendirilmekte olup glukoz ve lipit metabolizmasında rol alan bir miyokin olan irisin molekülünün de patogenezde rolü olduğu bildirilmiştir. Bu çalışma ile sağlıklı bireylerle tip 2 diyabetes mellitus hastalarının nefropati açısından farklı evreleri arasında serum irisin düzeyleri karşılaştırılarak irisinin henüz böbrek hasarı gelişmeden daha erken evrelerde hastalığın tanısında ve tedavisinde yol gösterici olabileceğinin ortaya konması amaçlanmıştır.

Gereç ve Yöntemler: Çalışma kapsamında, Çukurova Üniversitesi Balcalı Hastanesine başvuran ve sonraki dönemlerde periyodik kontrollere gelen 18 yaş üstü 101 tip 2 diyabetes mellitus tanılı hasta ve 28 sağlıklı gönüllü birey dahil edilmiştir. Çalışmaya alınanlar toplam beş gruba ayrılmıştır. Gruplar, diyabet öyküsü olmayan sağlıklı, gönüllü bireylerden oluşan birinci grup ve diyabetik nefropati hastalarından, Mogensen'in evreleme kriterleri dikkate alınarak oluşturulmuş dört ayrı gruptan meydana gelmiştir. Gruplarda serum irisin düzeyleri ile birlikte rutin biyokimyasal parametreler de değerlendirilmiştir. Grup verilerinin karşılaştırılmasında One Way Anova ve ki-kare testleri kullanılmıştır.

Bulgular: Serum irisin düzeyi, anlamlı olarak grup 1 için yüksek saptanmıştır ($p< 0.001$). Özellikle grup 2'de (Mogensen evre 1 hiperfiltrasyon ve hipertrofi evresinde) serum irisin düzeyinin birinci gruba göre anlamlı olarak düşük olduğu belirlenmiştir ($p< 0.001$). HOMA-IR, hemoglobin A1c, mikoralbumin/kreatinin değerleri ilk grupta anlamlı oranda düşük bulunmuştur (herbiri için $p< 0.001$). İrisin ile hemoglobin A1c, mikroalbumin/kreatinin ve glomerül filtrasyon hızı arasında zayıf negatif korelasyon saptanırken sırasıyla ($p= 0.001$, $r= -0.286$), ($p= 0.199$, $r= -0.015$), ($p= 0.142$, $r= -0.158$), HOMA-IR ile zayıf pozitif yönde korelasyon saptanmıştır ($p= 0.008$, $r= 0.308$).

Sonuç: Çalışmadan elde edilen verilere göre; serum irisin düzeylerinin diyabetik nefropatinin erken evrelerinde, henüz nefropatiye bağlı semptomlar ortaya çıkmadan ve glomerüler filtrasyon hızı düşmeden önce, erken tanıda yol gösterici olarak kullanılabileceği düşünülmüştür.

Anahtar Kelimeler: İrisin, diyabetik nefropati, tip 2 diyabetes mellitus, mikroalbuminüri

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INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a systemic disease characterized by impaired insulin receptor level, insulin resistance, insulin secretion defect, relative insulin deficiency, impaired blood glucose regulation and multi-organ involvement (1,2). In studies, T2DM has been shown to be directly related to adipokine disorders (3-6) in the pathogenesis of signal originating from adipose tissue and that the changes that occur with the secretion of molecules and inflammation play a role (7).

Irisin is a molecule identified in 2012 and has been defined as a hormone secreted from skeletal muscle in humans and mice due to exercise (8-14). It has been defined as a new myokine that enables the transformation of white adipose tissue into brown adipose tissue (15-17). Irisin is thought to increase energy consumption in this transformation and to be effective in systemic metabolism (8,18-20). It is predicted that irisin (21), which is also expressed among adipocytokines, may be a potential treatment agent in the fight against T2DM and atherogenesis, depending on the activation of brown adipose tissue (22).

Diabetic nephropathy (DN), which occurs with renal system involvement in diabetes, has become a common and important complication of end-stage renal disease in the global population (3-4) and is also seen as the main cause of diabetes-related mortality and morbidity (23). Microalbuminuria occurs in the first stage, macroalbuminuria and significant renal dysfunction occur in the later stage (24,25). The mechanism of DN formation has not been clearly revealed, but experimental studies suggest that inflammation plays a fundamental role in the progression of DN (26). Serum irisin level in the progressive process of DN reflects glucose intolerance through insulin resistance (27). In some studies, irisin has been reported to have a protective effect on the renal system. A study in rats has suggested the potential protective role of irisin on the kidney against harmful stimuli. Administration of recombinant irisin has been shown to improve kidney function by reducing kidney damage and fibrosis. This shows the direct renal protective activity of irisin, independent of its benefit on glycemic metabolism (28). In another study using the ischemia/reperfusion mouse model, it has been shown that irisin may have a renoprotective effect on acute kidney injury in tubular cells treated with hypoxia/recovery (29). It has also been shown that irisin is stimulated in renal ischemia-reperfusion and protects against tubular cell damage and apoptosis by suppressing p53 (30).

