Associations between serum JAML, nesfatin-1, and 25(OH)D and the risk of diabetic kidney disease in patients with type 2 diabetes

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Abstract

This study was designed to assess the associations between serum junctional adhesion molecule-like protein (JAML), nesfatin-1, and 25-hydroxy vitamin D (25(OH)D) and the incidence of diabetic kidney disease (DKD) in patients with type 2 diabetes mellitus (T2DM), as well as to explore their predictive value in DKD. Serum JAML, nesfatin-1, and 25(OH)D levels were measured in 227 patients with T2DM. All participants were categorized into tertiles based on their serum JAML, nesfatin-1, and 25(OH)D levels. For statistical analysis, multivariate logistic regression models and restricted cubic splines (RCS) were utilized, moreover, receiver operating characteristic (ROC) curves and the nomogram were developed. Of the 227 patients with T2DM, 114 (50.2%) were diagnosed with DKD. The RCS analysis showed an S-shaped association between the serum JAML and DKD occurrence, and an L-shaped association of serum nesfatin-1 or 25(OH)D with the risk of DKD. Multivariate logistic regression revealed that individuals in the highest tertile of serum JAML level had a significantly greater risk of developing DKD than those in the lowest tertile where confounders were controlled (JAML: OR 5.70, 95%CI 2.66–12.22, $P < 0.001$), in contrast, individuals in the highest tertile of serum nesfatin-1 or 25(OH)D had a significantly lower risk of DKD progression than those in the lowest tertile where confounders were controlled (nesfatin-1: OR 0.21, 95%CI 0.10–0.44, $P < 0.001$; 25(OH)D: OR 0.19, 95%CI 0.08–0.45, $P < 0.001$). The ROC curves showed that the serum JAML levels were better than nesfatin-1 or 25(OH)D at predicting DKD, with an optimal cutoff value of 289.47 pg/mL. Finally, a nomogram model based on the above three indicators combined with a history of hypertension, course of diabetes, and history of diabetic complications of retinopathy achieved a predictive accuracy of 87.2% in predicting DKD in T2DM population. Elevated serum JAML levels whereas decreased serum nesfatin-1 and 25(OH)D levels were associated with a greater risk of DKD in patients with T2DM. A nomogram model based on this could more accurately predict the risk of DKD in individuals with T2DM.

Introduction

The incidence of diabetes has been rising quickly worldwide in recent years with the growth of living standards, and the International Diabetes Federation (IDF) projects that 643 million people will have the disease by 2030 and 784 million people by 2045[1]. Chronic hyperglycemia with poor glycemic control over a long period can involve tissues or organs such as the heart, eyes, kidneys, and peripheral nerves, which can lead to a variety of complications, dysfunctions, and even organ failure[2]. Diabetic kidney disease (DKD) is one of the common microvascular complications of diabetes, and also a key cause of end-stage renal disease (ESKD)[3]. According to statistics, over 40% of diabetes patients may present with DKD, which brings a significant healthcare burden[4,5]. The typical clinical symptoms of DKD are persistent proteinuria and gradual decrease of renal function, with which doctors carry out clinical diagnosis and therapeutic interventions in their clinical work at present[6]. Nevertheless, current conventional diagnostic methods are not sufficient to meet the clinical needs because of the early atypical clinical symptoms of DKD and the unnecessarily abnormal results of estimated glomerular filtration rate (eGFR) and albuminuria[7]. Therefore, new biomarkers to predict the development of DKD are yet to be discovered to achieve early detection, diagnosis, and treatment of the disease.

