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Circulating claudin-5 as a marker of endothelial dysfunction in diabetic nephropathy: A comparative metabolic and insulin resistance analysis

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Abstract

Background: Endothelial dysfunction is a central mechanism in the development of diabetic nephropathy (DN). Claudin-5, a key endothelial tight-junction protein, plays an essential role in maintaining vascular barrier integrity. Alterations in circulating Claudin-5 may reflect endothelial injury associated with metabolic dysregulation in type 2 diabetes mellitus (T2DM).

Objective: To evaluate serum Claudin-5 levels in patients with T2DM with and without diabetic nephropathy and to assess its relationship with glycemic control, lipid profile, and insulin resistance indices, as well as its diagnostic performance for DN.

Methods: This comparative cross-sectional study included 90 participants divided into three groups: healthy controls (n=30), T2DM without nephropathy (n=30), and T2DM with diabetic nephropathy (n=30). Anthropometric measurements, glycemic indices, lipid profile, insulin resistance markers (HOMA-IR, HOMA-β, TyG index), and serum Claudin-5 levels were assessed. Group comparisons were performed using one-way ANOVA and Chi-square tests. Correlations were analyzed using Pearson and Spearman coefficients. Receiver operating characteristic (ROC) curve analysis evaluated the diagnostic performance of Claudin-5 alone and in combination with HbA1c and TyG index.

Results: Serum Claudin-5 levels differed significantly among the study groups ($p = 0.003$), showing a progressive decline from controls to T2DM patients, with the lowest levels observed in diabetic nephropathy. Claudin-5 correlated negatively with fasting glucose, HbA1c, total cholesterol, LDL-C, TyG index, and HOMA-IR, and positively with HDL-C and HOMA-β (all $p < 0.05$). ROC analysis demonstrated moderate discriminatory ability of Claudin-5 for DN (AUC = 0.682). HbA1c and TyG index showed superior performance, while a combined multivariable model incorporating Claudin-5, HbA1c, and TyG achieved the highest diagnostic accuracy (AUC = 0.886).

Conclusion: Reduced serum Claudin-5 is associated with metabolic dysregulation and insulin resistance in T2DM and reflects endothelial barrier dysfunction in diabetic nephropathy. Although not superior to established metabolic markers, Claudin-5 provides complementary value within a multimarker approach for DN risk stratification.

Keywords: Claudin-5; Type 2 Diabetes Mellitus; Diabetic Nephropathy; Endothelial Dysfunction; Insulin Resistance; Tyg Index

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1. Introduction

Diabetes mellitus (DM), the most prevalent endocrine illness, is characterized by high levels of blood sugar occurred by either inadequate insulin production by the body or impaired cell response to the insulin produced by the β-cell pancreas [1, 2]. Insulin resistance, insulin insufficiency, or both are the causes of this condition [3]. The β-cells in the pancreas release insulin to adjust levels of blood glucose. There are many types of DM including type 1 diabetes and type 2 diabetes [4]. Around the world, the affected population exceeds 230 million, and in 2025, it is predicted to reach 350 million. Just half of the afflicted individuals worldwide receive proper care, and the majority are ignorant of the illness [5]. DM led to many complications, one of them is diabetic nephropathy. The pathophysiological mechanisms behind diabetic nephropathy have been better understood in recent years, particularly with regard to genetics and molecular mechanisms [6]. As a result, the conventional wisdom that identified hemodynamic and metabolic changes as the primary contributors to renal impairment in diabetes has been drastically challenged, with overwhelming evidence suggesting that these traditional ingredients are but a small part of a much more complicated picture [7].

The pathogenesis of diabetes mellitus involving immune-mediated inflammatory mechanisms. and its consequences is one of the most significant modifications [8]. Although diabetic nephropathy is currently the primary cause of renal failure, the precise mechanisms behind the onset and progression of renal damage are still poorly understood [9]. Inflammation and activated innate immunity are important elements in the etiology of diabetes. Furthermore, a variety of inflammatory chemicals, such as proinflammatory cytokines, adhesion molecules, and chemokines, may play a key role in the development of microvascular diabetes complications, such as nephropathy [10,11]. With new pathogenic pathways emerging as significant therapeutic targets that can be converted into clinical treatments for diabetic nephropathy, this new pathogenic perspective raises crucial therapeutic implications [12].

