Serum Asprosin and Isthmin-1 Levels and the worsening of Albuminurea in Type 2 Diabetic Patients

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Abstract

Background: Diabetic Nephropathy (DN) is one of the most important microvascular complications of type 2 Diabetes Mellitus (T2DM) and one of the common causes of end-stage renal disease and T2DM death. The main distinguishing trait of DN is reduced glomerular filtration barrier, which is often assessed by estimated glomerular filtration rate (eGFR). Asprosin is a novel diabetogenic adipokine classified as a caudamin hormone protein. During fasting, white adipose tissue releases this adipokine, which has glucogenic and orexigenic actions. Isthmin-1 (Ism1) is an insulin-like adipokine that plays a dual effect in enhancing adipose glucose uptake and reducing hepatic lipid production.

Objectives: The objective of this study is to Investigate the effect of Asprosin and Isthmin 1 on diabetic nephropathy (DN) in patients with type 2 diabetes Miletus (T2D).

Patients and Methods: A case-control study was performed at specialized endocrine and diabetes research center in Baghdad, Iraq. for the period from October 2024 to March 2025. Ninety (90) patients diagnosed with T2DM, categorized into three groups based on their urine albumin/creatinine ratio, normal albuminuria, microalbuminuria, and macroalbuminuria. Asprosin and Isthmin-1 were tested using ELISA. Estimated glomerular filtrating rates were measure using CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) formula, blood urea and serum creatinine were tested in the laboratory.

ResultS: showed that The macroalbuminuria group exhibited the highest mean Asprosin concentration (69.53 ± 18.85 ng/mL), followed by the microalbuminuria group (50.65 ± 6.59 ng/mL), while the normoalbuminuria group had the lowest levels (25.75 ± 3.87 ng/mL). The macroalbuminuria group had the highest mean isthmin-1 concentration (729.19 ± 73.58 pg/mL), followed by the microalbuminuria group (479.26 ± 83.62 pg/mL), while the normoalbuminuria group exhibited the lowest levels (195.39 ± 48.80 pg/mL).

Conclusion: It was concluded that both Asprosin and Isthmin-1 levels increase significantly with worsening albuminuria (from normo- to micro- to macroalbuminuria).

Keywords: Diabetic nephropathy ,Albuminuria, Asprosin , Isthmin-1 , GFR , ACR .

INTRODUCTION

Type 2 Diabetes Mellitus accounts for approximately 90% of the world's diabetes prevalence, T2DM is characterized by the simultaneous presence of β-cell secretion failure and insulin resistance. [1]

One of the most common microvascular complications of diabetics and the leading cause of end-stage kidney disease (chronic kidney disease (CKD) is a diabetic nephropathy which is develops in 40% of people afflicted with diabetes mellitus [2] and it is characterized by synovuria and excessive filtration in the early stages followed by a progressive decline in kidney function. [3]

Diagnosing worsening of renal function in diabetic patients early on is critical, as it is considered a reversible situation. Markers such as urine albumin excretion (UAE), estimated glomerular filtration rate (eGFR), and albumin/creatinine ratio have little ability to discriminate different levels of organ injury severity in DNP. [4]

Asprosin acts as glucogenic via the olfactory receptor 4M1 (OR4M1) and also as appetite stimulant hormone via a cell surface receptor termed protein tyrosine phosphatase receptor δ (Ptprd). Plasma asprosin levels increased in both DN and non-DN patients when compared to the NGT group, with the highest level in patients suffering from DN . Furthermore, asprosin concentrations appear to be closely associated to early-stage DN clinical indicators such as estimated glomerular filtration rate (e-GFR) and urine albumin-creatinine ratio (UACR) (67). A favorable association was discovered between asprosin plasma levels and UACR, implying asprosin as an early clinical indication for DN progression. [5,6]

Isthmin-1 is another novel adipokine showing promising potential to be used as a biomarker in diabetic patients for monitoring diabetes related complications . Isthmin-1 (Ism-1) is a newly discovered insulin-like adipokine that promotes glucose uptake by adipocytes while inhibiting hepatic fat production.[7]

ISM-1 has two possible receptors: αvβ5 integrin (low affinity) and GRP78 (high affinity). Integrins are transmembrane receptors that regulate cell adherence to matrix molecules and play crucial roles in angiogenesis and inflammation. GRP78 is typically recognized as an ER lumen chaperon protein that facilitates protein folding and mediates the cellular stress response.[8]