Found by Moog-Lutz et al. in 2003, junctional adhesion molecule-like protein (JAML) is a secreted type I transmembrane glycoprotein characterized by junctional adhesion and belongs to the JAMs family[8]. Playing a role in mediating leukocyte adhesion and transendothelial migration, JAML is considered a new target for tumor immunotherapy, and the majority of research on JAML has focused on the field of oncology[9,10]. JAML was proved to be significantly upregulated in podocytes in a DKD mouse model, and its inhibition could enhance the silent information regulator 1 (SITR1) expression and the AMP-activated protein kinase α (AMPKα) activity, which in turn affected the sterol regulatory element binding protein-1 (SREBP1) and the downstream fatty acid and cholesterol synthesizing proteins, consequently improving lipid metabolism disorders in podocytes, reducing proteinuria, and alleviating kidney injury[11]. However, few JAML-related clinical studies have been reported so far. Nesfatin-1 is a newly discovered satiety molecule with functions such as food intake controlling[12], anti-inflammatory[13], anti-oxidative stress[14], and insulin resistance[15]. There has hitherto been no agreement on how serum nesfatin-1 levels fluctuate in patients with type 2 diabetes mellitus (T2DM)[16,17]. A meta-analysis claimed that serum nesfatin-1 levels were elevated in patients with newly diagnosed T2DM but decreased after drug treatment[18]. Moreover, to the best of our knowledge, few studies have focused on the variation of nesfatin-1 during the development of T2DM toward DKD, and the relationship between nesfatin-1 and DKD has not been elucidated yet. 25-hydroxy vitamin D (25(OH)D) is the major circulating form of vitamin D in the body that regulates bone and mineral metabolism[19]. 25(OH)D deficiency is highly prevalent among patients with T2DM, especially in the presence of DKD[20]. A number of studies manifested an association of low 25(OH)D level and albuminuria, indicating that 25(OH)D may be renoprotective in diabetes[21,22]; even though others disapproved this association[23].

Therefore, we aimed to disclose the underlying association of serum JAML, nesfatin-1 or 25(OH)D with DKD progression, as well as to assess their predictive value for DKD, and ultimately, benefit the clinic in preventing DKD or mitigate the burden of DKD in individuals with T2DM.

Results

• Demographic and baseline characteristics of the study population

A total of 227 patients who were diagnosed with T2DM met the inclusion criteria and participated in the study. The median age of the population was 60.0 (52.5–68.0) years, and 81 (35.7%) of them were female. Of all the participants, 114 were diagnosed with DKD during hospitalization. Table 1 outlined the demographic and baseline characteristics of the participants, categorized by the presence or absence of DKD. The results showed that compared to patients without DKD, those with DKD tended to be older ($P = 0.002$), have higher SBP ($P < 0.001$) and slightly lower HbA1c levels ($P = 0.014$). The prevalence of hypertension ($P < 0.001$), cardiovascular disease ($P = 0.016$) and complications of diabetic retinopathy ($P < 0.001$) was also significantly higher in the DKD group. Furthermore, individuals with DKD had lower eGFR levels and higher BUN, Scr, UA, UmALB and UACR levels (each $P < 0.001$), indicating impaired renal function. Notably, patients with DKD exhibited higher serum JAML but lower nesfatin-1 and 25(OH)D than the non-DKD cases (each $P < 0.001$), with the results of JAML in the DKD, median 315.79 pg/mL (IQR: 266.83-443.71) *vs.* in the non-DKD,

242.19 pg/mL (IQR: 162.04-295.31); nesfatin-1 in the DKD, median 563.22 pg/mL (IQR: 419.45-668.57) *vs.* in the non-DKD, median 665.75 pg/mL (IQR: 579.62-751.59) and 25(OH)D in DKD, median 19.57 ng/mL (IQR: 13.09–25.09) *vs.* in the non-DKD, median 23.60 ng/mL (IQR: 19.63–27.76).

Table 1
Demographic and baseline characteristics of patients with and without diabetic kidney disease.

Characteristic	Overall	Non-DKD	DKD	*P* value
	N = 227	N = 113	N = 114	
Gender (%)				0.714
Male	146 (64.3)	74 (65.5)	72 (63.2)	
Female	81 (35.7)	38 (34.5)	43 (36.8)	
Age (years)	60.0 (52.5, 68.0)	58.0 (50.0, 65.0)	61.0 (56.3, 69.0)	0.002**
BMI (kg/m^2)	24.14 (21.78, 25.87)	24.22 (21.45, 26.08)	23.88 (22.03, 25.57)	0.985
SBP (mmHg)	133 (120, 146)	129 (118, 139)	139 (125, 153)	< 0.001
DBP (mmHg)	79 (72, 85)	79 (70, 85)	79 (72, 85)	0.711
Hypertension (%)				< 0.001***
No	98 (43.2)	64 (56.6)	34 (29.8)	
Yes	129 (56.8)	49 (43.4)	80 (70.2)	
Hyperlipidemia (%)				0.638
No	121 (53.3)	62 (54.9)	59 (51.8)	
Yes	106 (46.7)	51 (45.1)	55 (48.2)	
Cardiovascular diseases (%)				0.016*
No	141 (62.1)	79 (69.9)	62 (54.4)	
Yes	86 (37.9)	34 (30.1)	52 (45.6)	
Smoking status (%)				0.357
Never smoker	151 (66.5)	73 (64.6)	78 (68.4)	
Former smoker	24 (10.6)	10 (8.8)	14 (12.3)	
Current smoker	52 (22.9)	30 (26.5)	22 (19.3)	
Alcohol consumption (%)				0.214