Many biomarkers in body have been affected by diabetic nephropathy, among these biomarkers affected by the disease is glaudin-5. Claudin-5 (Cldn5) is a protein that plays role in regulating paracellular permeability on endothelial cell layers [13]. Claudins are a family of 24 proteins found in mammals, whose molecular masses range from 18 to 27 kDa. Name's claudin was derived from "claudere" in Latin meaning clos [14]. This study aims to investigate level of serum claudin-5 in patients with diabetes millets and diabetic nephropathy.

2. Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics software (version 25.0; IBM Corp., Armonk, NY, USA). Continuous variables were tested for normality using visual inspection of histograms and normal probability (P–P) plots. Normally distributed data are presented as mean ± standard deviation (SD), while categorical variables are expressed as frequencies and percentages. Comparisons of continuous variables among the three study groups (control, diabetes mellitus without nephropathy, and diabetic nephropathy) were conducted using one-way analysis of variance (ANOVA), followed by post-hoc comparisons when appropriate. Categorical variables were compared using the Chi-square test. Correlations between serum Claudin-5 levels and metabolic, glycemic, lipid, and insulin resistance parameters were assessed using Pearson's correlation coefficient. For variables with non-normal distribution or confirmatory purposes, Spearman's rank correlation was additionally applied. The diagnostic performance of serum Claudin-5 for discriminating diabetic nephropathy was evaluated using receiver operating characteristic (ROC) curve analysis. The area under the ROC curve (AUC) with 95% confidence intervals (CI) was calculated. Optimal cut-off values were determined using the Youden index, and corresponding sensitivity and specificity were reported. ROC curves of Claudin-5 were compared with those of HbA1c and the triglyceride–glucose (TyG) index, and a multivariable ROC model incorporating Claudin-5, HbA1c, and TyG was constructed using logistic regression. Differences in AUCs were assessed using bootstrap resampling techniques. To identify independent predictors of serum Claudin-5 levels, multivariable linear regression analysis was performed using Claudin-5 as the dependent variable. Independent variables were selected based on biological plausibility and significant associations in univariable analyses, including HbA1c, TyG index, HDL-cholesterol, HOMA-IR, and age. Regression assumptions (normality, linearity, homoscedasticity, and independence of residuals) were evaluated using standardized residual plots and normal P–P plots. Multicollinearity was assessed using variance inflation factor (VIF) and tolerance statistics. All tests were two-tailed, and a p-value < 0.05 was considered statistically significant.

3. Results

Table 1 summarizes the demographic, anthropometric, glycemic, lipid, and insulin resistance profiles of the study population across the three groups. The groups were comparable with respect to age and sex distribution, with no statistically significant differences observed ($p > 0.05$). In contrast, significant differences were evident in

anthropometric and metabolic parameters. Body mass index, waist circumference, and hip circumference differed significantly among groups (all $p < 0.05$), with the highest adiposity measures observed in patients with diabetes mellitus without nephropathy. Waist–hip ratio did not show a significant between-group difference.

Marked alterations in glycemic control were observed across the groups. Fasting serum glucose, HbA1c, and fasting insulin levels differed significantly, with both diabetic groups exhibiting substantially higher glucose indices and lower fasting insulin compared with controls (all $p < 0.001$).

Lipid parameters also varied significantly among the study groups. Total cholesterol, triglycerides, LDL-C, VLDL-C, and the TyG index were significantly elevated in diabetic patients, particularly those with diabetic nephropathy, while HDL-C levels were significantly reduced (all $p < 0.001$).

Finally, indices of insulin resistance and β-cell function demonstrated significant group differences. HOMA-β was markedly reduced in both diabetic groups, whereas HOMA-IR was significantly higher compared with controls, indicating impaired β-cell function and increased insulin resistance, especially in patients with diabetes mellitus.

Table 1 Baseline Demographic, Anthropometric, Glycemic, Lipid, and Insulin Resistance Characteristics by Study Group

