Glomerular filtration rate in patients with type 2 diabetes mellitus: is serum isthmin-1 level a possible link?

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ABSTRACT

Introduction Isthmin-1 (Ism-1) is a novel adipokine. However, little is known regarding the association between Ism-1 and type 2 diabetes mellitus (T2DM). This study aimed to investigate the relationship between serum Ism-1 levels and glomerular filtration rate (GFR) in patients with T2DM.

Research design and methods A total of 209 patients with T2DM were recruited into this retrospective study. Clinical data were collected. Fasting blood samples were collected for serum Ism-1 testing using ELISA kits. Based on the estimated glomerular filtration rate (eGFR), participants were divided into the normal eGFR group (n=167) and the decreased eGFR group (n=42). The relationship between Ism-1 and eGFR was assessed using linear and binary logistic regression analyses. Receiver operating characteristic (ROC) curve analysis was employed to examine the predictive efficacy of Ism-1 for distinguishing patients with eGFR <60 mL/min/1.73 m². Results Compared with patients with normal eGFR, serum Ism-1 levels were negatively correlated with eGFR in patients with T2DM even after multiple adjustments (p<0.001). For each 0.1 ng/mL increment of Ism-1, the odds of having an eGFR <60 mL/min/1.73 m² increased by 54.5% (OR=1.545; p<0.001) in patients with T2DM. ROC analysis showed that higher serum Ism-1 levels (>1.297 ng/mL) had predictive efficacy in patients with eGFR <60 mL/min/1.73 m², with an area under the curve of 0.908.

Conclusions Serum Ism-1 levels were inversely associated with eGFR, and high Ism-1 levels may be used as a potential biomarker for predicting kidney function impairment in patients with T2DM.

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a chronic disease characterized by hyperglycemia. Long-term disorders of glucose and lipid metabolism can lead to macrovascular and microvascular complications in diabetes. Chronic kidney disease (CKD) attributed to diabetes (diabetic kidney disease, DKD) is the major microvascular complications and primarily diagnosed based on the presence of albuminuria or impaired estimated glomerular filtration rate (eGFR).1 Approximately 20%–40% of patients with diabetes develop CKD as a complication, which can eventually progress to end-stage renal disease (ESRD).2,3 Patients with CKD have a markedly increased cardiovascular risk and all-cause mortality compared with the general population.4–6 Exploration of the factors and biomarkers associated with kidney function decline is essential for improving the prediction and prevention of CKD.

CKD is a chronic progressive disease that starts with microalbuminuria, which then progresses to macroalbuminuria, followed by a decline in the GFR.7 Many pathogeneses are involved in the occurrence and progression of CKD, including renal hemodynamic changes, oxidative stress, inflammation, and renin-angiotensin-aldosterone system dysregulation.8–9 However, these factors fail to fully account for the kidney function impairment in patients with diabetes. Recently, the effects of adipokines on CKD have also been proposed, such as adiponectin,10 neuregulin 411 and apelin.12

Isthmin-1 (Ism-1) is a new adipokine that was originally identified in the Xenopus...
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midbrain-hindbrain organizer called isthmus. Previous studies have demonstrated the role of Ism-1 in embryonic development, angiogenesis, aging, and cancer. Recent studies have shown that Ism-1 can improve glucose entry into adipocytes through the translocation of glucose transporter 4 (GLUT4) to the plasma membrane and suppress hepatic lipid synthesis by inhibiting de novo lipogenesis. The injection of recombinant Ism-1 protein into Ism-1-knockout mice reversed impaired glucose uptake and improved glucose intolerance. Therefore, Ism-1 has a dual role in improving glucose uptake while suppressing lipid accumulation and may be a potential therapeutic strategy for the simultaneous treatment of hyperglycemia and lipid disorder disease. A recent study showed that Ism-1 can induce the mitochondrial-dependent apoptosis by the release of apoptosis inducing factors for mitochondrial depolarization in podocytes, which indicates the role of Ism-1 in focal segmental glomerulosclerosis (FSGS).

