Irisin and Visfatin Predicts Severity of Diabetic Nephropathy

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Abstract Although the roles of irisin and visfatin have been well established in diabetes mellitus, there are limited studies about their association in diabetic nephropathy. The present study was designed to assess the levels of irisin and visfatin and their association with the severity of diabetic nephropathy. 43 diabetic nephropathy cases and 43 diabetic subjects without nephropathy were enrolled in the study. Serum levels of irisin and visfatin were compared in both the groups. Irisin and visfatin were significantly increased in diabetic nephropathy cases when compared with diabetes subjects without nephropathy. eGFR was negatively correlated with visfatin ($r = -0.323$, $p = 0.034$), irisin ($r = -0.324$, $p = 0.034$), urine albumin ($r = -0.443$, $p = 0.003$) and albumin creatinine ratio ($r = -0.419$, $p = 0.005$) in patients with diabetic nephropathy. Visfatin was significantly elevated in stage IV nephropathy compared with stage III nephropathy. We conclude that irisin and visfatin are elevated in diabetic nephropathy and can be an index of its severity.

Keywords Diabetes mellitus · Nephropathy · Irisin · Visfatin · Adiponectin

Abbreviations

ESRD End stage renal disease
GFR Glomerular filtration rate
CKD Chronic kidney disease
ACR Albumin creatinine ratio

Introduction

Diabetic nephropathy (DN) is the most common cause of end-stage renal disease (ESRD) [1]. The prevalence of DN has been increasing worldwide and It has been estimated that 20% of type 2 diabetic patients reach ESRD during their lifetime [2]. A previous study from India has reported the prevalence of overt nephropathy and microalbuminuria of 2.2 and 26.9%, respectively among urban South Indians [3]. It has been hypothesized that duration of diabetes, smoking, hypertension, advanced age and poor glycemic control are common risk factors for the development of overt nephropathy [4].

Irisin is a novel myokine and exercise hormone, which may regulate energy homeostasis and insulin sensitivity [5]. Experimental studies have demonstrated the role of irisin in protecting mice against diabetes and obesity [6]. Previous investigators have demonstrated an increase in irisin levels in type 1 and type 2 diabetes subjects [7, 8], whereas others have reported reduced serum irisin levels in patients with type 2 diabetes mellitus [9]. Also a negative association of irisin with insulin resistance and oxidative stress has been documented indicating that irisin may have antidiabetic and antioxidant effects [10]. A longitudinal study from Korea has shown an independent association of high serum irisin levels with the development of diabetes and concluded that irisin can predict diabetes in adult Korean population [11]. But till date, the role of irisin in diabetic nephropathy is unclear.

Visfatin, secreted by visceral adipose tissue is a proinflammatory adipokine known to have insulin mimetic action [12]. Elevated plasma visfatin levels have been reported in patients with type 2 diabetes Mellitus and gestational diabetes [13, 14]. Yilmaz et al. [15] have reported a positive association of visfatin with insulin

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resistance and degree of albuminuria in type 2 diabetic patients. Experimental studies have shown that chronic administration of visfatin ameliorated nephropathy in experimental type 2 diabetic mice [16].

The present study was carried out since there are no studies about irisin and visfatin levels in diabetic nephropathy especially in Indian population. The objective of the study was to assess the levels of irisin and visfatin and their association with severity of diabetic nephropathy.

Materials and Methods

The present study was a cross sectional study conducted at the Department of Biochemistry, Medicine and Nephrology, JIPMER, Puducherry. This study was approved by Institute Ethics Committee (Human studies). Written informed consent was obtained from all subjects before participation.

A total of 43 consecutive diabetic nephropathy patients in the age group of 40–65 years admitted in nephrology ward were enrolled in the study. Diabetic nephropathy was diagnosed based on the presence of microalbuminuria or overt proteinuria or low GFR (eGFR < 60 mL/min), with evidence of diabetic retinopathy. The results were compared with 43 age, BMI and sex matched diabetic subjects without nephropathy. Diabetic nephropathy cases on dialysis, chronic renal disease due to other causes, those without retinopathy, blood pressure > 160/100, acute coronary syndrome in the past six months, infections and inflammatory conditions were excluded from the study.

Sample Collection

Five millilitre of fasting venous blood samples were collected from the subjects. 2 mL of whole blood was used for the estimation of glycated hemoglobin by turbidometry method. Serum was separated from rest of the sample and glucose, urea and creatinine were estimated immediately using automated clinical chemistry analyzer (Olympus AU400, San Diego, California, USA). The rest of the serum was stored at -80°C and used for further analysis of the test parameters namely insulin, adiponectin, irisin and visfatin. 2 mL of urine sample was collected for estimation of albumin by nephelometer.