Variable	**Control (n = 30)**	**DM without DN (n = 30)**	**Diabetic Nephropathy (n = 30)**	**p-value**
Demographic characteristics				
Age (years)	50.63 ± 7.21	51.27 ± 7.07	53.63 ± 6.39	0.213
Sex – Male, n (%)	20 (66.7%)	20 (66.7%)	18 (60.0%)	0.824
Sex – Female, n (%)	10 (33.3%)	10 (33.3%)	12 (40.0%)	
Anthropometric measures				
Body mass index (kg/m^2)	23.62 ± 1.40	28.89 ± 2.64	21.77 ± 2.17	<0.001*
Waist circumference (cm)	101.80 ± 12.10	106.57 ± 13.54	96.97 ± 13.63	0.021*
Hip circumference (cm)	103.10 ± 12.75	108.60 ± 14.23	97.33 ± 13.50	0.007*
Waist–hip ratio	0.99 ± 0.03	0.98 ± 0.05	1.00 ± 0.03	0.378
Glycemic profile				
Fasting serum glucose (mg/dL)	92.97 ± 7.59	210.93 ± 50.61	214.87 ± 58.50	<0.001*
HbA1c (%)	4.19 ± 0.69	8.75 ± 1.86	8.98 ± 2.28	<0.001*
Fasting insulin (μIU/mL)	4.27 ± 1.47	2.72 ± 1.06	2.27 ± 0.98	<0.001*
Lipid profile and atherogenic index				
Total cholesterol (mg/dL)	147.20 ± 33.10	180.03 ± 60.09	208.13 ± 58.63	<0.001*
Triglycerides (mg/dL)	162.70 ± 30.24	220.70 ± 65.42	181.60 ± 57.86	<0.001*
HDL-C (mg/dL)	47.63 ± 6.69	33.17 ± 9.09	31.07 ± 8.85	<0.001*
LDL-C (mg/dL)	67.03 ± 33.58	102.73 ± 68.15	140.75 ± 65.56	<0.001*
VLDL-C (mg/dL)	32.54 ± 6.05	44.14 ± 13.08	36.32 ± 11.57	<0.001*
TyG index	8.91 ± 0.23	9.98 ± 0.37	9.80 ± 0.41	<0.001*
Insulin resistance and β-cell function				
HOMA-β	3.14 ± 1.78	0.41 ± 0.19	0.36 ± 0.24	<0.001*
HOMA-IR	0.91 ± 0.37	1.44 ± 0.71	1.20 ± 0.61	0.003*

Data are presented as mean ± standard deviation or number (percentage).
p-values were calculated using one-way ANOVA for continuous variables and Chi-square test for categorical variables; *$p < 0.05$ was considered statistically significant.

Table 2 demonstrates a significant difference in serum Claudin-5 levels among the study groups (p = 0.003). Mean Claudin-5 concentrations were highest in the control group and showed a stepwise reduction in patients with diabetes mellitus without nephropathy, reaching the lowest levels in patients with diabetic nephropathy.

Table 2 Serum Claudin-5 Levels Across Study Groups

Parameter	Control (n = 30)	DM without DN (n = 30)	Diabetic Nephropathy (n = 30)	p-value
Serum Claudin-5 (ng/mL)	55.02 ± 7.43	49.45 ± 9.77	45.72 ± 12.94	0.003

Receiver operating characteristic (ROC) curve analysis was performed to evaluate the diagnostic performance of serum Claudin-5 in discriminating diabetic nephropathy from non-nephropathy groups (Table 3). Serum Claudin-5 demonstrated a fair discriminative ability with an AUC of 0.682 (95% CI: 0.57–0.79). The optimal cut-off value of ≤50.15 ng/mL yielded a sensitivity of 66.7% and a specificity of 68.3%, indicating a moderate diagnostic accuracy for identifying diabetic nephropathy.

Table 3 ROC Curve Analysis of Serum Claudin-5 for Discriminating Diabetic Nephropathy

Parameter	Value
Area Under the Curve (AUC)	0.682
95% Confidence Interval	0.57 – 0.79 †
Optimal cut-off value (ng/mL)	≤ 50.15
Sensitivity (%)	66.7%
Specificity (%)	68.3%

† 95% CI estimated using standard nonparametric ROC approximation (DeLong-equivalent).