ISM-1 can increase glucose entry into adipocytes by translocating glucose transporter 4 (GLUT4) to the plasma membrane and suppressing hepatic lipid production by blocking de novo lipogenesis . As a result, Ism-1 plays a dual role in enhancing glucose absorption while reducing lipid accumulation, and it may be an effective option for treating both hyperglycemia and lipid disorder disease. [9]

A recent study 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 αVβ5 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. Both podocyte impair and tubulointerstitial changes are important in DKD development.[10]

PATIENTS AND METHOD

A case-control study research, in which patients were recruited from the National Diabetes Centre for Treatment and Research, Mustansiriyah University, Baghdad, Iraq for the period from October 2024 to March 2025. Ethical approval was obtained from the local ethics committee according to national and international rules(number 65 on 31-10-2024) . a ninety (90) adult patients included in the study and they were classified according to there urinary ACR into three groups NormoAlb, microalbuminuria, and macroalbuminuria groups. A verbal consent was applied by each patient.

The Inclusion criteria: Adult patients with T2DM. The Exclusion criteria: Type 1Diabetes patients, Gestational Diabetes, An acute infection on the day of sample collection, chronic liver disease and chronic kidney disease .

Urine sample was obtained to measure albuminuria by using a special microalbumin auto-analyzer (Combilyzer-13, Human Company, Germany) . Blood sample (10 ml) was obtained from each patient via venipuncture , 2ml was transferred into EDTA tube for HbA1c estimation and 8ml was transferred into gel tube for others parameters analysis. The blood sample in the gel tube was left for 30 min then centrifuged at 3400 rpm for 10 minutes ,1.5 ml of the resultant serum was transferred into Eppendorf and stored at about – 20C to be used for Asprosin and Isthmin-1 detection and rest of this serum used for the detection of lipid profile parameters, Renal function tests, fasting blood sugar .

Asprosin and Isthmin-1 were assessessed using available enzyme-linked immune aorbent assay (ELISA) kits (human ELISA kit of china origin) and reading was obtained using a bioticELISA reader.

Statistical analyses were performed using GraphPad Prism (Version 9.0, GraphPad Software, San Diego, CA, USA) and MedCalc Statistical Software version 20.115 (MedCalc Software Ltd, Ostend, Belgium). Data were presented as mean ± standard deviation (SD) for normally distributed variables or median (interquartile range) for non-normally distributed variables. Categorical variables were compared using Chi-square or Fisher's exact test as appropriate. Both ANOVA and post-hoc tests were used to test the significance of the difference between groups after testing the normality of the data.

RESULTS

A significant difference in asprosin levels was observed across the study groups (**$p < 0.0001$**) as shown in Figure 1. The macroalbuminuria group exhibited the highest mean asprosin concentration (69.53 ± 18.85 ng/mL), followed by the microalbuminuria group (50.65 ± 6.59 ng/mL), while the normoalbuminuria group had the lowest levels (25.75 ± 3.87 ng/mL). Post-hoc analysis using the Tukey-Kramer test revealed that the asprosin levels in the macroalbuminuria group were significantly higher than in the normoalbuminuria group, indicating a potential association between increased asprosin levels and worsening albuminuria. This consistent and significant elevation in asprosin levels parallels the progression of albuminuria severity, suggesting a potential association between asprosin and the pathophysiology of diabetic kidney disease. Table 1 compares renal function parameters and biomarkers across albuminuria Cat egories.

Table 1 Comparison of Novel Biomarkers and Renal Function Parameters AcrossAlbuminuria groups

	Macroalbuminuria	Microalbuminuria	Normoalbuminuria	P #
Asprosin ng/mL	$69.53^{a,b} \pm 18.85$	$50.65^{a} \pm 6.59$	$25.75^{b} \pm 3.87$	<0.0001
Isthmin_1 pg/mL	$729.19^{a,b} \pm 73.58$	$479.26^{a} \pm 83.62$	$195.39^{b} \pm 48.80$	<0.0001
GFR mL/min/1.73m²	$53.67^{a,b} \pm 38.04$	$92.77^{a} \pm 25.05$	$99.00^{b} \pm 13.83$	<0.0001
ACR mg/mmol	$34.09^{a,b} \pm 0.00$	$11.57^{a} \pm 4.97$	$1.86^{b} \pm 1.90$	<0.0001
Blood Urea mg/dL	86.60a,b ± 64.47	34.17a ± 26.20	26.17b ± 12.33	<0.0001
Serum Creatinine mg/dL	2.03a,b ± 1.34	1.01a ± 0.80	0.71b ± 0.16	<0.0001

