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High Serum Levels of Irisin, Visfatin and Adiponectin as Potential Independent Risk Factors for Diabetic Nephropathy Progression in Patients With Type 2 Diabetes Mellitus

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Abstract

Background/Aim: Diabetic nephropathy (DN) is a common devastating complication in type 2 diabetes mellitus (T2DM). In order to investigate novel DN biomarkers, we evaluated serum levels of irisin, adiponectin, visfatin and interleukin 4 (IL-4) in patients with T2DM with normo-, micro- and macro-albuminuria and compared their means with non-diabetic controls.

Patients and Methods: Clinical data and routine laboratory parameters of metabolic and renal function status were determined in blood and urine samples obtained from 169 participants, divided into four groups according to the presence of diabetes and albuminuria using appropriate biochemical assays/calculations. Serum levels of irisin, adiponectin, visfatin and interleukin 4 (IL4) were assessed using enzyme-linked immunosorbent assay. Means of all tested parameters and biomarkers were compared using appropriate statistical methods. Logistic regression was used to determine albuminuria risk factors in T2DM as an indicator for DN.

continued

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Results: All tested parameters differed significantly among T2DM groups and controls (p<0.001). Irisin, adiponectin, visfatin and IL4 significantly increased in T2DM patients with significant increasing albuminuria. Along with hemoglobin A1C, irisin was the most highly significant risk factor for development and progression of albuminuria (p<0.001). Adiponectin was also a significant independent risk factor (p=0.009), whilst visfatin and IL4 conferred no significant risk.

Conclusion: High irisin levels in normo-albuminuric patients indicates their potential to develop DN even prior to detectable albuminuria. Both irisin and adiponectin may be considered as potential biomarkers indicating risk for DN progression in T2DM that might be therapeutically targeted.

Keywords: Irisin, adiponectin, T2DM, diabetic nephropathy, albuminuria.

Introduction

Diabetic nephropathy (DN) is a devastating microvascular complication of DM and is the leading cause of chronic kidney disease (CKD) and end-stage renal disease. It affects approximately 40% of patients within 10-20 years of diabetic onset, being clinically defined by the existence of proteinuria or declined renal function, although DN often develops years before it is diagnosed (1). While many important DN biomarkers have been discovered over the past two decades, advances in DN management and therapy are still limited (2). Therefore, defining new early predictive DN biomarkers would improve patients' health outcomes through therapeutic targeting of kidney-specific disease mechanisms (3-5).

Irisin is a newly discovered adipo-myokine derived from fibronectin type III domain-containing protein 5 (FNDC5) located in the plasma membrane, after cleavage of its extracellular portion. It is known to regulate energy homeostasis and insulin sensitivity (6). Previous investigators have reported increased serum irisin levels in patients with type 2 diabetes mellitus (T2DM) as compared to non-diabetic controls, finding positive associations with unfavorable diabetic parameters such as high body mass index (BMI), fasting plasma glucose (FPG) and triglyceride (TG) levels (7). On the contrary, other researchers reported significantly lower irisin levels in patients with T2DM (8). The role of irisin in DN is also still unclear as contradictory data were obtained among recent studies focusing on irisin level and its correlations with renal functions and DN predictors in T2DM (6, 9-11).

Adiponectin is a 30-kDa adipocyte-specific factor and a fat-derived hormone that contributes to beneficial metabolic actions in energy homeostasis. It improves insulin sensitivity, protects cells from apoptosis and reduces inflammation *via* receptor-dependent mechanisms. Deranged adiponectin levels were noted in obesity, diabetes, inflammation, atherosclerosis and cardiovascular disease (12). A reno-protective effect has been attributed to adiponectin in non-diabetic hypertensive patients based on its inverse association with albuminuria (13), however, there are various reports regarding its role in DN. Although adiponectin was described to be negatively correlated with the early features of nephropathy in T2DM, high levels were found to predict progression in established CKD (14).

Visfatin is a visceral fat-derived adipokine with insulin-mimicking action. It increases the activity of several matrix metalloproteinases and interleukins (ILs) in CKD. Elevated plasma visfatin levels have been reported in patients with DN, suggesting its pathogenetic role, although experimental studies have shown that chronic administration of visfatin ameliorated nephropathy in experimental T2DM mice, concluding it has a protective role against DN (15, 16). Moreover, interplay between several cytokines including IL4, which acts on podocytes to induce proteinuria, have been implicated in the pathogenesis of DN (17, 18). Thus, it may be of significance to study the input of visfatin and IL4 in DN.