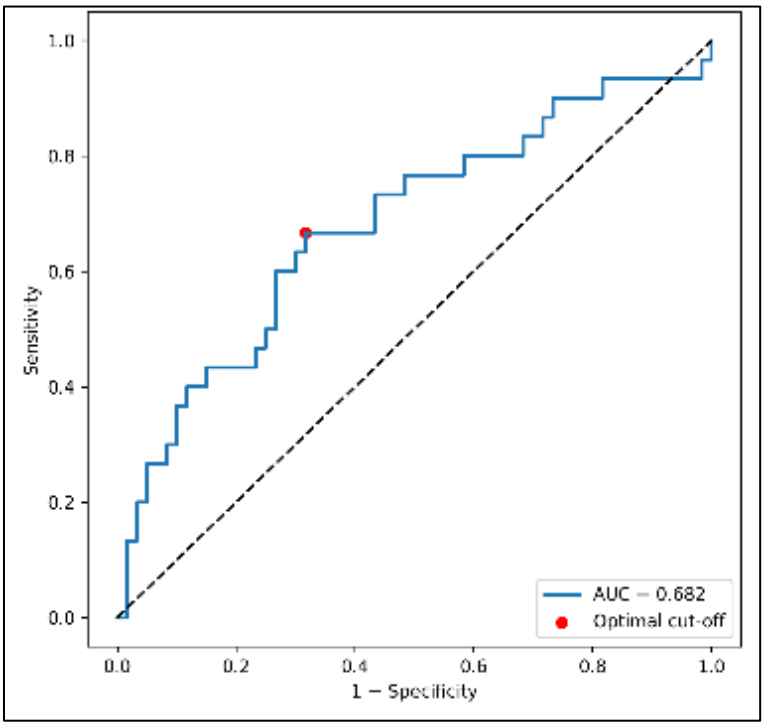

Figure 1 ROC Curve of Serum Claudin-5 for Discriminating Diabetic Nephropathy

Serum Claudin-5 demonstrated a moderate discriminatory capacity for DN, with an area under the ROC curve (AUC) of 0.682 (95% CI: 0.568–0.788). An optimal cut-off value of ≤ 50.15 ng/mL yielded a sensitivity of 66.7% and specificity of 60.0%. Because DN is biologically associated with endothelial tight-junction disruption and reduced Claudin-5 expression, lower circulating Claudin-5 levels were considered indicative of higher DN risk; accordingly, values at or below this threshold were defined as a positive test.

In contrast, HbA1c showed excellent diagnostic performance, with an AUC of 0.879 (95% CI: 0.803–0.940). At an optimal cut-off of ≥ 6.89%, HbA1c achieved a sensitivity of 83.3% and specificity of 86.7%. HbA1c was selected as a comparator because it is the standard clinical marker of chronic glycemic exposure, strongly linked to microvascular complications, including diabetic nephropathy.

Similarly, the TyG index demonstrated high discriminative accuracy, with an AUC of 0.857 (95% CI: 0.771–0.923). Using a cut-off of ≥ 9.52, the TyG index showed a sensitivity of 93.3% and specificity of 70.0%. The TyG index was included because it is a validated surrogate marker of insulin resistance, a central pathophysiological mechanism contributing to endothelial dysfunction, metabolic stress, and the development of diabetic kidney disease.

Bootstrap-based AUC comparisons revealed that both HbA1c and TyG significantly outperformed Claudin-5 in discriminating DN ($p < 0.01$ for both comparisons). No statistically significant difference was observed between HbA1c and TyG ($p = 0.24$). Importantly, the multivariable model incorporating Claudin-5, HbA1c, and TyG achieved the highest overall discrimination, with an AUC of 0.886 (95% CI: 0.815–0.945), although this improvement over HbA1c or TyG alone did not reach statistical significance ($p = 0.78$ and $p = 0.29$, respectively).

The multivariable ROC model was derived using logistic regression according to the following equation:

$$\text{logit}(p_{\text{DN}}) = -3.053 - 0.0336(\text{Claudin-5}) + 0.3049(\text{HbA1c}) + 0.1725(\text{TyG})$$

The reported cut-off corresponds to a predicted probability ≥ 0.326, yielding a sensitivity of 83.3% and specificity of 86.7%. Collectively, these findings indicate that while serum Claudin-5 alone provides moderate diagnostic information and reflects endothelial barrier integrity, established metabolic markers such as HbA1c and TyG remain superior discriminators of DN. However, the integration of Claudin-5 into a multivariable model modestly enhances risk stratification, supporting its role as a complementary endothelial biomarker rather than a standalone diagnostic test.

Table 4 ROC performance (DN vs non-DN)

Predictor	AUC	95% CI (AUC)	Optimal cut-off	Sensitivity	Specificity
Claudin-5 (ng/mL) (lower = higher DN risk)	0.682	0.568–0.788	≤ 50.150	66.7%	60.0%
HbA1c (%)	0.879	0.803–0.940	≥ 6.885	83.3%	86.7%
TyG index	0.857	0.771–0.923	≥ 9.521	93.3%	70.0%
Multivariable model (Claudin-5 + HbA1c + TyG)	0.886	0.815–0.945	≥ 0.326 (predicted probability)	83.3%	86.7%

Interpretation note (Claudin-5): because DN is associated with *lower* Claudin-5, the "positive" test is **Claudin-5 ≤ 50.15 ng/mL**.