Data are: Mean ± Standard deviation

ANOVA a groups with the same letter differ significantly in the post-hoc test according to Tukey-Kramer

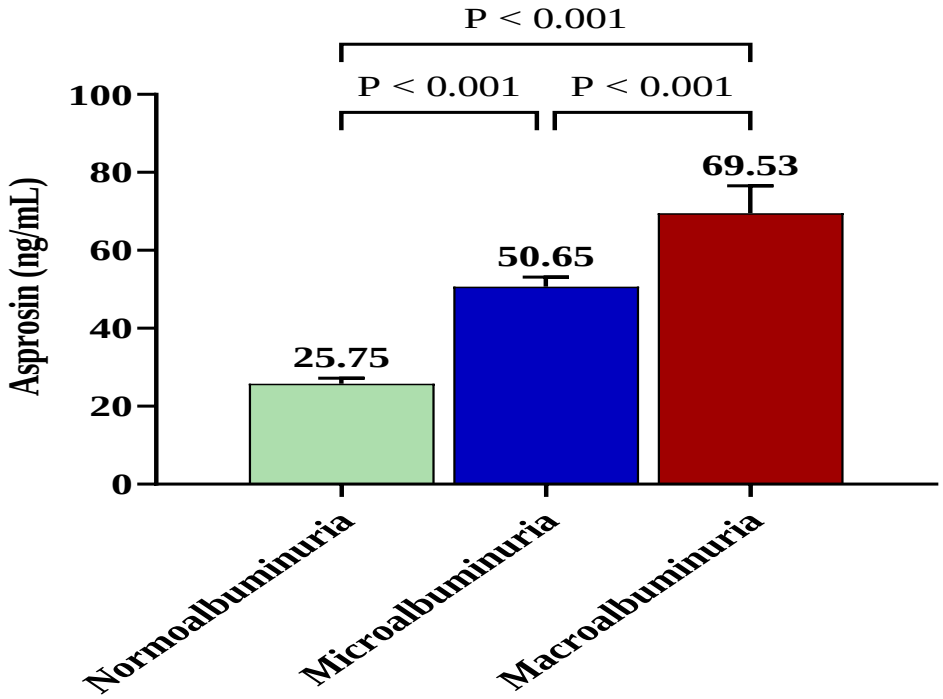

Figure 1 Asprosin Levels Across Albuminuria Categories

This bar graph presents the distribution of serum asprosin concentrations across three albuminuria groups, demonstrating a clear and statistically significant stepwise increase in asprosin levels with increasing severity of albuminuria. The error bars indicating the 95% CI of the mean

The data in Figure 2 showed a significant variation in isthmin-1 levels was also detected among the study groups ($p < 0.0001$) . The macroalbuminuria group had the highest mean isthmin-1 concentration (729.19 ± 73.58 pg/mL), followed by the microalbuminuria group (479.26 ± 83.62 pg/mL), while the normoalbuminuria group exhibited the lowest levels (195.39 ± 48.80 pg/mL). Post-hoc analysis demonstrated that the macroalbuminuria and microalbuminuria groups had significantly higher isthmin-1 levels compared to the normoalbuminuria group. The progressive increase in isthmin-1 levels from normoalbuminuria to macroalbuminuria suggests a potential role for this biomarker in the progression of renal dysfunction. The elevated isthmin-1 concentrations in individuals with higher degrees of albuminuria may indicate its involvement in endothelial dysfunction, inflammation, or metabolic disturbances associated with kidney disease progression and its possible utility as a biomarker.