Continuous variables are presented as the mean ± standard deviation or median (interquartile range), while categorical variables are expressed as the n (%). DKD, diabetic kidney disease; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; FBG, fasting blood glucose; 2h-PG, 2 hours plasma glucose; HbA1c, glycosylated hemoglobin; TG, triglyceride; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; BUN, blood urea nitrogen; Scr, serum creatinine; UA, uric acid; eGFR, estimated glomerular filtration rate; UmALB, urine albumin; UACR, urine albumin creatine ratio; JAML, junctional adhesion molecule-like protein; 25(OH)D, 25-hydroxy vitamin D. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Characteristic	Overall	Non-DKD	DKD	*P* value
	N = 227	N = 113	N = 114	
No	167 (73.6)	79 (69.9)	88 (77.2)	
Yes	60 (26.4)	34 (30.1)	26 (22.8)	
Course of diabetes (years)	10.00 (4.50, 16.00)	6.00 (2.00, 12.00)	10.00 (10.00, 18.75)	< 0.001***
Complications of diabetes (%)				
Peripheral neuropathy	186 (81.9)	90 (79.6)	96 (84.2)	0.371
Peripheral vascular disease	129 (58.6)	62 (54.9)	67 (58.8)	0.553
Retinopathy	84 (37.0)	22 (19.5)	62 (54.4)	< 0.001***
Diabetic foot	12 (5.3)	5 (4.4)	7 (6.1)	0.564
FBG (mmol/L)	7.55 (6.24, 9.49)	7.43 (6.20, 9.28)	7.59 (6.43, 9.61)	0.228
2h-PG (mmol/L)	15.28 ± 5.05	15.35 ± 4.97	15.21 ± 5.15	0.839
HbA1c (%)	8.60 (7.15, 9.90)	8.80 (7.30, 10.50)	8.30 (7.10, 9.30)	0.014*
TG (mmol/L)	1.78 (1.20, 2.73)	1.78 (1.08, 2.92)	1.82 (1.37, 2.63)	0.445
TC (mmol/L)	4.68 (3.91, 5.56)	4.77 (3.92, 5.61)	4.66 (3.78, 5.56)	0.729
HDL-C (mmol/L)	1.12 (0.95, 1.33)	1.14 (0.98, 1.33)	1.07 (0.91, 1.33)	0.320
LDL-C (mmol/L)	3.04 (2.37, 3.62)	3.07 (2.47, 3.72)	3.01 (2.27, 3.60)	0.526
BUN (mmol/L)	6.32 (5.01, 8.08)	5.67 (4.60, 6.44)	7.75 (5.82, 10.79)	< 0.001***
Scr (μmol/L)	79.00 (65.00, 110.50)	67.20 (58.70, 79.60)	102.55 (78.23, 157.50)	< 0.001***
UA (μmol/L)	353.50 (276.70, 420.65)	315.00 (264.30, 387.30)	383.85 (306.33, 449.28)	< 0.001***
eGFR (mL/min/1.73 m^2)	85.41 (55.11, 102.96)	99.71 (87.29, 114.61)	56.43 (33.23, 81.86)	< 0.001***
UmALB (mg/L)	35 (12, 328)	12 (9, 17)	328 (83, 1,530)	< 0.001***
UACR (mg/g)	31.90 (10.96,	11.00 (8.06,	482.74 (112.71,	< 0.001***