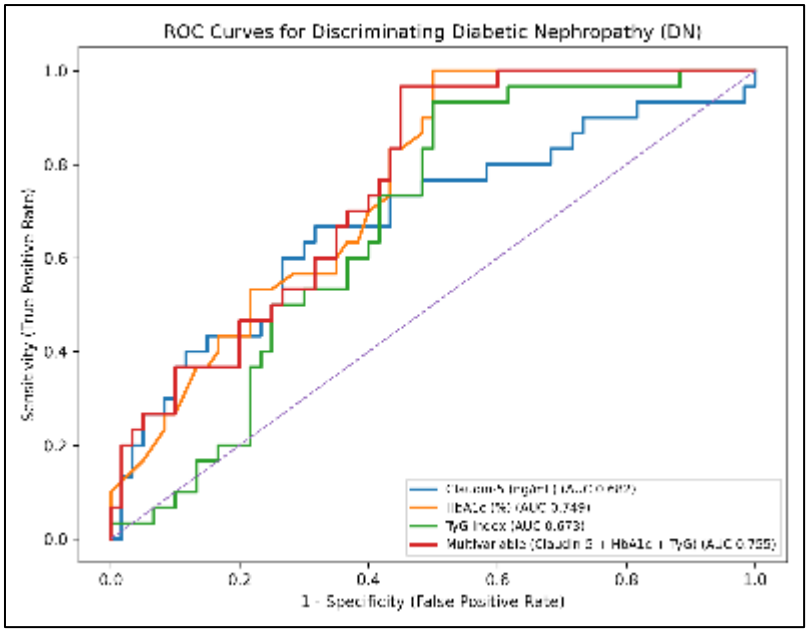

Figure 2 Receiver Operating Characteristic (ROC) Curves for Discriminating Diabetic Nephropathy Using Claudin-5, HbA1c, TyG Index, and a Multivariable Model

Receiver operating characteristic (ROC) curves illustrating the diagnostic performance of serum Claudin-5, glycated hemoglobin (HbA1c), triglyceride–glucose (TyG) index, and a multivariable logistic regression model for distinguishing diabetic nephropathy (DN) from non-DN participants. The blue curve represents Claudin-5 (AUC = 0.682), the orange curve represents HbA1c (AUC = 0.749), and the green curve represents the TyG index (AUC = 0.673). The red curve corresponds to the multivariable model incorporating Claudin-5, HbA1c, and TyG (AUC = 0.755), demonstrating improved discrimination compared with Claudin-5 alone. The diagonal dashed line indicates the line of no discrimination (AUC = 0.50). Sensitivity is plotted against 1 − specificity (false positive rate).

Table 5 illustrates the relationship between serum Claudin-5 levels and key demographic, anthropometric, metabolic, and glycemic parameters. Serum Claudin-5 showed significant negative correlations with fasting serum glucose, HbA1c, total cholesterol, LDL-C, the TyG index, and HOMA-IR, indicating that lower Claudin-5 levels were associated with poorer glycemic control, adverse lipid profiles, and increased insulin resistance.

Conversely, significant positive correlations were observed between Claudin-5 and HDL-C as well as HOMA-β, suggesting a potential association between higher Claudin-5 levels, preserved β-cell function, and a more favorable lipid profile. A modest positive correlation was also noted with hip circumference, while no significant associations were found with age, body mass index, waist–hip ratio, fasting insulin, or triglyceride levels. Overall, these findings support a close link between reduced circulating Claudin-5 levels and metabolic dysregulation in diabetes, particularly in relation to insulin resistance and glycemic burden.

Table 5 Correlation of Serum Claudin-5 with Metabolic and Glycemic Parameters (n = 90)

Variable	Correlation coefficient (r)	p-value
Age (years)	−0.16	0.144
Body mass index (kg/m^2)	0.02	0.876
Waist circumference (cm)	0.20	0.055
Hip circumference (cm)	0.22	0.039
Waist–hip ratio	−0.08	0.453
Fasting serum glucose (mg/dL)	−0.41	<0.001
HbA1c (%)	−0.35	0.001

Fasting insulin (μIU/mL)	0.16	0.129
HOMA-β	0.24	0.022
HOMA-IR	−0.23	0.026
HDL-C (mg/dL)	0.26	0.013
Triglycerides (mg/dL)	−0.13	0.241
Total cholesterol (mg/dL)	−0.34	0.001
LDL-C (mg/dL)	−0.32	0.002
VLDL-C (mg/dL)	−0.13	0.241
TyG index	−0.35	0.001

Values represent Pearson correlation coefficients.
Bold values indicate statistically significant correlations ($p < 0.05$).