Classically, albuminuria is regarded as a consequence of DM-induced glomerular damage; as an indicator for DN progression it has been used to categorize DN into two stages: micro-albuminuria and macro-albuminuria (19, 20). Thus, this work intended to evaluate serum levels of irisin, adiponectin, visfatin and IL4 in patients with T2DM as novel biomarkers predictive of DN, and to compare their levels with the stage of albuminuria (as an established indicator of DN progression). Therefore, this study may offer insights into developing new therapeutic targets in DN.

Patients and Methods

This case–control study was conducted at the departments of Physiology, Internal Medicine and Pathology upon approval by Ethics Review Committee of Al Azhar University Faculty of Medicine (165-4/2022). Informed consent was obtained from all patients before participation. All procedures were carried out in accordance with the current revision of the Helsinki Declaration principles for medical research involving human subjects (Declaration of Helsinki – The World Medical Association).

Participants, inclusion and exclusion criteria. A total of 169 participants were enrolled in the study. Normo-albuminuria was considered as an albumin to creatinine ratio of <30 mg/g; micro-albuminuria was indicated by a ratio between 30 and 300 mg/g; and macro-albuminuria was indicated by a ratio greater than 300 mg/g. Participants were divided into four groups: Non-diabetic healthy control group (41 non-diabetic volunteers: 14 males and 27 females); normo-albuminuric patients with T2DM (45 patients: 20 males and 25 females); patients with T2DM with micro-albuminuria (44 patients: 30 males and 14 females); and patients with T2DM with macro-albuminuria (39 patients: 27 males and 12 females).

Patients with T2DM were on medication for diabetes, including metformin, sulfonylureas, insulin and/or a combination of these drugs. None of the patients with T2DM were taking thiazolidinedione insulin sensitizers. Exclusion criteria were: Active urinary tract infection, obstructive uropathy or any renal disease other than DN (for patients with T2DM), T1DM, pregnancy, history of any thyroid or liver disease or malignancy, recent acute myocardial infarction or stroke, estimated glomerular filtration rate (eGFR) ≤30 ml/min/1.72 m^2, a history of any nephrotoxic medication, or a history of corticosteroid intake or smoking.

Anthropometric measurements and clinical evaluation. Collected data included age, sex and duration of diabetes. A detailed clinical history was taken, and complete general and systemic examinations were performed including, height, body weight, and blood pressure measurements. The body mass index (BMI) was calculated according to the standard formula (21) (Table I).

Biochemical assays. Fasting venous blood samples were withdrawn from all participants after a 12- to 14-h overnight fast. Each sample was distributed into three tubes. The first tube, containing sodium fluoride-potassium oxalate, was centrifuged at 1792 × *g* for 5 min. The separated plasma was used for determination of FPG by a glucose oxidase method. Blood in the second tube was allowed to clot for 30 min and then centrifuged at 1792 × *g* for 10 min. Then the serum was separated into aliquots and stored at −80˚C until analyzed. The third tube was mixed with EDTA, separated into aliquots then stored at −80˚C until analyzed. The following investigations were performed on serum samples: urea, creatinine, fasting lipid profile [including TG, total cholesterol (TC), low-density lipoproteins (LDL) and high-density lipoproteins (HDL)], total calcium and parathyroid (PTH) hormone levels using kits from Roche (Roche Diagnostics, Mannheim, Germany) on a BS-400 Mindray Chemistry analyzer (Shenzhen Mindray Bio-Medical Electronics Co., Ltd, Shenzhen, PR China). EDTA-treated whole blood was used for the estimation glycosylated hemoglobin (HbA1c) using high-performance liquid chromatography on a Bio-Rad D-10 system using kits from Bio-Rad Laboratories Inc. (Hercules, CA, USA).