Continuous variables are presented as the mean ± standard deviation or median (interquartile range), while categorical variables are expressed as the n (%). DKD, diabetic kidney disease; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; FBG, fasting blood glucose; 2h-PG, 2 hours plasma glucose; HbA1c, glycosylated hemoglobin; TG, triglyceride; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; BUN, blood urea nitrogen; Scr, serum creatinine; UA, uric acid; eGFR, estimated glomerular filtration rate; UmALB, urine albumin; UACR, urine albumin creatine ratio; JAML, junctional adhesion molecule-like protein; 25(OH)D, 25-hydroxy vitamin D. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Characteristic	Overall	Non-DKD	DKD	*P* value
	N = 227	N = 113	N = 114	
	482.74)	17.26)	2,599.88)	
GFR categories (%)				< 0.001***
G1 (≥ 90mL/min/1.73 m^2)	102 (44.9)	80 (70.8)	22 (19.3)	
G2 (60-89mL/min/1.73 m^2)	60 (26.4)	27 (23.9)	33 (28.9)	
G3a (45-59mL/min/1.73 m^2)	23 (10.1)	4 (3.5)	19 (16.7)	
G3b (40-44mL/min/1.73 m^2)	15 (6.6)	0	15 (13.2)	
G4 (15-29mL/min/1.73 m^2)	18 (7.9)	1 (0.9)	17 (14.9)	
G5 (<15mL/min/1.73 m^2)	9 (4.0)	1 (0.9)	8 (7.0)	
Albuminuria categories (%)				< 0.001***
A1 (<30mg/g)	110 (48.5)	106 (93.8)	4 (3.5)	
A2 (30-299mg/g)	52 (22.9)	6 (5.3)	46 (40.4)	
A3 (≥ 300mg/g)	65 (28.6)	1 (0.9)	64 (56.1)	
JAML (pg/mL)	278.48 (206.56, 348.44)	242.19 (162.04, 295.31)	315.79 (266.83, 443.71)	< 0.001***
Nesfatin-1 (pg/mL)	626.90 (515.53, 713.49)	665.75 (579.62, 751.59)	563.22 (419.45, 668.57)	< 0.001***
25(OH)D (ng/mL)	21.62 (16.69, 26.84)	23.60 (19.63, 27.76)	19.57 (13.09, 25.09)	< 0.001***

Continuous variables are presented as the mean ± standard deviation or median (interquartile range), while categorical variables are expressed as the n (%). DKD, diabetic kidney disease; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; FBG, fasting blood glucose; 2h-PG, 2 hours plasma glucose; HbA1c, glycosylated hemoglobin; TG, triglyceride; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; BUN, blood urea nitrogen; Scr, serum creatinine; UA, uric acid; eGFR, estimated glomerular filtration rate; UmALB, urine albumin; UACR, urine albumin creatine ratio; JAML, junctional adhesion molecule-like protein; 25(OH)D, 25-hydroxy vitamin D. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

• JAML and DKD

Spearman correlation analysis showed that serum JAML levels exhibited positive correlations with renal function indices of BUN (r = 0.20, $P = 0.002$), Scr (r = 0.29, $P < 0.001$), UmALB (r = 0.48, $P < 0.001$) and UACR (r = 0.44, $P < 0.001$), but negative correlations with HbA1c (r = -0.18, $P = 0.005$) and eGFR (r = -0.31, $P < 0.001$) (Fig. 1, **Supplemental Table 1**). Restricted cubic splines (RCSs) (Fig. 2A) revealed a non-linear association between JAML and the risk of DKD (P-Non-linear < 0.001); in contrast, the risk of DKD significantly increased

with increasing serum JAML levels when the level was under 553 pg/mL, whereas an upward first then downward slope was observed when JAML was ≥ 553 pg/mL (Fig. 2A). Table 2 displayed the results of the three multivariate logistic regression models in evaluating the correlations between JAML and DKD incidence (model 1: OR 1.81, 95%CI 1.38–2.38, $P < 0.001$; model 2: OR 1.79, 95%CI 1.36–2.36, $P < 0.001$ and model 3: OR 1.05, 95%CI 1.24–2.19, $P < 0.001$). As illustrated in model 3, when using gender, age, BMI, SBP, hypertension, hyperlipidemia, and cardiovascular disease as covariates, each 100 pg/mL increase in JAML was associated with a 5% increase in the risk of DKD. We further transformed JAML from a continuous variable to an ordered categorical one (tertiles) for sensitivity analysis and the results showed that, compared to those in the tertile 1, patients in the tertile 3 had more than 5-fold increased risk of DKD, and the statistical significances were in model 1: OR 6.45, 95% CI 3.18–13.09, $P < 0.001$; model 2: OR 6.47, 95%CI 3.15–13.28, $P < 0.001$ and model 3: OR 5.70, 95%CI 2.66–12.22, $P < 0.001$. Moreover, compared to those in the tertile 1, participants falling into the tertile 2 exhibited a 2-fold increased risk of DKD, however, this difference was statistically insignificant, as presented in model 3 (OR 2.00, 95%CI 0.97–4.14, $P = 0.062$). The ROC curve analysis revealed an optimal serum JAML cutoff value of 289.47 pg/mL and an AUC of 0.750 (95% CI = 0.687–0.812), with the sensitivity of 64.0%, specificity of 71.7%, positive predictive value of 69.5% and negative predictive value of 66.4% (Fig. 3A).