Analysis of Biochemical Parameters

Irisin (Cusabio, Hubei, China), visfatin (Ray Biotech, Inc. Georgia, United States) and adiponectin (AviBion, Finland) levels were estimated by ELISA. Insulin levels were estimated by chemiluminiscence method (ADVIA Centaur CP System, Siemens Healthcare, Germany) using reagents

from Siemens diagnostics. The severity of diabetic nephropathy was assessed by estimating eGFR by CKD-EPI Creatinine 2009 formula [17].

Statistical Analysis

The normality of the data was tested by Kolmogorov–Smirnov test. The data for the continuous variables were expressed as mean with Standard Deviation and median with range. Independent Student ‘*t*’ test or Mann–Whitney *U* test were used to compare the variables between cases and controls. Spearman’s correlation analysis was used to assess the association of eGFR with biochemical parameters. Multivariate linear regression analysis was performed to assess the predictors of severity of diabetic nephropathy with irisin, visfatin and adiponectin as variables. All statistical analyses were carried out for two tailed significance and *p* value < 0.05 was considered as significant.

Results

Table 1 shows the mean and standard deviation/median (range) of age, BMI, blood pressure and biochemical parameters in diabetic nephropathy and diabetic patients. Irisin, visfatin, urea, creatinine, blood pressure and albumin-creatinine ratio were significantly increased and eGFR was significantly reduced in diabetic nephropathy patients compared to those without nephropathy. There was no significant difference in adiponectin, insulin, glycated hemoglobin, blood glucose, age and BMI levels in both the groups.

The association of eGFR with various biochemical parameters is shown in Table 2. eGFR was negatively correlated with visfatin ($r = -0.323$, $p = 0.034$), irisin ($r = -0.324$, $p = 0.034$) and albumin creatinine ratio ($r = -0.419$, $p = 0.005$) in patients with diabetic nephropathy.

Also visfatin was positively correlated with albumin creatinine ratio ($r = 0.381$, $p = 0.012$) in patients with diabetic nephropathy.

Table 3 shows median (range) of biochemical parameters in stage 3 and stage 4 of diabetic nephropathy. Visfatin was significantly increased in stage 4 diabetic nephropathy cases compared to those with stage 3 nephropathy. There was no significant difference in adiponectin and visfatin levels in both the groups.

When multivariate linear regression analysis was performed to assess the predictors of severity of diabetic nephropathy with irisin, visfatin and adiponectin as variables, we found that visfatin significantly predicts the severity of diabetic nephropathy ($R^2 = 0.215$, $\beta = -0.325$, $p = 0.044$).

Table 1 Mean and standard deviation and median (range) of age, BMI, blood pressure and biochemical parameters in diabetics with and without nephropathy

Parameters	Diabetic without nephropathy (n = 43) (controls)	Diabetic with nephropathy (n = 43) (cases)	<i>p</i> value
Age (years)	52.2 ± 9.4	55.0 ± 7.8	0.12
BMI (kg/m ²)	25.3 ± 2.7	24.7 ± 3.0	0.34
Gender (M:F)	31:12	31:12	–
Duration of diabetes mellitus (years)	6.82 ± 4.90	8.73 ± 5.12	0.081
Age at onset of diabetes mellitus (years)	45.4 ± 9.67	46.36 ± 8.36	0.62
Systolic blood pressure (mmHg)	128.0 ± 13.1	138.7 ± 13.9	< 0.001
Diastolic blood pressure (mmHg)	82.42 ± 7.21	87.12 ± 8.71	< 0.001
Fasting serum glucose (mmol/L)	9.3 ± 4.5	10.27 ± 6.75	0.45
Postprandial serum glucose (mg/dL)	15.18 ± 6.40	16.28 ± 8.24	0.42
Glycated hemoglobin (%)	8.74 ± 2.2	8.22 ± 2.1	0.27
Fasting serum Insulin (pmol/L)	127.23 (35.5–634.6)	125.01 (35.84–552.9)	0.89
Serum creatinine (μmol/L)	79.56 ± 12.20	217.50 ± 78.36	< 0.001
Serum urea (mmol/L)	7.84 ± 1.70	29.44 ± 8.44	< 0.001
eGFR	87.8 ± 8.4	30.5 ± 12.7	< 0.001
U. ACR (mg/g Cr)	21.38 (7.9–29.5)	2223.91 (98–10,328)	< 0.001
Adiponectin (mg/L)	439 (13–3620)	450 (4–3395)	0.99
Visfatin (mg/L)	8.75 (2.52–17)	10.39 (2.07–36.48)	0.002
Irisin (mg/L)	492.14 (222–1614)	959.55 (405–1664)	< 0.001

Table 2 Correlation of eGFR with biochemical parameters like irisin, visfatin, adiponectin, creatinine and urine ACR