Table I. *Formulae and definition criteria for calculated study parameters.*

Parameter	Calculation formula/definition criteria
Body mass index	Body weight (kg)/squared height (m).
Albuminuria	Normo-albuminuria: UAE <30 mg/day. Micro-albuminuria: UAE ≥30 and ≤300 mg/day. Macro-albuminuria: UAE >300 mg/day.
eGFR	Chronic Kidney Disease Epidemiology Collaboration equation (23): eGFR (ml/min/1.73 m^2)=141 × min (serum creatinine/k, 1) α × max (serum creatinine/k, 1) −1.209 × 0.993Age × 1.018 (if female) × 1.159 (if Black), where k is 0.7 for females and 0.9 for males, α is −0.329 for females and −0.411 for males, min indicates minimum serum creatinine/k or 1, and max indicates maximum serum creatinine/k or 1.
Albumin/creatinine ratio	Urinary albumin concentration (mg in 24 h)/urinary creatinine concentration (g in 24 h).

eGFR: Estimated glomerular filtration rate; UAE: urinary albumin excretion.

The rest of the serum stored at −80˚C was used to determine total serum irisin (Cusabio, Hubei, PR China), visfatin (Ray Biotech, Inc., Peachtree Corners, GA, USA), IL4 (Abcam, Inc., Waltham, MA, USA) and adiponectin (Thermo Fisher Scientific, Bengaluru, India) concentrations using these commercially available enzyme-linked immunosorbent assay kits according to the manufacturers' instructions. Inter-assay and intra-assay variabilities were kept below 10% for all tests (6, 21).

Urine tests. Albuminuria (measured by the immuno-turbidimetric method; Beckman Coulter IMMAGE, Brea, CA, USA) was used to separate healthy controls and normo-albuminuric (normal or mildly increased) patients with T2DM from those with albuminuria and to categorize patients with DN into two stages: Micro-albuminuria (moderately increased) and macro-albuminuria (severely increased) as defined in Table I (19, 22). Spot urine samples were collected for the determination of urinary micro-albumin and urinary creatinine to calculate albumin/creatinine ratio (ACR) using strip and laboratory quantitative tests (21). The severity of DN was assessed with the eGFR using the Chronic Kidney Disease Epidemiology Collaboration equation (23) (Table I).

Statistical analysis. Data were recorded and analyzed using IBM SPSS Statistics for Windows version 20.0 (IBM Corp., Armonk, NY, USA). Continuous variables are presented as the mean±standard deviation. Data for categorical variables are presented as numbers/percentages. Normality of the distribution of continuous variables was tested using the Kolmogorov–Smirnov method. Associations between continuous variables were described by Pearson's or Spearman's correlation coefficients. Comparisons of categorical variables among groups were performed using the chi-square test. One-way analysis of variance was used to determine whether there were any statistically significant differences between the means of two or more samples. Least significant difference was used for *post hoc* testing for multiple comparisons of statistically significant results between different variables. Logistic regression analysis with odds ratios were used to determine independent risk factors of albuminuria in patients with T2DM. A *p*-value of less than 0.05 was considered significant and of 0.001 or less as highly significant, at a confidence interval of 95%.

Results

Anthropometric measurements and clinical evaluation. As demonstrated in Table II, the study group ranged in age from 22-75 years. The mean age of the macro-albuminuria group (49.76 years) was significantly higher than the three other groups and that of the micro-albuminuria group was higher (46.33 years) than the normo-albuminuria T2DM group. Male patients more frequently had either micro- or macro-albuminuria compared to female patients with T2DM and female controls (p=0.003). Albuminuric

Table II. *Comparison of the tested parameters between the non-diabetic control group and groups of patients with type 2 diabetes mellitus (T2DM) with different stages of albuminuria*.*