The development of DN consists of five stages according to the criteria defined by Mogensen. Stage 1 is the stage of hyperfiltration and hypertrophy. Most patients diagnosed with diabetes are at this stage. Stage 2 is known as the asymptomatic silent stage. There may be no clinical signs. This quiet period can last 10-15 years. Glomerular filtration rate (GFR) is high at the beginning of this period, and hyperfiltration can be seen as the process progresses. Over time, the findings may slowly decrease and return to normal values. Excretion of albumin in the urine and blood pressure may be within normal limits. Stage 3 refers to the microalbuminuric stage. Persistence of microalbuminuria (albumin in urine; 20-200 µg/min, 30-300 mg/day) can be seen in the urine. There is a slight increase in blood pressure. On average, diabetes begins 6-15 years after onset. It indicates the detectable early phase of kidney dysfunction. GFR can be normal, high, or low. Stage 4 is called overt diabetic nephropathy or azotemic stage. It is the phase of irreversible changes. Histological changes are evident and hypertension has become permanent. Stage 5 is the last stage and is known as end-stage renal disease or uremic stage. During this period, high levels of urea and creatinine lead to advanced resistant hypertension (31).

Identification of new biomarkers in early stage DN is of great clinical importance (32). Although there is no definitive cure for DN, there are treatment approaches that slow down the disease. With this study, it was aimed to compare serum irisin levels between different stages of healthy individuals and patients with DN and to demonstrate that irisin can be a guide in the diagnosis and treatment of the disease in the early stages before end-stage kidney damage develops.

MATERIALS and METHODS

The scope of the study included 101 patients with T2DM over the age of 18 years and 28 healthy volunteers who applied to the internal medicine outpatient clinic of our hospital and came for periodic controls in the following periods. The study started with the approval of Çukurova University Faculty of Medicine Non-Invasive Clinical Research Ethics Committee (Decision Number: 15, Date: 14.02.2020). Consent was obtained from all individuals participating in the study. Routine biochemical parameters were evaluated within the scope of the study. Serum irisin level was studied from a total of 129 samples. Of the patients participating in the study, patients with malignancy, liver disease, rheumatic and autoimmune disease, pregnancy, decompensated heart failure, and cerebrovascular disease were excluded from the study. Diabetes duration and medication information of the patients were evaluated together with their demographic data.

The study was carried out in Çukurova University Medical Faculty Education Research and Application Hospital Central Laboratory. Within the routine sampling procedures, five cc venous blood samples from the antecubiteal region of the patients were taken into Becton Dickinson vacuum-gel and Ethylenediamine Tetraacetic Acid (EDTA) tubes. Complete blood count and hemoglobin A1c (HbA1c) test parameters were studied from EDTA tubes. For biochemical test parameters, the samples in gel tubes were centrifuged in the Nüve-NF200 centrifuge (three minutes-five thousand revolutions). After the routine biochemical test parameters were studied, the serums were taken into Eppendorf tubes to be studied and stored in a deep freezer at -80°C. Frozen sera were analyzed at room temperature after thawing and mixing by vortex on the study day.

From biochemical test parameters, hemogram, fasting blood glucose, GFR, creatinine, blood urea nitrogen (BUN), total protein, albumin, urine microalbum/creatinine ratio, high-density lipoprotein (HDL), triglyceride (TG), total cholesterol, low-density lipoprotein (LDL), aspartate aminotransferase (AST), HbA1c, alanine aminotransferase (ALT), sodium (Na), phosphorus (P), chlorine (Cl), potassium (K), calcium (Ca), insulin, homeostatic model evaluation of insulin resistance (HOMA-IR) levels were evaluated within the scope of the study. Among the biochemical test parameters, serum Cl, K, Na, glucose, Ca, creatinine, AST, albumin, BUN, P, total cholesterol, ALT, LDL cholesterol, HDL cholesterol and TG by ISE (Ion selective electrode) method; Beckman coulter AU5800, hemogram (complete blood count) by photometric method, Beckman Coulter DXH-800, HbA1c by autoanalyzer technique; Premier USA HB9210, insulin by HPLC (High Performance Liquid Chromatography) method; Beckman Coulter DXI-800 by chemiluminescence method, microalbumin and creatinine in spot urine were studied on Beckman Coulter AU5800 devices with immunoturbidimetric and photometric methods. GFR was calculated by the chronic kidney disease epidemiology collaboration (CKD-EPI) formula and HOMA-IR (insulin resistance): glucose (plasma) x insulin (plasma)/405. Moreover, microalbumin/creatinine ratios were calculated in spot urine. Serum irisin level was analyzed with enzyme-linked immunosorbent assay (ELISA method-Sunred ELISA kit catalogue no: 201-12-5328) in accordance with the kit protocols.

Statistical analysis

The variables were divided into two as continuous and categorical and evaluated. By testing whether continuous variables fit the normal distribution with the Kolmogrov-Smirnov test, the variables were shown with standard deviation and mean values. Categorical data were calculated as numbers and percentages. Normally distributed variables were tested with One Way ANOVA, and non-normally distributed variables were tested with Kruskal-Wallis tests. Results are presented with mean and standard deviation values. Median and IQR values are given as summary criteria for irisin. Categorical data were compared with the Chi-square test and evaluated. Correlation analysis was performed between irisin level and other parameters, and the variables were expressed with Pearson and Spearman correlation coefficients. Data analyzes were statistically evaluated in the Windows operating system with SPSS 20.0 (SPSS Inc. Chicago, IL, United States). $P< 0.05$ was considered significant.