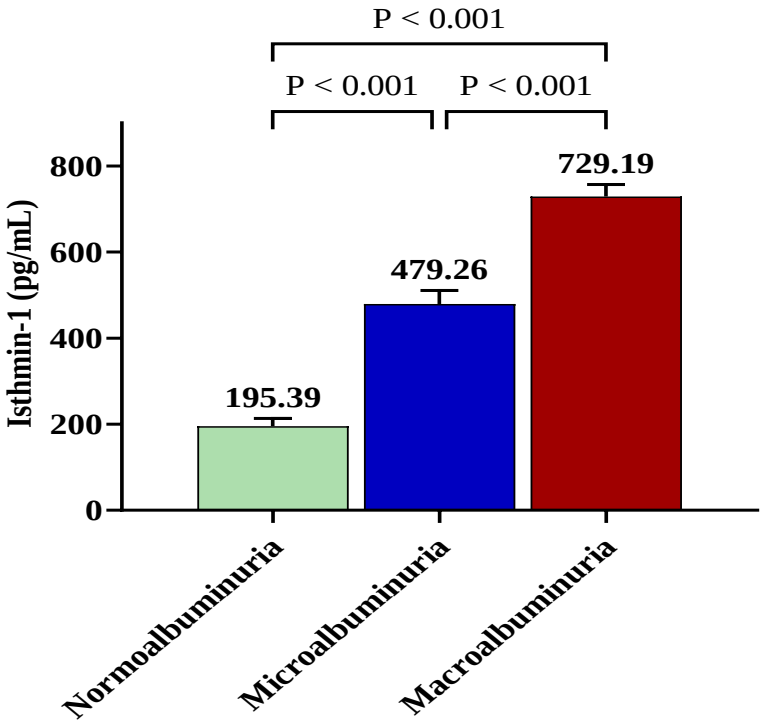

Figure 2 Isthmin-1 Concentrations Across Albuminuria Groups

This bar graph shows serum isthmin-1 levels across albuminuria categories, demonstrating a significant stepwise increase Statistical analysis shows highly significant differences between all groups ($p < 0.001$). Error bars represent the 95% CI of the mean.

The data in table 2 suggests a strong positive correlation between serum asprosin and isthmin-1 in patients with normoalbuminuria, indicating these adipokines may be closely linked at earlier stages of kidney involvement. However, as albuminuria progresses to micro- and macroalbuminuria, the correlation weakens and becomes statistically insignificant, which could imply a disrupted or altered relationship between these biomarkers during advancing diabetic nephropathy (DN). Figure 3

Table 2 Correlation Analysis Between Asprosin and Isthmin-1 in Different Albuminuria Groups

Group	n	Correlation Coefficient (r)	P-value	Interpretation
Normoalbuminuria	30	0.638	<0.001*	Strong positive correlation
Microalbuminuria	30	0.051	0.7890	No significant correlation
Macroalbuminuria	30	0.305	0.1007	Weak positive correlation

**Statistically significant at p < 0.05.*

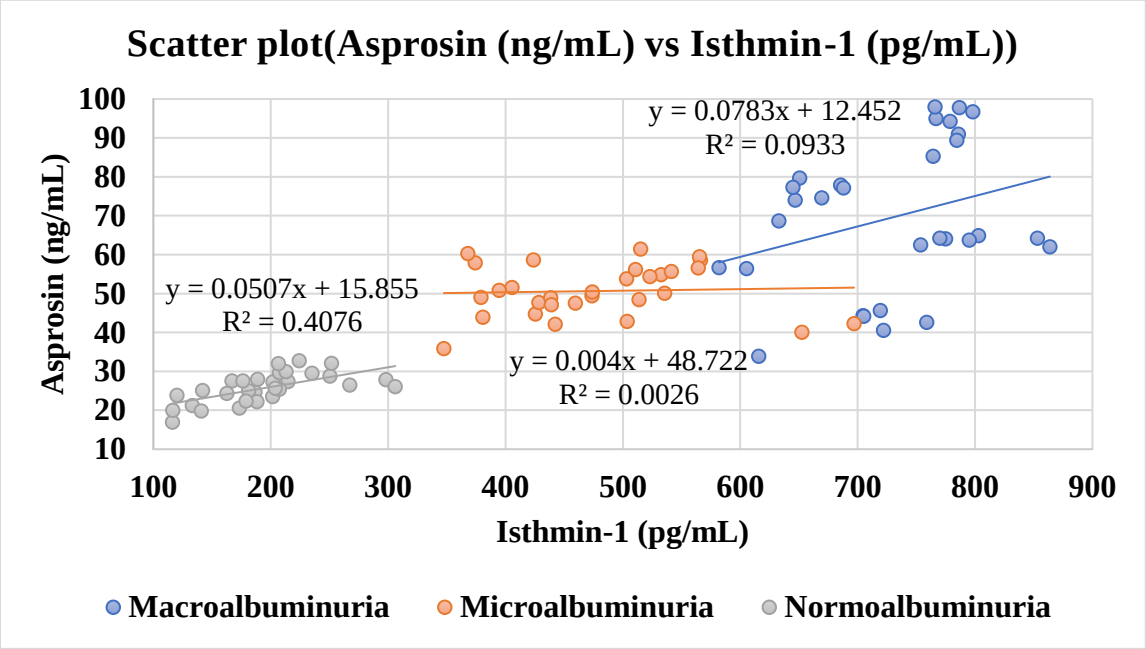

Figure **Error! No text of specified style in document.** Correlation Analysis between Asprosin and Isthmin-1 Levels

This scatter plot demonstrates distinct correlations between asprosin and isthmin-1 across albuminuria groups.