Parameter		Control (n=41)	T2DM		
			Normo-albuminuria (n=45)	Micro-albuminuria (n=44)	Macro-albuminuria (n=39)
Age, years	Mean±SD	44.10±4.16	42.32±6.98[a]	46.33±7.85[b]	49.76±13.43[abc]
	Range	36-53	22-54	22-53	28-75
Sex, n, %	Male	14 (34.1%)	20 (44.4%)	30 (68.18%)[ab]	27 (69.2%)[ab]
	Female	27 (65.9%)	25 (55.6%)	14 (31.81%)	12 (30.8%)
BMI, kg/m^2	Mean±SD	26.31±1.37	27.62±1.60	28.75±1.7[a]	30.55±1.98[ab]
	Range	23.69-27.81	22.66-28.84	24.72-29.87	25.75-32.96
Duration of DM, years	Mean±SD	--	6.60±2.54	8.85±1.36[b]	10.30±1.49[bc]
	Range	--	2.45-11.96	5.76-11.22	5.76-11.96
Glucose, mg/dl	Mean±SD	85.19±13.49	161.39±13.93[a]	177.56±15.45[ab]	230.76±40.42[abc]
	Range	63.87-114.98	135.98-190.54	157.66-230.87	176.99-366.98
HbA1c, mg/dl	Mean±SD	4.68±1.3	7.34±1.03[a]	8.52±1.48[a]	9.67±1.19[ab]
	Range	3.65-7.01	6.01-9.54	6.44-10.84	7.27-12.42
TG, mg/dl	Mean±SD	120.52±11.19	206.30±18.85[a]	277.22±9.45[ab]	286.69±21.93[abc]
	Range	107.78-147.62	110.99-223.88	211.86-243.77	230.43-368.56
Creatinine, mg/dl	Mean±SD	0.81±0.29	0.98±0.46	1.20±0.56[a]	1.46±0.37[ab]
	Range	0.49-1.40	0.47-1.81	0.49-1.90	0.89-1.70
	Mean±SD	108.46±8.39	100.87±8.48	99.81±9.72	86.44±11.99[abc]
eGFR, ml/min	Range	83-120	77-121	88-121	65-120
	Mean±SD	148.72±14.46	227.24±10.23[a]	265.95±11.13[a]	288.30±57.76[abc]
TC, mg/dl	Range	120.55-170.88	200-247.11	233.78-265.75	235.86-430.77
	Mean±SD	59.11±7.70	136.52±9.86[a]	182.76±14.61[ab]	263.73±9.80[abc]
LDL, mg/dl	Range	45.35-76.55	122.88-165.76	170.45-210.76	231.65-260.34
	Mean±SD	61.95±7.52	50.28±8.44a	44.35±9.53[ab]	39.53±7.96[abc]
HDL, mg/dl	Range	50.78-84.56	38.75-65.89	33.89-59.54	30.55-53.85
	Mean±SD	18.48±2.95	16.74±3.14	80.56±8.43[ab]	455.38±77.27[abc]
ACR, mg/g creatinine	Range	12.56-23.55	11.85-23.43	61.94-95.43	320.87-600.43
	Mean±SD	9.11±0.61	8.94±0.57	8.65±1.22[ab]	8.12±1.81[abc]
Total Ca, mg/dl	Range	7.78-10.46	8.56-10.32	5.76-10.32	5.76-9.89
	Mean±SD	32.76±4.2	34.67±3.91	39.12±5.10[a]	42.65±6.17[abc]
PTH, ng/l	Range	24.65-43.65	22.65-45.23	22.87-49.76	26.76-50.06
Urea, mg/dl	Mean±SD	19.59±3.17	22.89±4.09[a]	27.89±7.52[ab]	34.98±5.21[abc]
	Range	11.66-23.54	17.98-32.65	18.71-40.87	24.89-45.32
IL4, pg/ml	Mean±SD	3.75±1.27	32.13±6.55[a]	67.41±7.87[ab]	91.23±7.59[abc]
	Range	2.11-6.98	20.54-45.89	50.68-70.54	79.45-105.98
Visfatin, mg/l	Mean±SD	1.65±1.01	8.61±1.43[a]	28.30±6.77ab	44.62±8.36[abc]
	Range	0.31-3.55	5.97-10.76	15.54-36.87	26.76-69.43
Irisin, mg/l	Mean±SD	89.61±20.07	422.51±28.55[a]	491.75±69.87[ab]	943.85±43.58[abc]
	Range	60-140	370-491	390.76-749	849-1001
Adiponectin, pg/ml	Mean±SD	4.53±1.73	29±7.01[a]	59.25±9.67[ab]	168.09±9.51[abc]
	Range	14.56-37.54	41.86-76.56	123-176.56	1.99-8.54

ACR: Albumin-to-creatinine ratio; BMI: body mass index; eGFR: estimated glomerular filtration rate; HbA1c: hemoglobin A1c; HDL: high-density lipoprotein; IL4: interleukin 4; LDL: low-density lipoprotein; PTH: parathyroid hormone; SD: standard deviation; T2DM: type 2 diabetes mellitus; TC: total cholesterol; TG: triglycerides. *Chi-squared test showed all parameters differed between groups at $p<0.001$, except for sex which was $p=0.003$. Significantly different from: [a]control group; [b]normo-albuminuria group; [c]micro-albuminuria group by analysis of variance.

patients with T2DM had significantly higher BMI ($p<0.001$) compared to normo-albuminuric patients and controls. They had also highly significant longer duration of DM (8.85 and 10.3 years for those with micro- and macro-albuminuria respectively) compared to diabetic patients without albuminuria (6.6 years, $p<0.001$).