RESULTS

In our study, the first group was formed with 28 healthy volunteers without a history of diabetes. In the other groups of the study, patients diagnosed with T2DM, under treatment and followed-up were included. Thirty diabetic patients with creatinine within the reference range and urine microalbumin <30 mg/day were in group 2 (Mogensen stage 1-2), 25 patients with 30-300 mg/day were in group 3 (Mogensen stage 3), >300 mg/day 24 patients were determined as group 4 (Mogensen stage 4). Twenty-two patients with high microalbumin and creatinine levels (>300 mg/day, >1.1 mg/dL), respectively, were in group 5 (Mogensen stage 5).

Demographic data for the groups included in the study are given in Table 1. Serum irisin level was found to be significantly higher in group 1 ($p< 0.001$). Especially in group 2 (Mogensen stage 1 hyperfiltration and hypertrophy stage), serum irisin level was found to be significantly lower than the control group ($p< 0.001$) (Figure 1). HbA1c, micoralbumin/creatinine and HOMA-IR values were found to be significantly lower in group 1 ($p< 0.001$ for each) (Table 2). There were weak negative correlations between irisin and HbA1c, microalbumin/creatinine, and GFR, respectively ($p= 0.001$, $r= -0.286$), ($p= 0.199$, $r= -0.015$), ($p= 0.142$, $r= -0.158$), but there was a weak positive correlation between ($p= 0.008$, $r= 0.308$).

DISCUSSION

T2DM is a metabolic disease with a rapidly increasing global prevalence. In the literature, it is seen that there are conflicting results in studies on serum irisin levels in T2DM patients.

In our study, serum irisin level was found to be significantly lower in diabetic nephropathic patients than in healthy individuals. In some of the studies, serum irisin levels have been found to be high in patients with T2DM (33,34), and in many studies, it has been found to be low, similar to our study (35,36). In most clinical studies, lower irisin levels

Table 1. Comparison of the general demographic data of the study groups

	*Group 1 n= 28	Group 2 n= 30	Group 3 n= 25	Group 4 n= 24	Group 5 n= 22	**p
Age (year)	40.045 ± 11.05	59.047 ± 11.61	53.10 ± 10.60	57.90 ± 8.42	57.10 ± 9.19	**<0.001**
Weight (kg)	71.09 ± 11.14	79.29 ± 10.72	85.95 ± 16.29	79.60 ± 11.88	82.25 ± 15.42	**0.006**
Height (cm)	166.95 ± 9.48	166.62 ± 9.27	160.79 ± 21.66	160.10 ± 8.72	166.55 ± 7.21	0.357
Body mass index (kg/m^2)	24.5 ± 3.6	28.9 ± 5.2	35.2 ± 13.2	30.8 ± 4.5	30.5 ± 6.3	**<0.001**
Systolic blood pressure (mmHg)	111.36 ± 9.90	126.9 ± 18.06	125.26 ± 11.23	138.50 ± 19.80	144.50 ± 26.25	**<0.001**
Diastolic blood pressure (mmHg)	74.09 ± 6.66	78.33 ± 9.91	79.74 ± 5.39	82.00 ± 8.94	84.0 ± 13.53	**0.004**
Follow-up duration of diabetes (year)	-	14.57 ± 9.41	10.57 ± 7.79	13.95 ± 8.53	16.65 ± 7.82	**<0.001**
Male sex, n (%)	18 (64.2)	22 (73.3)	23 (96.0)	11 (45.8)	11 (50.0)	**0.055**
Hypertension, n (%)	4 (14.3)	20 (66.6)	23 (92.0)	22 (91.6)	18 (81.8)	**<0.001**
Hyperlipidemia, n (%)	0 (0)	7 (23.3)	8 (32.0)	2 (8.3)	1 (4.5)	0.109
Coronary artery disease, n (%)	0 (0)	8 (26.6)	7 (28.0)	10 (41.6)	6 (27.2)	**0.006**
Smoking, n (%)	7 (25.0)	6 (20)	20 (80.0)	12 (50.0)	14 (63.6)	**0.006**
Asthma, n (%)	1 (3.5)	3 (10)	0 (0)	3 (10)	2 (9.0)	0.264
Hyperthyroidism, n (%)	0 (0)	1 (3.3)	0 (0)	0 (0)	0 (0)	0.402
Hypothyroidism, n (%)	0 (0)	2 (6.6)	5 (20.0)	1 (4.1)	1 (4.5)	0.168

*Group 1: Healthy control, Group 2: <30 mg/day albuminuria, Group 3: 30-300 mg/day albuminuria, Group 4: >300 mg/day albuminuria, Group 5: >300 mg/day albuminuria, high creatinine.
**p< 0.05: Accepted as statistically significant.