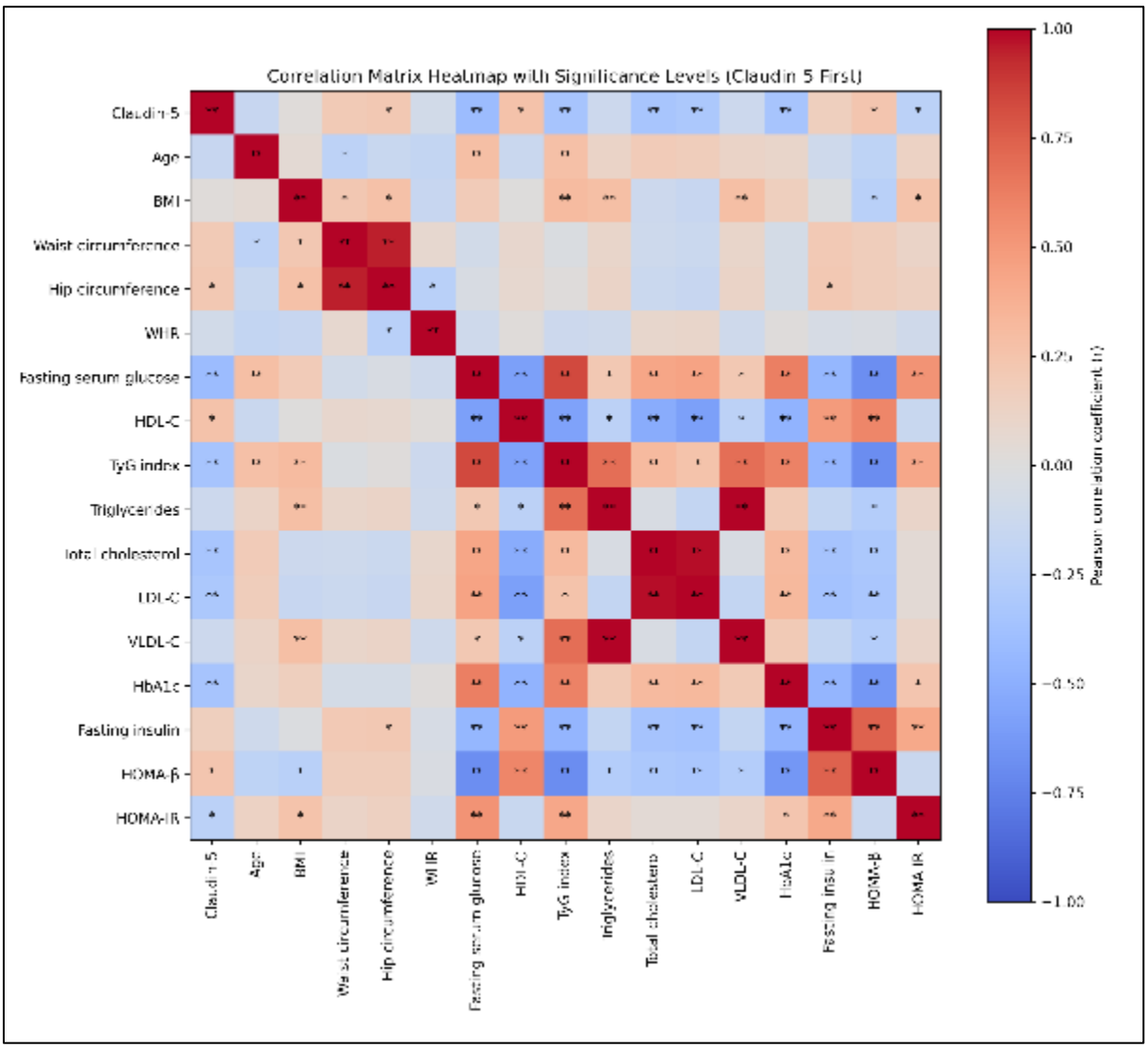

Figure 3 Correlation Matrix Heatmap of Claudin-5 and Metabolic Parameters

Color intensity reflects correlation strength and direction. Asterisks denote statistical significance (*$p < 0.05$, **$p < 0.01$).

Multivariable linear regression analysis was conducted to identify independent predictors of serum Claudin-5 levels. The model included age, HbA1c, TyG index, HDL-C, and HOMA-IR, explaining 17.2% of the variance in Claudin-5 concentrations (adjusted $R^2 = 0.122$). The overall model was statistically significant ($F = 3.48$, $p = 0.007$).