Metabolic parameters. Tested metabolic parameters including FPG, HbA1c, TG, TC and LDL were significantly increased in patients with T2DM with macro-albuminuria, followed by those with micro-albuminuria compared to controls and patients with T2DM with normo-albuminuria, while the mean HDL was significantly lower in the former two groups compared to the latter two (p<0.001 for all tested parameters, Table II).

Indicators of renal function. Concerning the patients with T2DM with macro-albuminuria, the mean ACR, PTH and urea levels were significantly higher and calcium level and eGFR significantly lower when compared to the other three groups; meanwhile, they had a significantly higher mean creatinine level compared to the controls and the normo-albuminuria group, but with no difference from the T2DM microalbuminuria group. Patients with T2DM with micro-albuminuria demonstrated a significantly higher mean ACR and urea levels and lower total calcium level compared to both the control and the normo-albuminuric groups, and significantly higher mean creatinine and PTH levels compared to controls, but no difference was noticed in the eGFR (Table II).

ELISA. Comparison of patients with controls: As demonstrated in Table II, patients with T2DM had IL4, visfatin, irisin, and adiponectin ranges of 20.54-105.98 pg/ml, 5.97-69.43 mg/l, 370-1001 mg/l, and 14.56-176.56 pg/ml respectively, highly significantly greater than those of the control group (ranges of 2.11-6.98 pg/ml, 1.31-3.55 mg/l, 60-140 mg/l, 4.56-37.54 pg/ml, respectively).

Comparison of patients with T2DM with different stages of albuminuria: Among patients with T2DM, those with macro-albuminuria demonstrated statistically significantly higher mean IL4, visfatin, irisin, and adiponectin levels (91.23 pg/ml, 44.62 mg/l, 943.85 mg/l, 168.09 pg/ml, respectively) compared to patients with normo- and micro-albuminuria. Moreover, patients with micro-albuminuria had significantly higher mean IL4, visfatin, irisin, and adiponectin levels (67.41 pg/ml, 28.30 mg/l, 491.75 mg/l, 59.25 pg/ml, respectively) when compared to the normo-albuminuria T2DM group (32.13 pg/ml, 8.61 mg/l, 422.51 mg/l, 29±7.01 pg/ml respectively, p<0.001 for each biomarker) (Table II).

Table III. *Logistic regression analysis of albuminuria risk factors in type 2 diabetes mellitus.*

Parameter	Odds ratio	95% Confidence interval		*p*-Value
		Lower	Upper	
Age, years	1.001	0.475	2.107	0.599
Sex	1.641	0.794	3.394	0.295
Duration of DM, years	1.061	0.503	2.234	**0.034**
BMI, kg/m^2	0.906	0.833	0.984	**0.027**
Glucose, mg/dl	1.618	1.442	1.816	**0.014**
HbA1c, mg/dl	2.568	1.997	3.308	**<0.001**
TG, mg/dl	1.880	1.467	2.409	**0.023**
Creatinine, mg/dl	1.740	0.843	3.598	0.312
eGFR, ml/min	1.093	0.518	2.300	0.654
TC, mg/dl	2.959	0.356	7.782	0.212
LDL, mg/dl	1.179	1.146	1.213	**0.004**
HDL, mg/dl	1.459	1.379	1.544	0.213
ACR, mg/g creatinine	10.394	1.247	10.282	0.399
Total Ca, mg/dl	1.724	1.576	1.882	0.003
PTH, ng/l	1.098	1.035	1.166	0.109
Urea, mg/dl	2.022	1.849	2.207	0.124
IL4, pg/ml	1.562	1.392	1.753	0.134
Visfatin, mg/l	1.680	1.633	1.729	0.401
Irisin, mg/l	2.190	1.702	2.820	**<0.001**
Adiponectin, pg/ml	1.163	1.095	1.234	**0.009**

ACR: Albumin-to-creatinine ratio; BMI: body mass index; eGFR: estimated glomerular filtration rate; HbA1c: glycosylated hemoglobin; HDL: high-density lipoprotein; IL4: interleukin 4; LDL: low-density lipoprotein; PTH: parathyroid hormone; TC: total cholesterol; TG: triglycerides. Bold values indicate statistical significance.