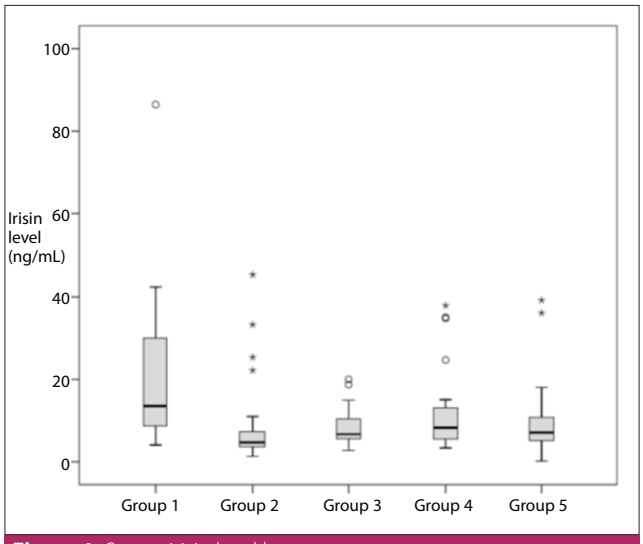

Figure 1. Serum irisin level by groups.

have been reported in patients with prediabetes or T2DM compared to the control group (37-42). In a study conducted with patients with T2DM, it has been determined that the serum irisin level was higher in diabetic patients than in the control group. It has been stated that the reason insulin increase in serum irisin level is to compensate insulin resistance seen in the muscle tissue in diabetics (43).

In the study by Shelbaya et al., irisin levels have been found to be significantly lower in patients with T2DM (44). It has been shown that serum irisin limits are lower in patients with newly diagnosed T2DM than in individuals with normal glucose tolerance (15,45). The fact that it is lower in patients with T2DM for a long time suggests that the iris may be a T2DM marker (46,47). In another study, the fact that serum irisin level has been found to be significantly lower in the T2DM group than in the control group suggests that irisin may be a protective factor for T2DM (48). It has been suggested that this increase may be associated with insulin resistance (49).

Ebert et al. have evaluated the serum irisin levels of 532 patients with chronic kidney disease, stage 1-5. It has been determined that the serum irisin level of the patients in stage five was considerably lower than that of the patients in the other stages. As the stage of the disease progresses, the serum irisin level decreases, while a positive correlation of irisin with insulin resistance and kidney functions has been reported (50). The relationship between serum irisin level and metabolic parameters and kidney functions has been shown. However, it has been emphasized that low levels of irisin in diabetic individuals also cause nephropathy (51). In our study, it was shown that irisin level was lower in nephropathic patients than in healthy individuals, and our result also supports this study.

Table 2. Comparison of complete blood count, biochemical parameters and irisin levels of the study groups

	*Group 1 n= 28	Group 2 n= 30	Group 3 n= 25	Group 4 n= 24	Group 5 n= 22	**p
Glucose (mg/dL)	80.12 ± 8.84	160.5 ± 39.98	181.8 ± 92.13	181.47 ± 89.39	172.0 ± 70.39	**<0.001**
Urea (mg/dL)	11.35 ± 3.23	14.3 ± 3.4	13.77 ± 4.96	18.66 ± 7.30	40.51 ± 15.10	**<0.001**
Creatinine (mg/dL)	0.72 ± 0.12	0.67 ± 0.11	0.72 ± 0.25	0.89 ± 0.26	4.35 ± 7.09	**<0.001**
GFR (ml/min. (m^2)	I I 1.8 1 ± 8.68	99.6 ± 19.33	90.43 ± 31.84	87.36 ± 14.0	29.78 ± 15.50	**<0.001**
Sodium (mEq/L)	140.43 ± 1.26	138.83 ± 1.46	129.60 ± 33.1	137.52 ± 37.20	138.82 ± 2.51	0.714
Potassium (mEq/L)	4253 ± 0.27	4.51 ± 0.48	4.69 ± 0.37	4.64 ± 0.49	4.86 ± 0.53	**<0.001**
ALT (U/L)	21.43 ± 8.37	26.83 ± 30.83	22.4 ± 11.35	22.57 ± 6.97	15.65 ± 8.00	0.665
AST (U/L)	21.75 ± 5.19	20.41 ± 6.51	20.20 ± 8.94	18.78 ± 5.12	17.34 ± 6.03	0.354
Uric acid (mg/dL)	5.30 ± 1.39	5.46 ± 1.78	12.64 ± 30.14	5.6 ± 1.5	6.77 ± 1.68	0.493
Total protein (g/dL)	71.08 ± 4.28	70.69 ± 4.73	71.30 ± 6.5	69.15 ± 6.71	69.32 ± 6.03	0.572
Albumin (g/dL)	40.37 ± 2.52	41.34 ± 7.74	40.30 ± 4.03	39.05 ± 5.77	37.40 ± 4.08	**0.045**
Calcium (mg/dL)	9.63 ± 0.66	9.45 ± 0.52	9.65 ± 0.54	9.38 ± 0.71	9.06 ± 0.79	0.003
Phosphorus (mg/dL)	3.29 ± 0.49	3.47 ± 0.50	3.68 ± 0.50	3.49 ± 0.49	4.01 ± 0.94	**0.001**
Total cholesterol (mg/dL)	210.5 ± 45.67	194.35 ± 65.34	203.80 ± 54.34	183.00 ± 36.13	196.44 ± 56.15	0.345
Triglyceride (mg/dL)	145.31 ± 66.92	168.03 ± 83.24	196.00 ± 96.03	201.57 ± 125.93	206.28 ± 133.41	0.050
LDL cholesterol (mg/dL)	131.54 ± 34.86	118.96 ± 46.21	120.11 ± 41.17	103.84 ± 27.89	121.43 ± 46.34	0.165
HDL cholesterol (mg/dL)	49.27 ± 13.72	54.15 ± 17.09	48.80 ± 23.08	41.05 ± 10.76	39.88 ± 10.65	**0.015**
White blood cell (10^3/uL)	6.70 ± 1.57	7.96 ± 1.86	8.20 ± 1.75	9.08 ± 2.22	7.94 ± 2.13	**<0.001**
Hb (g/dL)	14.25 ± 1.47	13.41 ± 1.59	13.21 ± 1.76	13.08 ± 1.93	12.02 ± 1.33	**<0.001**
Hematocrit (%)	40.98 ± 4.02	38.80 ± 3.93	37.69 ± 9.18	36.07 ±	34.71 ± 3.92	**<0.001**
Thrombocyte (10^3/mm^3)	230.68 ± 48.27	265.07 ± 68.8	280.15 ± 74.96	257.31 ± 70.80	266.08 ± 100.24	0.123
HbA1c (mg/dL)	5.6 ± 0.3	7.68 ± 1.75	8.66 ± 2.32	9.15 ± 2.38	8.72 ± 1.86	**<0.001**
Microalbumin/cr	4.9 ± 3.0	9.24 ± 6.64	105.47 ± 84.64	693.04 ± 541.94	2176.00 ± 2118.6	**<0.001**
HOMA-IR	1.20 ± 0.50	3.51 ± 2.40	3.78 ± 1.90	4.92 ± 1.81	5.2 ± 1.3	**<0.001**
Irisin (ng/mL)	19.71 ± 17.40 13.5 (8.7-30)	8.35 ± 10.06 4.7 (3.6-7.3)	8.22 ± 4.56 6.7 (5.6-10.4)	12.24 ± 9.98 8.3 (5.5-13.1)	8.53 ± 7.28 7.1 (5.1-10.8)	**<0.001**