Correlation Analysis Between Asprosin and Isthmin-1 and other parameters in Different Albuminuria Groups

The analysis of correlations between Asprosin and Isthmin-1 levels and various clinical and biochemical parameters revealed that In the Macroalbuminuria group, as presented in table 3 Asprosin exhibited significant positive correlations with blood urea ($r = 0.453$, $p = 0.0083$) and serum creatinine ($r = 0.348$, $p = 0.0495$), indicating that higher levels of Asprosin are associated with increased blood urea and serum creatinine levels. These findings suggest a potential link between Asprosin and renal function markers in this group.

Isthmin-1 demonstrated a significant negative correlation with systolic blood pressure (SBP) (r = -0.408, p = 0.0179), suggesting that higher Isthmin-1 levels are associated with lower SBP. This finding may indicate a potential role of Isthmin-1 in blood pressure regulation.(table3).

Table **Error! No text of specified style in document.**- Correlations Between Asprosin and Isthmin-1 and other parametes in
Macroalbuminuria Group (n=30)

Variable	Asprosin		Isthmin-1	
	R	p-value	r	p-value
SBP	0.047	0.8039	-0.408	0.0179*
Blood Urea	0.453	0.0083*	0.071	0.7118
Sr. Creatinine	0.348	0.0495*	-0.132	0.4821
GFR	-0.321	0.0730	0.051	0.7856
ACR	0.000	1.0000	0.000	1.0000

ROC Analysis Results for Novel Biomarkers in Distinguishing Albuminuria Groups:

The ROC analysis demonstrated that Asprosin and Isthmin-1 had distinct diagnostic capabilities as potential albuminuria biomarkers (Table 4). Both Asprosin and Isthmin-1 have perfect discriminatory ability (AUC=1.00) to differentiate macro- and microalbuminuria from normoalbuminuria with 100% sensitivity and specificity at thresholds >32.66 ng/mL for Asprosin and >306.119 pg/mL for Isthmin-1.

When distinguishing macroalbuminuria from microalbuminuria, the diagnostic performance is slightly lower but still excellent with AUCs of 0.813 (Asprosin) and 0.981 (Isthmin-1), high sensitivity, and specificity.

Table 4 ROC Analysis Results for Novel Biomarkers in Distinguishing Albuminuria Groups

Biomarker & Comparison	AUC	Criterion	Sensitivity (%)	Specificity (%)	+LR	-LR	+PV (%)	-PV (%)
Asprosin								
Macro vs Normo	1.00	>32.66	100.00	100.00	∞	0.00	100.0	100.0
Micro vs Normo	1.00	>32.66	100.00	100.00	∞	0.00	100.0	100.0
Macro vs Micro	0.813	>61.401	73.33	100.00	∞	0.27	100.0	78.9
Isthmin-1								
Macro vs Normo	1.00	>306.119	100.00	100.00	∞	0.00	100.0	100.0
Micro vs Normo	1.00	>306.119	100.00	100.00	∞	0.00	100.0	100.0
Macro vs Micro	0.981	>566.048	100.00	93.33	15.00	0.00	93.7	100.0

AUC: Area Under Curve; PPV: Positive Predictive Value; NPV: Negative Predictive Value Normo: Normoalbuminuria; Micro: Microalbuminuria; Macro: Macroalbuminuria

Discussion

Diabetic nephropathy remains a leading cause of chronic kidney disease in patients with type 2 diabetes mellitus .Hyperglycemia triggers excessive formation of advanced glycation end-products and activation of their receptor (RAGE), leading to oxidative stress, inflammation, and progressive glomerular and tubular injury. The findings in this study support this pathophysiologic framework and identify the adipokines Asprosin and Isthmin-1 (Ism-1) as potential biomarkers reflecting renal involvement in diabetes.