Parameters	Rho value	<i>p</i> value
Adiponectin (mg/L)	– 0.030	0.848
Visfatin (mg/L)	– 0.323	0.034
Irisin (mg/L)	– 0.324	0.034
Urine ACR (mg/g Cr)	– 0.419	0.005

Discussion

Irisin has been a focus of study in diabetes mellitus owing to its association with insulin resistance. A meta analysis by Qiu et al. [18] has demonstrated a positive association between irisin and insulin resistance in non diabetic adults. Even though the alteration in irisin levels in diabetes is

well established, the data regarding the same in kidney disease associated with diabetes mellitus is limited. Previous investigators have shown reduced plasma irisin levels in patients with albuminuria and chronic kidney disease with or without diabetes [19]. Two recent studies have reported low irisin levels in diabetic nephropathy cases and demonstrated negative association of irisin with serum creatinine and albumin-creatinine ratio in these patients [20, 21]. Also irisin was found to be negatively correlated with different stages of chronic kidney disease [22, 23]. In the present study serum irisin levels were significantly increased in diabetic nephropathy cases compared to those without nephropathy. Irisin was negatively correlated eGFR indicating high irisin levels are associated with severity of diabetic nephropathy. These findings are in contrast with a recent study by Hu et al. [24] which reported reduced irisin levels in diabetic patients with

Table 3 Mean and standard deviation and median (range) of visfatin, adiponectin and irisin in stage 3 and 4 of diabetic nephropathy

Parameters	Stage 3 of diabetic nephropathy (n = 21)	Stage 4 of diabetic nephropathy (n = 22)	<i>p</i> value
Urine ACR (mg/g Cr)	799.26 (98–9536)	3399.91 (190–10,328)	0.02
Serum adiponectin (mg/L)	380 (7–2352)	661.50 (4–3395)	0.308
Serum visfatin (mg/L)	9.354 ± 2.97	13.19 ± 6.17	0.017
Serum irisin (mg/L)	730.30 (405–1624)	1127.24 (450–1664)	0.114

macroalbuminuria compared to those with microalbuminuria and normoalbuminuria. Also we didn't found any significant association between irisin and insulin resistance in diabetic nephropathy patients.

The role of visfatin in diabetes is well established by earlier studies. Experimental studies have shown an increase in visfatin synthesis in the renal glomeruli of type 2 diabetic animals [25]. Previous studies have hypothesized that visfatin is up-regulated in diabetic kidney and is associated with the progression of diabetic nephropathy [12]. El Samahi et al. [26] have demonstrated reduced visfatin level in diabetic patients with microalbuminuria and suggested that visfatin plays a role in the pathogenesis of diabetic nephropathy. A recent experimental study has shown that visfatin administration ameliorated diabetic nephropathy in type 2 diabetic mice and concluded that visfatin has a protects against diabetic nephropathy [27]. In the present study, visfatin levels were significantly increased in diabetic nephropathy cases compared to those without nephropathy. Also visfatin was positively associated with albumin Creatinine ratio and negatively associated with eGFR indicating a link between elevated visfatin and severity of diabetic nephropathy. This was further justified when subgroup analysis was done in diabetic nephropathy cases based on different stages (stage 3 and stage 4). Visfatin was significantly increased ($p = 0.012$) in stage 4 nephropathy patients when compared with stage 3 patients in contrast with a study by Rodriguez et al. [28], which reported reduced visfatin levels in advanced stages of diabetic kidney disease. Multivariate linear regression analysis showed that visfatin predicts the severity of nephropathy in diabetes patients. Even though irisin was increased in stage 4 diabetic nephropathy cases, it was not significant when compared with stage 3 nephropathy cases ($p = 0.114$).

There are contradictory reports regarding adiponectin levels in patients with diabetic nephropathy. In some cross-sectional studies, serum adiponectin was found to be inversely associated with albuminuria, indicating a renoprotective role of adiponectin [29, 30], where as in other studies, higher adiponectin levels were associated with increased albuminuria and worsening of renal function [31]. In the present study, there was no significant difference in adiponectin levels in diabetic patients with nephropathy compared to those without nephropathy.

The main limitation of the study was small sample size and non inclusion of healthy control groups and early stages of nephropathy cases. Since the patients were on treatment for dyslipidemia and hypertension, the effect of drugs on these parameters was not investigated. Also urinary irisin and visfatin levels were not estimated due to financial constraints. Future studies with large number of samples are needed to investigate the mechanism through

which irisin and visfatin are linked with the severity of nephropathy in diabetes patients.

Conclusion

The findings from the present study conclude that irisin and visfatin are elevated and associated with disease severity in diabetic nephropathy. Further studies are needed to investigate whether they can be used as prognostic markers in these patients.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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