Table 2
Logistic regression models for the association of JAML, nesfatin-1 and 25(OH)D with diabetic kidney disease.

	Model 1			Model 2			Model 3		
	OR	95% CI	*P* value	OR	95% CI	*P* value	OR	95% CI	*P* value
JAML (pg/mL)									
per 100pg/mL increase	1.81	1.38–2.38	< 0.001***	1.79	1.36–2.36	< 0.001***	1.05	1.24–2.19	< 0.001***
Tertile 1	Reference			Reference			Reference		
Tertile 2	2.02	1.03–3.93	0.039*	2.05	1.04–4.06	0.039*	2.00	0.97–4.14	0.062
Tertile 3	6.45	3.18–13.09	< 0.001***	6.47	3.15–13.28	< 0.001***	5.70	2.66–12.22	< 0.001***
P for trend			< 0.001***			< 0.001***			< 0.001***
Nesfatin-1 (pg/mL)									
per 100pg/mL increase	0.86	0.75–0.98	0.021*	0.88	0.77–0.99	0.041*	0.85	0.74–0.98	0.029*
Tertile 1	Reference			Reference			Reference		
Tertile 2	0.38	0.19–0.74	0.004**	0.40	0.20–0.78	0.007**	0.33	0.16–0.71	0.004**
Tertile 3	0.23	0.11–0.45	< 0.001***	0.24	0.12–0.48	< 0.001***	0.21	0.10–0.44	< 0.001***
P for trend			< 0.001***			< 0.001***			< 0.001***
25(OH)D (ng/mL)	Reference								
per 1ng/mL increase	0.94	0.91–0.98	0.001**	0.91	0.87–0.95	< 0.001***	0.92	0.87–0.96	0.001***
Tertile 1				Reference			Reference		
Tertile 2	0.30	0.15–	<	0.21	0.09–	<	0.29	0.12–	0.004**

Model 1: Crude model; Model 2: Gender- and age-adjusted model; Model 3: Model adjusted by gender, age, BMI, SBP, hypertension, hyperlipidemia and cardiovascular disease. JAML, junctional adhesion molecule-like protein; 25(OH)D, 25-hydroxy vitamin D; BMI, body mass index; SBP, systolic blood pressure; OR, odds ratio; CI, confidence interval. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

	Model 1			Model 2			Model 3		
		0.60	0.001***		0.45	0.001***		0.67	
Tertile 3	0.26	0.13–0.53	< 0.001***	0.16	0.07–0.37	< 0.001***	0.19	0.08–0.45	< 0.001***
P for trend			< 0.001***			< 0.001***			< 0.001***

Model 1: Crude model; Model 2: Gender- and age-adjusted model; Model 3: Model adjusted by gender, age, BMI, SBP, hypertension, hyperlipidemia and cardiovascular disease. JAML, junctional adhesion molecule-like protein; 25(OH)D, 25-hydroxy vitamin D; BMI, body mass index; SBP, systolic blood pressure; OR, odds ratio; CI, confidence interval. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