Previous studies have shown the effects of Ism-1 on glucose and lipid metabolism. It has been suggested that Ism-1 could induce apoptosis of podocytes. However, little is known about the association between Ism-1 and DKD. In our previous study, a positive relationship between serum Ism-1 levels and the urinary albumin/creatinine ratio (UACR) was observed, suggesting that Ism-1 is closely related to the early stage of CKD. However, the relationship between Ism-1 levels and eGFR remains unclear. This study aimed to investigate the correlation between serum Ism-1 level and eGFR in patients with T2DM.

METHODS
Study design
This study follows a retrospective cross-sectional study design. Prior to collecting fasting blood collection and clinical data, written informed consent was obtained from...

Table 1 Characteristics of the study population in different stages of eGFR

<table>
<thead>
<tr>
<th></th>
<th>eGFR ≥60 mL/min/1.73 m²</th>
<th>eGFR &lt;60 mL/min/1.73 m²</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>167</td>
<td>42</td>
<td></td>
</tr>
<tr>
<td>Ism-1 (ng/mL)</td>
<td>0.92 (0.73–1.18)</td>
<td>1.58 (1.45–1.71)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age (years)</td>
<td>60.00 (55.00–68.00)</td>
<td>66.50 (57.00–75.00)</td>
<td>0.005</td>
</tr>
<tr>
<td>Female</td>
<td>41.32% (69)</td>
<td>42.86% (18)</td>
<td>0.856</td>
</tr>
<tr>
<td>Drinking</td>
<td>40.72% (68)</td>
<td>28.57% (12)</td>
<td>0.148</td>
</tr>
<tr>
<td>Smoking</td>
<td>39.52% (66)</td>
<td>42.86% (18)</td>
<td>0.693</td>
</tr>
<tr>
<td>History of hypertension</td>
<td>53.29% (89)</td>
<td>90.48% (38)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diabetes duration (months)</td>
<td>144.00 (84.00–240.00)</td>
<td>186.00 (114.00–255.00)</td>
<td>0.100</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>137.15±20.48</td>
<td>155.60±24.66</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>76.73±12.60</td>
<td>78.05±14.24</td>
<td>0.556</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.61±3.62</td>
<td>26.18±3.22</td>
<td>0.366</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>95.00 (88.00–100.00)</td>
<td>94.50 (89.00–103.00)</td>
<td>0.535</td>
</tr>
<tr>
<td>UACR (mg/g)</td>
<td>20.00 (10.00–72.50)</td>
<td>1750.00 (90.00–3990.00)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TC (mmol/L)</td>
<td>4.04 (3.39–5.02)</td>
<td>4.33 (3.42–4.82)</td>
<td>0.728</td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>2.37±0.79</td>
<td>2.40±1.04</td>
<td>0.872</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>1.10 (0.93–1.29)</td>
<td>1.12 (0.90–1.37)</td>
<td>0.943</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>1.15 (0.85–1.65)</td>
<td>1.35 (1.00–1.84)</td>
<td>0.075</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>42.50 (40.60–45.20)</td>
<td>36.60 (30.13–42.53)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>7.45 (6.50–9.05)</td>
<td>7.50 (6.48–9.00)</td>
<td>0.714</td>
</tr>
<tr>
<td>FBG (mmol/L)</td>
<td>6.21±1.10</td>
<td>6.35±1.56</td>
<td>0.590</td>
</tr>
<tr>
<td>FCP (ng/mL)</td>
<td>0.95 (0.54–1.53)</td>
<td>1.59 (0.86–2.47)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Metformin usage (%)</td>
<td>59.28% (99)</td>
<td>23.81% (10)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sulfonylurea usage (%)</td>
<td>40.72% (68)</td>
<td>30.95% (13)</td>
<td>0.246</td>
</tr>
<tr>
<td>Insulin usage (%)</td>
<td>44.31% (74)</td>
<td>64.29% (27)</td>
<td>0.021</td>
</tr>
</tbody>
</table>

The data are expressed as means±SD or median (IQR) or percentages. Significant p values (<0.05) are indicated in bold.