In this study, serum Asprosin and Ism-1 concentrations increased significantly and step-wise from normo- to micro- to macroalbuminuria, paralleling the decline in eGFR. This pattern agrees with previous reports linking Asprosin with insulin resistance, inflammation, and renal dysfunction [5,11] and identifying Ism-1 as a regulator of glucose uptake and lipid metabolism. [7]

Elevated Asprosin may enhance hepatic gluconeogenesis and promote inflammatory cytokine release, whereas higher Ism-1 concentrations may reflect compensatory metabolic stress or podocyte apoptosis induced by binding to αvβ5 integrin and GRP78. [10]

According to this study, The data showed that asprosin levels increase progressively from normoalbuminuria (mean ~25.75 ng/mL) to microalbuminuria (~50.65 ng/mL) and macroalbuminuria (~69.53 ng/mL), with statistically significant differences between all groups ($P < 0.001$). Similarly, isthmin-1 levels exhibit a marked increase across the albuminuria spectrum, from ~195.39 pg/mL in normoalbuminuria to ~479.26 pg/mL in microalbuminuria and ~729.19 pg/mL in macroalbuminuria. These consistent rises highlight both biomarkers' relationship with worsening kidney injury in diabetes.

The strong positive correlation between Asprosin and Ism-1 observed in the normoalbuminuria group suggests coordinated early metabolic regulation. As DN progresses, this correlation weakens, possibly due to differential activation of inflammatory and apoptotic pathways that dysregulate adipokine secretion. Such divergence may mark transition from functional metabolic adaptation to irreversible structural renal injury.

The glomerular filtration rate is an important indication of renal function and is used to evaluate kidney health, especially in people with diabetes or chronic kidney disease. [12] In this study a significant difference in GFR was observed across the groups ($p < 0.0001$). Post-hoc analysis showed that the macroalbuminuria group had the lowest mean GFR (53.67 ± 38.04 mL/min/1.73m²), which was significantly lower than both the microalbuminuria group (92.77 ± 25.05 mL/min/1.73m²) and the normoalbuminuria group (99.00 ± 13.83 mL/min/1.73m²). These findings highlight a strong association between worsening albuminuria and declining renal function, as measured by GFR.

Both biomarkers exhibited excellent diagnostic ability in ROC analysis; however, the apparent perfect discrimination (AUC = 1.00) likely reflects sample-size limitation and lack of cross-validation. Future studies should include larger, multi-center cohorts and validation sets or bootstrapped ROC analyses to confirm sensitivity and specificity.

In the context of distinguishing between normal albumin excretion and pathological states (both micro- and macroalbuminuria), both biomarkers exhibited exceptional diagnostic performance. Asprosin demonstrated perfect discrimination (AUC = 1.00) between normoalbuminuria and both micro- and macroalbuminuria groups, with an optimal cutoff value of >32.66 ng/mL. At this threshold, the marker achieved 100% sensitivity and specificity, resulting in perfect positive and negative predictive values. This indicates Asprosin's robust capability to identify the presence of albuminuria with high

precision. these results were seen in previous studies. [13]

Similarly, Isthmin-1 showed equivalent discriminatory power in detecting abnormal albumin excretion, achieving an AUC of 1.00 for both micro- and macroalbuminuria versus normoalbuminuria comparisons. The optimal diagnostic threshold was established at >306.119 pg/mL, yielding perfect sensitivity, specificity, and predictive values. This suggests that Isthmin-1 possesses comparable efficacy to Asprosin in initial albuminuria detection.

Both biomarkers show significant rise with albuminuria severity ($p<0.0001$). Asprosin correlates positively with renal markers (urea, creatinine) in macroalbuminuria.

Isthmin-1 shows inverse relation with SBP, suggesting vascular-protective behavior So this shows that Asprosin may reflect metabolic stress and renal injury, while Isthmin-1 may represent endothelial adaptation.This dual action strengthens their potential as complementary biomarkers of diabetic nephropathy

Clinically, these adipokines could serve as early indicators for renal microvascular injury, allowing intervention before irreversible nephron loss. Nonetheless, mechanistic studies are warranted to clarify whether modulation of Asprosin or Ism-1 pathways might slow nephropathy progression.

CONCLUSION

our data demonstrate that rising Asprosin and Ism-1 levels accompany worsening albuminuria and declining renal function in T2DM. While promising, these findings should be interpreted cautiously until validated in larger, longitudinal cohorts.

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