Cr: Creatinine, GFR: Glomerular filtration rate, ALT: Alanine aminotransferase, AST: Aspartate transaminase, LDL: Low density lipoprotein, HDL: High density lipoprotein, Hb: Hemoglobin, HbA1c: Hemoglobin A1C, HOMA-IR: Homeostatic model assessment for insulin resistance-insulin resistance. Presented as mean ± standard deviation.
*Group 1: Healthy control, Group 2: <30 mg/day albuminuria, Group 3: 30-300 mg/day albuminuria, Group 4: >300 mg/day albuminuria, Group 5: >300 mg/day albuminuria, high creatinine
**p< 0.05: Accepted as statistically significant.
***Mean ± standard deviation presented as median (IQR).

Overall, according to a meta-analysis of thirteen matched case-control studies involving 1735 T2DM patients, serum irisin levels have been found to be significantly lower in patients with microalbuminuria (10 studies) compared to T2DM patients with normoalbuminuria. In addition, serum levels of irisin in T2DM patients with macroalbuminuria have been found to be significantly lower than those with microalbuminuria (10 studies). In addition, serum irisin level has been found to be lower in patients with 1.73 m^2 GFR< 60 mL/min compared to patients with 1.73 m^2 GFR≥ 60 mL/min (four studies). These studies show that serum irisin level may be associated with albuminuria and decreased GFR in T2DM patients (52). In our study, a negative correlation was found between irisin and GFR.

In some studies, the protective effect of irisin on the renal system has been evaluated. Irisin inhibits inflammation and apoptosis in HK-2 cells treated with lipopolysaccharide, suggesting that the anti-inflammatory effect of irisin contributes to its potential renal protective effect (53). The potential protective efficacy of irisin against acute kidney injury

induced by ischemia reperfusion has been demonstrated in another study (54).

Future studies are needed to determine the potential independent relationship between irisin and DN. In addition, the longitudinal relationship between decreased irisin and the incidence of DN in T2DM patients should be determined in prospective cohort studies (49).

Although it seems premature to evaluate the benefit of irisin as a therapeutic agent against obesity or T2DM, it has been emphasized in a study that it should be the main research target for the near future (26).

There are also weaknesses of our study; one of them is that the mean ages between the control and study groups were not similar. In addition, due to patients who should be excluded, the number of patients in the study groups differed from those in the control group. The other is the weak correlation between serum irisin level and other compared parameters. Irisin is secreted in many tissues and organs in the body (heart, ovary, testis, stomach, neuronal cells, muscle and adipose tissue), and the effect of these tissues and organs on serum irisin levels in diabetic patients could not be predicted in our study.

CONCLUSION

This study showed that there were significant differences in serum irisin levels between healthy individuals and patients with DN. It is demonstrated that irisin can be used as a guide in order to guide the diagnosis, especially in the early stage of renal complications of diabetes. It is seen that more comprehensive studies are needed to reveal the low level of irisin and to evaluate the irisin level with close follow-up, especially during the period of hyperfiltration and hypertrophy in stage 1 nephropathy, when quality of life does not decrease.