• Nesfatin-1 and DKD

In addition to JAML, spearman correlation analysis concluded that serum nesfatin-1 had significantly positive correlations with HbA1c (r = 0.15, $P = 0.027$) and eGFR (r = 0.24, $P < 0.001$), but significantly negative correlations with renal function indicators including BUN (r = -0.14, $P = 0.033$), Cr (r = -0.19, $P = 0.003$), UA (r = -0.14, $P = 0.038$), UmALB (r = -0.28, $P < 0.001$) and UACR (r = -0.33, $P < 0.001$) (Fig. 1, **Supplemental Table 2**). Figure 2B illustrated an apparent L-shaped association between the nesfatin-1 level and the incidence of DKD (*P*-Non-linear < 0.001). The risk of DKD significantly decreased with increasing serum nesfatin-1 until its level ≥ 657 pg/mL, the curve made a sharp turn and became very flat. Multifactorial logistic regression analysis revealed that nesfatin-1, when treated as a continuous variable, was strongly negatively correlated with DKD (model 1: OR 0.86, 95% CI 0.75–0.98, $P = 0.021$; model 2: OR 0.88, 95% CI 0.77–0.99, $P = 0.041$; and model 3: OR 0.85, 95% CI 0.74–0.98, $P = 0.029$). On the other hand, when nesfatin-1 was inputed as an ordered categorical variable in tertiles, participants in the tertile 3 yielded a significant low risk of DKD compared to those in the tertile 1 (model 1: OR 0.23, 95% CI 0.11–0.45, $P < 0.001$; model 2: OR 0.24, 95% CI 0.12–0.48, $P < 0.001$; and model 3: OR 0.21, 95% CI 0.10–0.44, $P < 0.001$). Likewise, participants in the tertile 2 also demonstrated significantly lower risk of DKD than those in the tertile 1. The ROC curve demonstrated an optimal serum nesfatin-1 cutoff value of 513.35 pg/mL and an AUC of 0.704 (95% CI = 0.636–0.771), with the sensitivity of 41.2%, specificity of 92.0%, positive predictive value of 83.9% and negative predictive value of 60.8%. (Fig. 3B).

• 25(OH)D and DKD

Apart from JAML and nesfatin-1, spearman correlation analysis confirmed significant positive correlations of serum 25(OH)D level with DKD risk indicators including FBG (r = 0.14, $P = 0.050$), 2-PG (r = 0.20, $P = 0.004$) and eGFR (r = 0.16, $P = 0.020$), as well as significant positive correlations with BUN (r = -0.25, $P < 0.001$), Scr (r = -0.24, $P < 0.001$), UA (r = -0.29, $P < 0.001$), UmALB (r = -0.39, $P < 0.001$) and UACR (r = -0.35, $P < 0.001$), which are indicators of renal function (Fig. 1, **Supplemental Table 3**). The L-shaped RCS curve plotted for 25(OH)D (*P*-Non-linear = 0.008) was similar to that for nesfatin-1, depicting a significant reduction in the risk of DKD with 25(OH)D elevation, while the slope turned to be flat when 25(OH)D ≥ 21.5 ng/mL (Fig. 2C). Multifactorial logistic regression analysis explicated a significant negative correlation of 25(OH)D, when introduced as a

continuous variable, with DKD (model 1: OR 0.94, 95% CI 0.91–0.98, $P = 0.001$; model 2: OR 0.91, 95% CI 0.87–0.95, $P < 0.001$; model 3. OR 0.92, 95%CI 0.87–0.96, $P = 0.001$). Adjustment of gender, age, BMI, SBP, hypertension, hyperlipidemia, and cardiovascular disease as covariates in model 3 manifested that each 1 ng/mL increase in 25(OH)D was associated with an 8% reduction in the risk of DKD. Remarkedly, when 25(OH)D was treated as an ordered categorical variable in tertiles, participants in the tertile 3 presented a significantly low risk of DKD compared with those in the tertile 1 (model 1: OR 0.26, 95% CI 0.13–0.53, $P < 0.001$; model 2: OR 0.16, 95% CI 0.07–0.37, $P < 0.001$; model 3: OR 0.19, 95% CI 0.08–0.45, $P < 0.001$). In addition, participants in the tertile 2 also demonstrated significantly low risk of DKD in contrast to those in the tertile 1. The ROC curve analysis for 25(OH)D resulted in an optimal cutoff value of 18.93 ng/mL and an AUC of 0.638 (95% CI = 0.562–0.714), with a sensitivity of 47.6%, specificity of 80.4%, positive predictive value of 71.4%, and negative predictive value of 59.9% (Fig. 3C). Serum JAML presented better predictive value than did the serum 25(OH)D (Delong's test: JAML vs. 25(OH)D, $P = 0.019$), and serum nesfatin-1 was not statistically significant (Delong's test: nesfatin-1 vs. 25(OH)D, $P = 0.196$)

• Nomogram model for predicting the risk of DKD

A nomogram model for predicting the risk of DKD in T2DM population based on serum JAML, nesfatin-1 and 25(OH)D was built. The dataset was randomly split in the ratio of 7:3 to construct the training and validation cohorts, and by performing LASSO logistic regression analysis on the training cohorts, we screened seven potential predictors (SBP, hypertension, course of diabetes, retinopathy, JAML, nesfatin-1 and 25(OH)D) (Fig. 4A-B, **Supplementary Table 4**). Study subjects were excluded if there were difficulties in standardizing their SBP data, which did not contribute to the model construction. The final constructed nomogram risk assessment model was shown in Fig. 4C. The ROC results showed that the AUC of the nomogram model in the training and validation cohorts were 0.872 and 0.854, respectively (Fig. 4D-E). The calibration curves of the nomogram model in the training and validation cohorts presented significant correlation between the observed and predicted groups (Fig. 4F-G), indicating that the model predicted probability had a high degree of consistency with the actual outcome. The DCA curves showed that the DKD prediction model provided better clinical gains in both the training and validation cohorts of patients with T2DM (Fig. 4H-J).