Logistic regression analysis of albuminuria risk factors in T2DM. In the univariate logistic regression analysis (Table III), albuminuria was used as the dependent variable and the all the other tested parameters were used as independent variables. Duration of DM, BMI, and elevated serum FPG, TG, LDL, and adiponectin levels were significant risk factors for albuminuria (p<0.05 for all). Increasing levels of HbA1c and irisin were the only highly significant risk factors for albuminuria (p<0.001 for both, odds ratio=2.568 and 2.190, respectively). Insignificant albuminuria risk factors were: age, sex, eGFR, creatinine, TC, HDL, ACR, PTH, urea, IL4 and visfatin levels (p>0.05 for all).

Discussion

DN is a clinical syndrome characterized by persistent albuminuria and a progressive decline in renal function. It is associated with increased cardiovascular morbidity and mortality in diabetic patients, and the outcomes for individuals with DN are significantly worse than those without (22). As T2DM accounts for 90% of all cases of diabetes, so the majority of people who develop DN do so because of T2DM (1). For these reasons, it was important to testify the validity of new biomarkers directed towards early detection, progression monitoring and therapeutic targeting of DN in T2DM. In the current study, we investigated the differences between patients with T2DM at different stages of albuminuria, as well as non-diabetic controls, in regard to several parameters and indicators of metabolic diabetic status and renal function with particular regard to the newly addressed serum biomarkers irisin, adiponectin, visfatin and IL4.

Since being discovered in 2012, irisin has been the subject of extensive research that proved its participation in several pathological conditions, such as inflammation, obesity and carcinogenesis beside its role in normal metabolism. T2DM has been linked to the pathophysiology of irisin, a new adipokine. Prior research assessing the correlation between irisin and DN produced contradictory findings (24), being positively associated with insulin resistance, which is the hallmark of T2DM (25). It is also hypothesized that patients with CKD usually have abnormal irisin levels (8). In the current study, we reported a significant increase in mean serum irisin level in patients with T2DM compared to controls, thus confirming its role in promoting insulin resistance in this disease. Moreover, a significant difference was observed between normo-albuminuric patients with T2DM and non-diabetic controls, with higher levels of irisin in normoalbuminuric diabetic patients, indicating its potential ability to portray DN even prior to the occurrence of detectable albuminuria. Among our patients with T2DM, the irisin level was found to increase substantially in concordance with progression in albuminuria and was noted as a significant independent risk factor for albuminuria, signifying that it may well be an indicator of DN progression in T2DM. Along with HA1c, the established contributor to the pathogenesis of diabetic complications, irisin was found to be the most highly significant risk factor for albuminuria in logistic regression analysis ($p<0.001$), thus the irisin level might be used to reflect the long-term status of diabetic control, or other diabetic complications; however, this issue may be considered in further studies. Comparable studies also detected high irisin levels in patients with T2DM, and significantly lower levels in non-diabetic subjects, with irisin levels correlating positively with BMI, FPG and TG levels in the T2DM group (7, 26). In the same vein, Berezina *et al.* (6) reported elevated irisin in DN and confirmed it as a predictor of nephropathy severity, being negatively associated with the eGFR. It seems logical that under conditions of oxidative stress, inflammation and high levels of free fatty acids, such as in obesity, metabolic syndrome or diabetes, the expression of peroxisome proliferator-activated receptor gamma coactivator-1 α in muscle may stimulate increasing expression of FNDC5 and subsequently serum irisin levels (7). Yet contradictory data exist in a number of studies that reported decreased serum irisin level in patients with T2DM (8, 27-29), with even more significant reduction in patients with DN (21, 28) and an inverse correlation between irisin level and creatinine, FPG, DM duration, BMI, ACR and HbA1c (28), as well as with urinary albumin excretion level (30). Explanations for such inconsistencies lie in the considerable variation in irisin range across different ethnic populations (8, 25) because high levels were reported in Caucasian and Saudi Arabian patients with T2DM (26). Besides, unknown physical factors, differences in age, BMI, DM duration, severity of insulin resistance or other associated diseases and the method used to measure irisin among studies may also have contributed to this (8, 13). Moreover, single nucleotide polymorphisms in the FNDC5 gene constitute a factor that may modulate irisin levels. For example, certain genotypes (rs157069 TT) are associated with lower circulating irisin levels and higher fasting levels of insulin (26), while other alleles are associated with elevated

insulin resistance and dyslipidemia without any effect on the circulating irisin level (21).