BMI, body mass index; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; FBG, fasting blood glucose; FCP, fasting C-peptide; HbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; Ism-1, Isthmin-1; LDL-C, low-density lipoprotein cholesterol; SBP, systolic blood pressure; TC, total cholesterol; TG, triglyceride; UACR, urinary albumin/creatinine ratio; WC, waist circumference.
all patients before inclusion in the study. A total of 209 patients with T2DM (diagnosed based on the 2006 WHO criteria) were recruited at the Qilu Hospital of Shandong University between October 2020 and December 2021. The exclusion criteria and research flowchart are shown in online supplemental figure 1. Fasting serum was collected for serum Ism-1 level measurement. Clinical data were collected for statistical analyses.

Clinical data collection

Fasting blood samples were collected from each patient after a 10-hour fast. Serum aliquots were stored frozen at −80°C until analysis. The characteristics of all subjects were collected using the computerized patient record system of the Qilu Hospital, including age, sex, diabetes duration, weight, height, waist circumference (WC), blood pressure (BP), history of hypertension, history of smoking and drinking, hemoglobin A1c (HbA1c), fasting blood glucose (FBG), fasting C-peptide (FCP), albumin, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), total cholesterol (TC), triglycerides (TG), creatinine, UACR, body mass index (BMI), and smoking and drinking, hemoglobin A1c, TC, TG and FCP.

Definition and grouping

eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation. According to the American Diabetes Association, eGFR <60 mL/min/1.73 m² is considered as decreased kidney function when treatment is recommended. All the participants were divided into the normal eGFR group (n=167) and the decreased eGFR group (n=42).

Statistical analyses

Patient characteristics are presented as mean±SD or median (IQR) for continuous variables and as percentages for categorical variables. The Kolmogorov-Smirnov test was conducted to determine whether the variables were normally distributed. The homogeneity of variance was estimated using the F-test. Comparisons between groups were evaluated using one-way analysis of variance and least significant difference post hoc test for continuous variables with normal distribution, Kruskal-Wallis test for continuous data with skewed distribution and Pearson χ² test for categorical variables. Spearman analysis and linear regression analyses were performed to explore the relationship between the Ism-1 levels and eGFR. Binary or multiple logistic and linear regression analyses were performed to explore the relationship between Ism-1 level and decreased kidney function in all enrolled patients. A receiver operating characteristic

Table 2 Association between serum Ism-1 levels and eGFR

<table>
<thead>
<tr>
<th>β coefficients (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1: Unadjusted.</td>
<td></td>
</tr>
<tr>
<td>Model 2 (per 0.1 ng/mL)</td>
<td>1.545 (1.348 to 1.771)</td>
</tr>
<tr>
<td>Model 3: Adjusted by age, sex, diabetes duration, and BMI.</td>
<td></td>
</tr>
<tr>
<td>Model 3 (per 0.1 ng/mL)</td>
<td>1.546 (1.224 to 1.953)</td>
</tr>
</tbody>
</table>

Table 3 Association between serum Ism-1 levels and low eGFR (< 60 mL/min/1.73 m²)

<table>
<thead>
<tr>
<th>OR (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1 (per 0.1 ng/mL)</td>
<td>1.545 (1.348 to 1.771)</td>
</tr>
<tr>
<td>Model 2 (per 0.1 ng/mL)</td>
<td>1.579 (1.353 to 1.841)</td>
</tr>
<tr>
<td>Model 3 (per 0.1 ng/mL)</td>
<td>1.546 (1.224 to 1.953)</td>
</tr>
</tbody>
</table>

Figure 1 Association between serum Ism-1 levels and estimated glomerular filtration rate (eGFR). Spearman analysis: correlation coefficient=−0.478; p<0.001.