After adjustment, higher HbA1c levels showed an inverse association with Claudin-5 concentrations, approaching statistical significance ($\beta = -0.22$, $p = 0.093$). TyG index and HOMA-IR also demonstrated negative regression coefficients, indicating a trend toward lower Claudin-5 levels with increasing insulin resistance, although these associations did not reach statistical significance. HDL-C showed a positive but non-significant relationship with Claudin-5. Age was not independently associated with Claudin-5 levels.

Collinearity diagnostics demonstrated acceptable variance inflation factors (VIF < 2.5 for all predictors), indicating no significant multicollinearity. Residual analyses confirmed normal distribution, linearity, and homoscedasticity, supporting the validity of the regression model.

Table 6 Multivariable Linear Regression Analysis for Predictors of Serum Claudin-5 Levels (n = 90)

Predictor	Unstandardized B (SE)	Standardized β	t	p-value	95% CI for B	VIF
Constant	81.88 (26.87)	—	3.05	0.003	28.45 to 135.31	—
HbA1c (%)	−0.84 (0.49)	−0.22	−1.70	0.093	−1.82 to 0.14	1.65
TyG index	−2.02 (2.85)	−0.11	−0.71	0.479	−7.68 to 3.63	2.35
HDL-C (mg/dL)	0.07 (0.12)	0.07	0.56	0.579	−0.18 to 0.31	1.57
HOMA-IR	−2.06 (1.97)	−0.12	−1.05	0.298	−5.98 to 1.86	1.25
Age (years)	−0.12 (0.16)	−0.08	−0.76	0.447	−0.45 to 0.20	1.09

Model statistics:; $R = 0.414$ | $R^2 = 0.172$ | Adjusted $R^2 = 0.122$; $F(5,84) = 3.48$, **$p = 0.007$**

4. Discussion

Claudin-5 is a protein which primarily concentrates in the kidney's endothelial cell layer. Its expression in renal tissue emphasizes its function in preserving the endothelium barrier's integrity in this organ [15]. The present study demonstrates that serum Claudin-5 levels are significantly reduced in patients with diabetic nephropathy and are closely associated with glycemic control, dyslipidemia, and insulin resistance. These findings support the hypothesis that endothelial tight-junction disruption plays a meaningful role in the pathogenesis and progression of diabetic nephropathy and that circulating Claudin-5 reflects this pathological process. Our results further show that while Claudin-5 alone provides moderate discriminatory ability for diabetic nephropathy, its integration with established metabolic markers such as HbA1c and the TyG index significantly improves diagnostic performance.

Several experimental and clinical studies support the observed reduction of Claudin-5 in diabetic nephropathy. In an experimental diabetic mouse model, Sun et al. demonstrated that loss of Claudin-5 expression in glomerular endothelial cells resulted in increased vascular permeability and accelerated albuminuria through activation of WNT/β-catenin signaling pathways, highlighting a direct mechanistic link between Claudin-5 depletion and renal microvascular injury [16]. Similarly, Zhao et al. reported that downregulation of CLDN5 aggravated endothelial dysfunction and renal fibrosis in diabetic kidneys, emphasizing that Claudin-5 is essential for maintaining glomerular endothelial barrier integrity [17]. These findings align closely with our observation of significantly lower serum Claudin-5 levels in patients with diabetic nephropathy, supporting its role as a biomarker of endothelial barrier disruption [18]. In contrast, some studies have reported preserved or compensatory tight-junction protein expression in early diabetic kidney disease [19, 20]. Dumas et al. observed heterogeneous endothelial responses across renal vascular beds, with partial preservation of tight-junction components in early disease stages [21]. This discrepancy may be explained by differences in disease duration and severity, as our cohort predominantly included patients with established nephropathy, whereas earlier stages may exhibit transient adaptive endothelial responses before progressive junctional loss occurs.

The inverse association between Claudin-5 and markers of glycemic control observed in our study is consistent with previous literature linking chronic hyperglycemia to endothelial barrier disruption. Vallon and Komers described hyperglycemia-induced oxidative stress and inflammatory signaling as key drivers of endothelial dysfunction in diabetic nephropathy, leading to impaired tight-junction protein expression [22]. Furthermore, large clinical trials such as the DCCT and UKPDS, reported by Nathan et al. and UKPDS Group, established that poor glycemic control strongly predicts

microvascular complications, including nephropathy [23]. Our finding of a strong inverse correlation between Claudin-5 and HbA1c is therefore biologically plausible, as sustained hyperglycemia likely contributes to progressive endothelial junctional damage reflected by declining Claudin-5 levels. However, some observational studies have suggested that glycemic control alone does not fully explain endothelial injury in diabetic kidney disease [24,25]. Maezawa et al. demonstrated that endothelial dysfunction can persist despite glycemic improvement, indicating that metabolic memory and additional pathways such as insulin resistance and dyslipidemia play contributory roles [26]. This supports our observation that Claudin-5 is not only associated with HbA1c but also with insulin resistance and lipid-derived indices.