As a multifunctional protein, adiponectin is assumed to influence renal physiology and pathological change relating to obesity and diabetes through interaction with its receptor AdipoR1 expressed in renal tissues (31). By the virtue of this role, our study demonstrated the mean serum adiponectin level in patients with T2DM to be significantly higher compared to non-diabetic controls. Its levels increased significantly with increasing albuminuria and it was detected as a significant (although not highly significant, p=0.009) independent risk factor for albuminuria in T2DM in logistic regression analysis. Thus, the serum adiponectin level can be a good predictor of DN in patients with T2DM. Our data support a series of previous studies (32-34) that confirmed higher serum adiponectin concentrations in patients with T2DM and CKD, and its association with the duration of diabetes, increasing albuminuria, and several indicators of renal insufficiency (*e.g.*, high ACR and creatinine, and reduced eGFR) and adverse lipid profiles (high TC and LDL levels). Other studies demonstrated no significant difference in serum adiponectin between patients with T2DM with nephropathy and those without (6), or even lower serum adiponectin level in patients with T2DM when compared to controls, with inverse associations with the probability of worsening albuminuria, DN progression or the development of other general diabetic complications (35, 36). However, in diabetics with advanced albuminuria and concomitantly reduced GFR, adiponectin was claimed to increase due to limited renal clearance (37). To authenticate our findings, we note that an increased adiponectin level in DN seems to be mediated by upregulation of adiponectin production in adipose tissue and its secretion into the bloodstream through an as yet unknown compensatory mechanism. This is in order to alleviate renal injury *via* mitigating disrupted renal endothelial function, lowering oxidative stress, and increasing expression of endothelial nitric oxide synthase and peroxisome proliferator-activated receptor-α (33, 34, 37, 38). Increased AdipoR1 expression in patients with DN may stimulate adiponectin production, despite adiponectin resistance, suggesting the presence of a positive feedback system of adiponectin (38). In view of the clearance of serum adiponectin *via* glomerular filtration, a higher level of serum adiponectin may also be related to impaired urinary excretion (34).

Results of this study disclosed high mean visfatin and IL4 levels in patients with T2DM that increased with progression of albuminuria, but both biomarkers bore no significant risk for the development of albuminuria in T2DM. Although a contradictory study reported lowered visfatin levels in advanced stages of DN (39), others reported significantly higher visfatin levels in nephropathic diabetic patients, with positive correlations with creatinine and DN stage and a negative correlation with eGFR (6, 16). For emphasis, high blood glucose stimulates synthesis of intracellular visfatin that hastens glucose uptake by renal cells, thus activating inflammatory pathways leading to injury in DN (16). Furthermore, it promotes insulin resistance and causes dysfunction of pancreatic beta cells at later stages (38). Concerning IL4, although significant in situ production was observed in renal tissues of patients with T2DM with DN compared to controls (18) and its overexpression was sufficient to induce kidney injury and proteinuria in mice (17), its circulating levels were not different in patients with albuminuric and non-albuminuric diabetic kidney in another study (39), suggesting that this cytokine acts locally within the diabetic kidney more effectively than systemically. Therefore, serum visfatin and IL4 may be biomarkers of renal involvement in T2DM rather than true risk factors in DN progression.

Conclusion

Patients with T2DM have elevated serum irisin and adiponectin levels compared to controls. High irisin and adiponectin levels in normo-albuminuric patients indicates their potential ability to portray DN even prior to detectable albuminuria. Both biomarkers were positively associated with increasing albuminuria and

were significant independent risk factors for it, thus both might be considered as potential independent risk factors for DN progression in patients with T2DM that might be therapeutically targeted.

Conflicts of Interest

None.

Authors' Contributions

BE, AE, OM, EO contributed to the conceptualization and design of the study, generated, curated, and analyzed the data. AA interpreted the results, wrote the original draft and the final manuscript. MK & SA edited and revised the manuscript. All Authors revised and approved the final version submitted for publication.

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