Ethics Committee Approval: The study started with the approval of Çukurova University Faculty of Medicine Non-Invasive Clinical Research Ethics Committee (Decision Number: 15, Date: 14.02.2020)

Author Contributions: Concept/Design: GD, YD, Gİ, ME, FK; Analysis/Interpretation: GD, YD, ÖGÖ; Data Acquisition: ME, Gİ, GD; Writting: GD, ÖGÖ, YD, FK; Critical Revision: FK, ME, ÖGÖ, GD, YD, Gİ; Final Approval: GD, YD, Gİ, FK, ME, ÖGÖ.

Conflict of Interest: All authors declare that they have no conflict of interest.

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REFERENCES

1. *Alberti KG, Zimmet P. Definition, diagnosis and classification of diabetes mellitus and its complications. I. Diagnosis and classification of diabetes mellitus: Provisional report of a WHO consultation. Diabet Med 1998;15:539-53. https://doi.org/10.1002/(SICI)1096-9136(199807)15:7<539::AID-DIA668>3.0.CO;2-S*
2. *Bennett PH, Knowler WC. Definition, diagnosis, and classification of diabetes mellitus and glucose homeostasis. In: Kahn, CR, King, GL, Moses AC, Weir GC, Jacobson AM, Smith RJ eds. Joslin's Diabetes Mellitus (14th ed). 2010. New Delhi: Walter Kluwer Pvt. Ltd. p. 331-9.*
3. *Pugliese G, Penno G, Natali A, Barutta F, Di Paolo S, Reboldi G, et al. Italian Diabetes Society and the Italian Society of Nephrology. Diabetic kidney disease: New clinical and therapeutic issues. Joint position statement of the Italian Diabetes Society and the Italian Society of Nephrology on "The natural history of diabetic kidney disease and treatment of hyperglycemia in patients with type 2 diabetes and impaired renal function". Nutr Metab Cardiovasc Dis 2019;29(11):1127-50. https://doi.org/10.1016/j.numecd.2019.07.017*
4. *Bonner R, Albajrami O, Hudspeth J, Upadhyay A. Diabetic kidney disease. Prim Care 2020;47:645-59. https://doi.org/10.1016/j.pop.2020.08.004*
5. *Kershaw EE, Flier JS. Adipose tissue as an endocrine organ. J Clin Endocrinol Metab 2004;89(6):2548-56. https://doi.org/10.1210/jc.2004-0395*
6. *IDF Diabetes Atlas Key Findings 2014. Available from: http://www.idf.org/diabetesatlas/update-2014.*
7. *Akgul Balaban Y, Yilmaz N, Kalayci M, Unal M, Turhan T. Irisin and chemerin levels in patients with type 2 diabetes mellitus. Acta Endocrinol (Buchar) 2019;15(4):442-6. https://doi.org/10.4183/aeb.2019.442*
8. *Boström P, Wu J, Jedrychowski MP, Korde A, Ye L, Lo JC, et al. PGC1-a-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. Nature 2012;481(7382):463-8. https://doi.org/10.1038/nature10777*
9. *Schumacher MA, Chinnam N, Ohashi T, Shah RS, Erickson HP. The structure of irisin reveals a novel intersubunit β-sheet fibronectin type III (FNIII) dimer: Implications for receptor activation. J Biol Chem 2013;288(47):33738-44. https://doi.org/10.1074/jbc.M113.516641*
10. *Ferrer-Martínez A, Ruiz-Lozano P, Chien KR. Mouse PeP: A novel peroxisomal protein linked to myoblast differentiation and development. Dev Dyn 2002;224(2):154-67. https://doi.org/10.1002/dvdy.10099*
11. *Teufel A, Malik N, Mukhopadhyay M, Westphal H. Frcp1 and Frcp2, two novel fibronectin type III repeat containing genes. Gene 2002;297(1-2):79-83. https://doi.org/10.1016/S0378-1119(02)00828-4*
12. *Irving BA, Still CD, Argyropoulos G. Does irisin have a brite future as a therapeutic agent in humans? Curr Obes Rep 2014;3(2):235-41. https://doi.org/10.1007/s13679-014-0091-1*
13. *İnci A, Aypak ÜS. İrisin ve metabolik etkileri. Turkiye Klinikleri J Endocrin 2016;11(1):15-21. https://doi.org/10.5336/endocrin.2016-49995*
14. *Novelle MG, Contreras C, Romero-Pıco A, Lopez M, Dieguez C. Irisin, two years later. Int J Endocrinol 2013;2013:746281. https://doi.org/10.1155/2013/746281*
15. *Choi YK, Kim MK, Bae KH, Seo HA, Jeong JY, Lee WK, et al. Serum irisin levels in new-onset type 2 diabetes. Diabetes Res Clin Pract 2013;100(1):96-101. https://doi.org/10.1016/j.diabres.2013.01.007*