Discussion

Diabetes is a major disease that seriously endangers the health of the global population. In recent years, the incidence and prevalence of diabetes have sharply increased because of changes in diet and lifestyle, and there is a trend toward a younger age of onset[24]. As one of the most common complications of diabetes, DKD affects 30%-50% of patients with T2DM and remains the leading cause of end-stage kidney disease, but its exact mechanism is not yet fully understood. It may be a combined result of damage to multiple kidney targets under hyperglycemic conditions[25,26]. Early detection, diagnosis and treatment are the keys to improve the life quality and expectancy of patients with DKD[27]. eGFR and UACR are routinely measured in DKD diagnosis for clinical practitioners, but due to the compensatory function of the kidney, these conventional diagnostic indicators are not sufficient to meet the clinical needs[7,28]. Therefore, hunting for biomarkers that are more sensitive and efficient is urgent for the clinic.

JAML belongs to the family of JAMs, whose protein structure is mostly made up of two extracellular immunoglobulin-like structural domains (D1, D2), a stem region, a transmembrane helical structural domain, and an intracellular structural domain[8]. JAML was initially used as a specific co-stimulatory molecule for the activation of epithelial γδ T cells, activated by coxsackie virus-adenovirus receptor (CXADR)[29]. Its intracellular structural domain can mediate intracellular signaling through the PI3K/MAPK/Akt pathway, leading to cell activation, proliferation and production of cytokines and growth factors[30], which play roles in immune-inflammatory response and tissue homeostasis, as well as in transendothelial migration of neutrophils and monocytes[31]. In our study, patients with DKD had significantly higher serum JAML levels than those without DKD ($P < 0.001$). Serum JAML showed a significant positive correlation with the renal function indicators, including BUN, Scr, UmALB, and UACR, and a significant negative correlation with eGFR. The risk of DKD significantly increased with increasing serum JAML levels, and this growth slowed down when JAML levels were greater than 553 pg/mL (P-Non-linear = < 0.001). The association between the serum JAML level and the increased risk of DKD remained after further adjustment of the potential confounders. The serum JAML threshold for predicting DKD was 289.47 pg/mL, with a sensitivity of 64.0%, a specificity of 71.7%, a positive predictive value of 69.5% and a negative predictive value of 66.4%. These findings suggest that serum JAML can be used as a monitoring indicator for DKD.

In hyperglycemic and dyslipidemic environments, Fu et al. discovered that JAML expression was significantly upregulated in renal tissues and serum of patients with DKD, which is consistent with our findings. Further studies revealed that JAML could regulate lipid metabolism in podocytes through action on SREBP1, a transcription factor participating in the synthesis of fatty acids and cholesterol, interfering with downstream signaling pathways by regulating the expression of SITR1/AMPK[11]. Indeed, JAML was found to participate in macrophage phenotypic polarization and efferocytosis, which was pivotal to renal inflammatory and repair processes[32]. Therefore, it is reasonable to assume that increased serum JAML level in the development of DKD may be the result of the dual role of podocytes and inflammatory cells.

Nesfatin-1, an ingestion-regulating peptide derived from nucleobindin-2, attracted our attention for its regulatory role in food intake and glucose homeostasis[33]. Our previous studies disclosed downregulation of serum nesfatin-1 in prediabetes and patients with T2DM, and its strong association with β-cell insulin secretion[34,35]. In this study, the patients with DKD exhibited significantly low changes in serum nesfatin-1 levels compared to the individuals with non-DKD ($P < 0.001$). Serum nesfatin-1 demonstrated a significant positive correlation with eGFR, meanwhile, a significant negative correlation with BUN, Scr, UA, UmALB or UACR. Moreover, a distinctive L-shaped association was observed between the serum nesfatin-1 level and the risk of developing DKD (P-Non-linear = < 0.001). The critical serum nesfatin-1 value for predicting DKD was 513.35 pg/mL, with a sensitivity of 41.2%, a specificity of 92.0%, a positive predictive value of 83.9%, and a negative predictive value of 60.8%. It suggested that nesfatin-1 as a diagnostic marker for DKD, performed excellent in the specificity, but the sensitivity needs to be further improved. Notably, another report gave evidence of that serum nesfatin-1 levels were slightly higher in early DKD than that in T2DM[36], which was not in accordance with our findings. Taken together, our results on the relationship of nesfatin-1 level with DKD conflict with prior work and deserve deep exploration.