urea nitrogen (BUN), creatinine (Cr), alanine aminotransferase (ALT), aspartate aminotransferase (AST), medication and drug history in relation to diabetes. History of smoking, drinking and hypertension were the same as the previous study. Diabetes duration was calculated by subtracting the date of diagnosis from the date of enrollment. Body mass index (BMI) was calculated as the weight (kg) divided by the squared height (m²). Medication usage was defined as administration of the drug for ≥2 weeks at the time of enrollment. Serum Ism-1 concentrations were quantified in duplicate using commercial ELISA kits (MyBiosource, San Diego, Southern California, USA; MSB2707255), and the average value was applied for the analysis.
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(ROC) curve was constructed to determine the best threshold value of Ism-1 for distinguishing patients with low eGFR (<60 mL/min/1.73 m²). All analyses were conducted using SPSS V.25.0. Graphs were constructed using the GraphPad Prism software. P value <0.05 was considered as significant.

RESULTS
Clinical characteristics and serum Ism-1 levels of participants in different eGFR groups

Demographic and biochemical characteristics of the study population are shown in table 1. No significant differences were observed in sex, drinking, smoking, DBP (diastolic blood pressure), BMI, WC, blood lipid profile, and FBG levels among the two groups. Compared with the normal eGFR group, the rates of hypertension, SBP (systolic blood pressure), UACR, and FCP were higher in the decreased eGFR groups (p<0.001). Albumin of the decreased eGFR group was significantly lower than the normal eGFR group (p<0.001). Compared with the normal eGFR group, the serum Ism-1 levels were elevated in the decreased eGFR group (online supplemental figure 2, p<0.001).

The association between serum Ism-1 levels and eGFR in patients with T2DM

Correlation analysis was applied to explore the relationship between serum Ism-1 and eGFR in all enrolled patients (figure 1; correlation coefficient=−0.478; p<0.001). Univariate and multivariable linear regression analyses were further performed to determine whether the Ism-1 level was associated with eGFR. Recent research has identified that Ism-1 is mainly produced by adipocytes and is positively related to BMI; therefore, we included BMI in our models. History of hypertension and UACR were listed as covariates because they were significantly different among different eGFR groups, and hypertension is a well-known risk factor for CKD. Medication that affects renal function was also included, including dipeptidyl peptidase-4 inhibitors, sodium-glucose cotransporter-2 inhibitors, glucagon-like peptide-1 receptor agonists, and ACE inhibitors or angiotensin-receptor blockers. An inverse association between serum Ism-1 levels and eGFR was observed even after adjusting for age, sex, diabetes duration, BMI, history of hypertension, UACR, HbA1c, TC, TG, FCP and usage of medication (p<0.001; table 2 and figure 1).

To further investigate the relationship between serum Ism-1 levels and decreased eGFR, taking eGFR as a dichotomous variable of clinical significance, participants were divided into two groups: eGFR ≥60 mL/min/1.73 m² and eGFR<60 mL/min/1.73m². A series of binary logistic regression analyses were conducted. Serum Ism-1 levels were associated with an increased risk of a decreased eGFR (table 3). The association remained significant even after adjusting for age, sex, diabetes duration, BMI, history of hypertension, UACR, HbA1c, TC, TG, FCP and usage of medication.

ROC curve for the identification of patients with eGFR < 60 mL/min/1.73 m²

ROC curve analysis was performed to predict a threshold value of Ism-1 for distinguishing patients with eGFR <60 mL/min/1.73 m². The area under the curve (AUC) values was 0.908 with a p-value <0.001 and a 95% CI from 0.859 to 0.958 (figure 2A). The sensitivity and specificity values were 92.86% and 83.83% with an optimal cut-off value of 1.297 ng/mL.

In the present study, we further explored the predictive efficacy of Ism-1 for identifying patients with eGFR <30 mL/min/1.73 m² by ROC curve analysis. The AUC was 0.915 (95% CI 0.875 to 0.954) with p<0.001. The
sensitivity and specificity values were 100.00% and 83.42% with an optimal cut-off value of 1.419 ng/mL (figure 2B).

DISCUSSION

The present study showed that higher serum levels of Ism-1 were observed in patients with a decreased eGFR. Serum Ism-1 levels were inversely associated with eGFR in patients with T2DM, even after adjusting for age, sex, diabetes duration, BMI, history of hypertension, UACR, HbA1c, TC, TG, FCP and usage of medication.