16. Jeremic N, Chaturvedi P, Tyagi SC. Browning of white fat: Novel insight into factors, mechanisms, and therapeutics. J Cell Physiol 2017;232(1):61-8. https://doi.org/10.1002/jcp.25450
17. Pahlavani M, Razafimanjato F, Ramalingam L, Kalupahana NS, Moussa H, Scoggin S, et al. Eicosapentaenoic acid regulates brown adipose tissue metabolism in high-fat-fed mice and in clonal brown adipocytes. J Nutr Biochem 2017;39:101-9. https://doi.org/10.1016/j.jnutbio.2016.08.012
18. Aydin S. Three new players in energy regulation: Preptin, adropin and irisin. Peptides 2014;56:94-110. https://doi.org/10.1016/j.peptides.2014.03.021
19. Villarroya F. Irisin, turning up the heat. Cell Metab 2012;15(3):277-8. https://doi.org/10.1016/j.cmet.2012.02.010
20. Perakakis N, Triantafyllou GA, Fernández-Real JM, Huh JY, Park KH, Seufert J, et al. Physiology and role of irisin in glucose homeostasis. Nat Rev Endocrinol 2017;13(6):324-37. https://doi.org/10.1038/nrendo.2016.221
21. Coelho M, Oliveira T, Fernandes R. Biochemistry of adipose tissue: An endocrine organ. Arch Med Sci 2013;9(2):191-200. https://doi.org/10.5114/aoms.2013.33181
22. Sanchez-Delgado G, Martinez-Tellez B, Olza J, Aguilera CM, Gil Á, Ruiz JR. Role of exercise in the activation of brown adipose tissue. Ann Nutr Metab 2015;67(1):21-32. https://doi.org/10.1159/000437173
23. Phillips AO, Steadman R. Diabetic nephropathy: The central role of renal proximal tubular cells in tubulointerstitial injury. Histol Histopathol 2002;17(1):247-52.
24. Lin YC, Chang YH, Yang SY, Wu KD, Chu TS. Update of pathophysiology and management of diabetic kidney disease. J Formos Med Assoc 2018;117(8):662-75. https://doi.org/10.1016/j.jfma.2018.02.007
25. Jerums G, Panagiotopoulos S, Premaratne E, MacIsaac RJ. Integrating albuminuria and GFR in the assessment of diabetic nephropathy. Nat Rev Nephrol 2009;5(7):397-406. https://doi.org/10.1038/nrneph.2009.91
26. Sakai N, Wada T. Revisiting inflammation in diabetic nephropathy: The role of the Nlrp3 inflammasome in glomerular resident cells. Kidney Int 2015;87(1):12-4. https://doi.org/10.1038/ki.2014.322
27. Kawada T. Serum irisin and diabetic nephropathy in patients with diabetes mellitus. Horm Metab Res 2021;53(12):825. https://doi.org/10.1055/a-1676-4118
28. Peng H, Wang Q, Lou T, Qin J, Jung S, Shetty V, et al. Myokine mediated muscle-kidney crosstalk suppresses metabolic reprogramming and fibrosis in damaged kidneys. Nat Commun 2017;8(1):1493. https://doi.org/10.1038/s41467-017-01646-6
29. Zhang R, Ji J, Zhou X, Li R. Irisin pretreatment protects kidneys against acute kidney injury induced by ischemia/reperfusion via upregulating the expression of uncoupling protein 2. Biomed Res Int 2020;2020:6537371. https://doi.org/10.1155/2020/6537371
30. Liu Y, Fu Y, Liu Z, Shu S, Wang Y, Cai J, et al. Irisin is induced in renal ischemia-reperfusion to protect against tubular cell injury via suppressing p53. Biochim Biophys Acta Mol Basis Dis 2020;1866(7):165792. https://doi.org/10.1016/j.bbadis.2020.165792
31. Mogensen CE, Schmitz O. The diabetic kidney: From hiperfiltration and microalbuminuria to end-stage renal failure. Med Clin North Am 1988;72(6):1465-92. https://doi.org/10.1016/S0025-7125(16)30717-9
32. Fu H, Liu S, Bastacky SI, Wang X, Tian XJ, Zhou D. Diabetic kidney diseases revisited: A new perspective for a new era. Mol Metab 2019;30:250-63. https://doi.org/10.1016/j.molmet.2019.10.005
33. García-Fontana B, Reyes-García R, Morales-Santana S, Ávila-Rubio V, Muñoz-Garach A, Rozas-Moreno P, et al. Relationship between myostatin and irisin in type 2 diabetes mellitus: A compensatory mechanism to an unfavourable metabolic state? Endocrine 2016;52(1):54-62. https://doi.org/10.1007/s12020-015-0758-8
34. Al-Daghri NM, Alokail MS, Rahman S, Amer OE, Al-Attas OS, Alfawaz H, et al. Habitual physical activity is associated with circulating irisin in healthy controls but not in subjects with diabetes mellitus type 2. Eur J Clin Invest 2015;45(8):775-81. https://doi.org/10.1111/eci.12468
35. Moreno-Navarrete JM, Ortega F, Serrano M, Guerra E, Pardo G, Tinahones F, et al. Irisin is expressed and produced by human muscle and adipose tissue in association with obesity and insulin resistance. J Clin Endocrinol Metab 2013;98(4):E769-78. https://doi.org/10.1210/jc.2012-2749
36. Liu JJ, Wong MD, Toy WC, Tan CS, Liu S, Ng XW, et al. Lower circulating irisin is associated with type 2 diabetes mellitus. J Diabetes Complications 2013;27(4):365-9. https://doi.org/10.1016/j.jdiacomp.2013.03.002
37. Zhang M, Chen P, Chen S, Sun Q, Zeng QC, Chen JY, et al. The association of new inflammatory markers with type 2 diabetes mellitus and macrovascular complications: A preliminary study. Eur Rev Med Pharmacol Sci 2014;18(11):1567-72.
38. Hu W, Wang R, Li J, Zhang J, Wang W. Association of irisin concentrations with the presence of diabetic nephropathy and retinopathy. Ann Clin Biochem 2016;53(1):67-74. https://doi.org/10.1177/0004563215582072
39. Wang HH, Zhang XW, Chen WK, Huang QX, Chen QQ. Relationship between serum irisin levels and urinary albumin excretion in patients with type 2 diabetes. J Diabetes Complications 2015;29(3):384-9. https://doi.org/10.1016/j.jdiacomp.2015.01.001
40. Alis R, Sanchis-Gomar F, Pareja-Galeano H, Hernández-Mijares A, Romagnoli M, Víctor VM, et al. Association between irisin and homocysteine in euglycemic and diabetic subjects. Clin Biochem 2014; 47: 333-5. https://doi.org/10.1016/j.clinbiochem.2014.08.017
41. Sanchis-Gomar F, Alis R, Pareja-Galeano H, Sola E,Victor MV, Rocha M, et al. Circulating irisin levels are not correlated with BMI, age, and other biological parameters in obese and diabetic patients. Endocrine 2014; 46: 674-7. https://doi.org/10.1007/s12020-014-0170-9
42. Zhang C, Ding Z, Lv G, Li J, Zhou P, Zhang J. Lower irisin level in patients with type 2 diabetes mellitus: A case-control study and meta-analysis. J Diabetes 2016;8(1):56-62. https://doi.org/10.1111/1753-0407.12256
43. Rodrigues KF, Pietrani NT, Bosco AA, Ferreira CN, Gomes KB. Circulating irisin is increased in type 2 diabetes mellitus and correlates with fasting glucose levels. Apollo Med 2016;3(13):152-5. https://doi.org/10.1016/j.apme.2016.02.010
44. Shelbaya S, Abu Shady MM, Nasr MS, Bekhet MM, Mageed YA, Abbas M. Study of irisin hormone level in type 2 diabetic patients and patients with diabetic nephropathy. Curr Diabetes Rev 2018;14(5):481-6. https://doi.org/10.2174/1573399813666170829163442
45. Wang L, Song J, Wang C, Lin P, Liang K, Sun Y, et al. Circulating levels of betatrophin and irisin are not associated with pancreatic β-cell function in previously diagnosed type 2 diabetes mellitus patients. J Diabetes Res 2016;2016:2616539. https://doi.org/10.1155/2016/2616539
46. Chen JQ, Huang YY, Gusdon AM, Qu S. Irisin: A new molecular marker and target in metabolic disorder. Lipids Health Dis 2015;14:2. https://doi.org/10.1186/1476-511X-14-2