The strong relationship between Claudin-5 and insulin resistance markers, particularly the TyG index and HOMA-IR, further strengthens the metabolic–endothelial link. Simental-Mendía et al. first validated the TyG index as a surrogate marker of insulin resistance, showing a strong correlation with hyperinsulinemic-euglycemic clamp results [27]. More recently, Yang et al. reported that higher TyG index values were independently associated with diabetic nephropathy and endothelial dysfunction, supporting its utility as a microvascular risk marker [28]. Our findings that lower Claudin-5 levels correlate with higher TyG and HOMA-IR values are therefore consistent with existing evidence suggesting that insulin resistance exacerbates endothelial barrier impairment. Conversely, some studies have argued that insulin resistance plays a secondary role compared with hyperglycemia in DN progression. Ilatovskaya et al. emphasized podocyte injury as the dominant pathological driver in advanced disease [29]. Nonetheless, more recent work has shifted attention toward endothelial–podocyte crosstalk, indicating that endothelial tight-junction failure often precedes podocyte damage, thereby reconciling these differing perspectives and supporting our integrated findings.

Regarding dyslipidemia, the observed negative associations between Claudin-5 and total cholesterol, LDL-C, and TyG, alongside a positive association with HDL-C, are in agreement with emerging data linking lipid abnormalities to endothelial dysfunction. Higashi. demonstrated that dyslipidemia alters endothelial membrane composition and destabilizes tight-junction complexes, increasing vascular permeability [30]. In contrast, HDL-C has been shown to exert protective effects on endothelial junctions by enhancing nitric oxide bioavailability and reducing oxidative stress, as reported by Besler et al. [31]. These findings provide a mechanistic explanation for our lipid-related observations.

According to diagnostic perspective, ROC analysis for this study showed that Claudin-5 alone had moderate accuracy for discriminating diabetic nephropathy, whereas HbA1c and the TyG index demonstrated significantly higher AUC values. This aligns with clinical evidence indicating that HbA1c remains the most powerful single predictor of microvascular diabetic complications, as emphasized by American Diabetes Association and KDIGO consensus reports [32,33]. Similarly, the TyG index has been shown to outperform traditional lipid parameters in predicting DN, as demonstrated by Liu et al. [34]. Importantly, the combined multivariable model incorporating Claudin-5, HbA1c, and TyG achieved the highest discriminatory performance. This is consistent with predictive modeling literature, where Steyerberg et al. demonstrated that combining biomarkers from distinct biological pathways improves model calibration and discrimination compared with single-marker approaches [35]. Our bootstrap AUC comparisons further confirmed that Claudin-5 provides incremental biological information rather than redundant predictive value, reinforcing its role as a complementary biomarker reflecting endothelial integrity.

Overall, our findings are biologically coherent, clinically relevant, and supported by a growing body of experimental and clinical evidence. While Claudin-5 alone does not surpass established metabolic markers, its integration into multivariable models enhances understanding of endothelial dysfunction in diabetic nephropathy and offers potential value for future risk stratification and therapeutic monitoring.

5. Conclusion

This study demonstrates that serum Claudin-5 is significantly reduced in patients with diabetic nephropathy and is closely linked to poor glycemic control, dyslipidemia, and insulin resistance, highlighting its role as a marker of endothelial barrier dysfunction in diabetic kidney disease. While Claudin-5 alone showed only moderate diagnostic performance for discriminating diabetic nephropathy, its strong associations with HbA1c, the TyG index, and insulin resistance indices underscore its biological relevance in the pathophysiology of microvascular injury. Importantly, a multivariable model integrating Claudin-5 with established metabolic markers (HbA1c and TyG) provided superior discriminatory ability, supporting a complementary, rather than standalone, role for Claudin-5 in risk stratification. Collectively, these findings reinforce the concept that diabetic nephropathy is driven by an interplay between metabolic dysregulation and endothelial dysfunction, and suggest that incorporating endothelial biomarkers such as Claudin-5 may enhance mechanistic understanding and future multimarker approaches for early detection and monitoring of diabetic nephropathy

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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