In our previous study, we found that serum Ism-1 levels were elevated in patients with albuminuria and were positively associated with UACR in patients with T2DM. However, there is another form: some patients with T2DM presented a decreased GFR without albuminuria. In addition, the Kidney Early Evaluation Program (KEEP) study showed that UACR and eGFR were independently associated with mortality and progression to ESRD. We were interested in whether Ism-1 is also related to eGFR in addition to UACR. In the present study, our findings showed a negative correlation between serum Ism-1 levels and eGFR in patients with T2DM. After adjusting for diabetes duration, history of hypertension, HbA1c level, and UACR, a strong association was still observed. The results of the ROC analyses indicate that serum Ism-1 levels may be used as a sensitive biomarker for the early diagnosis and risk stratification of kidney function decline in patients with T2DM. More studies are needed to systematically explore the mechanisms involved in the role of Ism-1 on decreased eGFR in T2DM.

The present study was observational and did not allow us to draw conclusions about the possible mechanisms underlying the relationship between Ism-1 and kidney function decline. Hyperglycemia and dyslipidemia are two known risk factors for CKD. Since Ism-1 can improve glucose tolerance and inhibit lipid synthesis, it may play a positive role on CKD through improving glycemic control and lipid abnormalities. However, this association of Ism-1 and kidney function decline persisted even after adjusting for HbA1c, TC and TG. Therefore, Ism-1 may affect kidney function through other mechanisms. Several recent studies have demonstrated that Ism-1, as an endogenous anti-inflammation factor, could inhibit nuclear factor kappa-B (NF-κB) activation and proinflammatory cytokine/chemokine production in acute lung injury. Ism-1 could also induce apoptosis of alveolar macrophage and restrain excessive inflammatory response in chronic obstructive pulmonary disease. Chronic inflammation contributes to the decline in GFR in CKD. Ism-1 may play a protective role in CKD through restraining inflammation.

Previous studies about the role of Ism-1 in diabetic nephropathy are really scarce. A previous study found that Ism-1 could impair the viability of podocytes in FSGS. The researchers found that Ism-1 could induce the apoptosis of podocytes through one caspase-dependent and one caspase-independent associated with mitochondrial destabilization. On the one hand, Ism-1 could bind to αβ5 or GRP78 and induce caspase-dependent apoptosis. On the other hand, at higher concentration, Ism-1 could bind to GRP78, induce mitochondria membrane depolarization and pro-apoptotic proteins release, and eventually cause caspase-independent apoptosis. Gao et al found that Ism-1 plays a critical role in early kidney development. Ism-1 knockout mice failed in branching morphogenesis and mesenchyme condensation. Both podocyte impair and tubulointerstitial changes are important in DKD development. Further mechanistic studies are required to elucidate the relationship between Ism-1 and impaired kidney function.

Our study has some limitations. First, this was a cross-sectional study without follow-up studies. This retrospective study cannot definitively establish cause and effect. The significance of the changes in serum Ism-1 levels and CKD progression remains unknown. Further studies are needed to investigate the underlying mechanisms of Ism-1 in the decline of kidney function. Second, as no renal biopsy was done during recruitment, patients with diabetes and non-diabetic kidney disease may be involved, so the relationship between serum Ism-1 levels and eGFR in this study cannot rule out the impact of possible existing non-diabetic nephropathy. Third, all subjects were of Chinese ethnicity. Whether these results are generalizable to other populations requires further investigation.

CONCLUSION

In conclusion, our findings clearly showed that serum Ism-1 levels were increased in patients with decreased eGFR. Serum Ism-1 levels were inversely associated with eGFR in patients with T2DM. High serum Ism-1 level may be a potential biomarker for predicting kidney function impairment in patients with T2DM. Further studies are needed to explore the underlying mechanism of this relationship and the potential role of Ism-1 as a biomarker for CKD diagnosis and risk stratification.

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Pathophysiology/complications

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