47. *Liu JJ, Liu S, Wong MD, Tan CS, Tayintharan S, Sum CF, et al. Relationship between circulating irisin, renal function and body composition in type 2 diabetes. J Diabetes Complications 2014;28(2):208-13. https://doi.org/10.1016/j.jdiacomp.2013.09.011*
48. *Xuan X, Lin J, Zhang Y, Zhou L, Xu L, Jia J, et al. Serum irisin levels and clinical implication in elderly patients with type 2 diabetes mellitus. J Clin Med Res 2020;12(9):612-7. https://doi.org/10.14740/jocmr4261*
49. *Pedersen BK, Steensberg A, Fischer C, Keller C, Keller P, Plomgaard P, et al. Searching for the exercise factor: Is IL-6 a candidate? J Muscle Res Cell Motil 2003;24(2-3):113-9. https://doi.org/10.1023/A:1026070911202*
50. *Ebert T, Focke D, Petroff D, Wurst U, Richter J, Bachmann A, et al. Serum levels of the myokine irisin in relation to metabolic and renal function. Eur J Endocrinol 2014;170(4):5016. https://doi.org/10.1530/EJE-13-1053*
51. *Khidr EG, Ali SS, Elshafey MM, Fawzy OA. Association of irisin and FNDC5 rs16835198 G>T gene polymorphism with type 2 diabetes mellitus and diabetic nephropathy. An Egyptian pilot study. Gene 2017;626:26-31. https://doi.org/10.1016/j.gene.2017.05.010*
52. *Wang R, Liu H. Association between serum irisin and diabetic nephropathy in patients with type 2 diabetes mellitus: A meta-analysis. Horm Metab Res 2021;53(5):293-300. https://doi.org/10.1055/a-1475-4444*
53. *Jin YH, Li ZY, Jiang XQ, Wu F, Li ZT, Chen H, et al. Irisin alleviates renal injury caused by sepsis via the NF-κB signaling pathway. Eur Rev Med Pharmacol Sci 2020;24(11):6470-6.*
54. *Zhang J, Bi J, Ren Y, Du Z, Li T, Wang T, et al. Involvement of GPX4 in irisin's protection against ischemia reperfusion-induced acute kidney injury. J Cell Physiol 2021;236(2):931-45. https://doi.org/10.1002/jcp.29903*