Numerous studies have demonstrated that vitamin D insufficiency or deficiency is independently associated with an elevated risk of DKD events in diabetic patients[37–39]. A cross-sectional study uncovered a significant correlation between chronic kidney disease and vitamin D deficiency, where individuals with chronic kidney disease were 1.7 times more likely to have vitamin D deficient than those without chronic kidney disease[40]. Serum 25(OH)D had a nonlinear connection with UACR, and a negative correlation between them was observed when 25(OH)D < 67 nmol/L, according to a recent retrospective study on patients with T2DM[41]. Interestingly, the similar results were given in our study: Serum 25(OH)D in DKD was significantly lower than in non-DKD (P< 0.001); renal function indicators such as BUN, Scr, UA, UmALB, and UACR showed a significant negative connection with serum 25(OH)D but a significant positive correlation with eGFR. Furthermore, the RCS curve for 25(OH)D was very similar to that for nesfatin-1, where the risk of DKD significantly decreased with increasing serum 25(OH)D levels, and the slope of this change became flat to unchanged when 25(OH)D ≥ 21.5 ng/mL (P-Non-linear = 0.008). The serum 25(OH)D threshold for predicting DKD was 18.93 ng/mL, with a sensitivity of 47.6%, specificity of 80.4%, positive predictive value of 71.4%, and negative predictive value of 59.9%. The mechanisms regarding the reversal of DKD progression by vitamin D are multifaceted which include the role of vitamin D in assisting glucose processing, protecting podocytes from apoptosis, reducing renin-angiotensin activation, attenuating fibrosis, and activating anti-inflammation[42]; nevertheless, more work needs to be done to clarify the mechanism and benefit the clinic.

In conclusion, we constructed a nomogram risk assessment model based on serum JAML, nesfatin-1, and 25(OH)D to predict the risk of developing DKD in the T2DM population. The combination of the above three metrics with easily obtainable records of history of hypertension, course of diabetes, and history of diabetic complications of retinopathy ultimately resulted in good performance of the nomogram model. Through internal validation, the model has a high agreement between the predicted and the actual occurrence of DKD, which would positively benefit the clinic. However, this study has some limitations. First, our study sample were all from Xiangya Hospital of Central South University, which lacks external validation with broad significance. Follow-up should be done to enlarge the sample size and conduct multicenter validation. Second, as a cross-sectional study, this study failed to observe the dynamic changes of JAML, nesfatin-1, and 25(OH)D levels during the disease progression process or determine their causal relationship with DKD. Third, since the clinical treatment data of the subjects were incomplete in this cross-sectional study, how the clinical strategy would affect serum JAML, nesfatin-1, and 25(OH)D in T2DM and DKD was unknown and deserves further exploration. Last but not least, although we revealed the associations of JAML, nesfatin-1, and 25(OH)D with UACR and eGFR, and their potential predictive value for DKD, more efforts should be made to elucidate the mechanism by which they are involved in DKD progression.

Methods

• Participants

This cross-sectional study comprised 227 patients with T2DM who were aged between 18 and 85 years old, met the inclusion and exclusion criteria, attended Xiangya Hospital of Central South University from Apr. 2023 to Nov. 2024 and admitted to the hospital for poor glycemic control or severe diabetic complications. According to the American Diabetes Association (ADA) guidelines for diabetes and studies[43], T2DM status is

defined as (1) a diagnosis previously reported by a healthcare professional, or (2) fasting blood glucose (FBG) ≥ 7.0 mmoL/L, or (3) glycosylated hemoglobin (HbA1c) ≥ 6.5%, or (4) 2 hours plasma glucose (2h-PG) ≥ 11.1 mmol/L during the oral glucose tolerance test (OGTT), or (5) random blood glucose ≥ 11.1 mmol/L for patients with classic symptoms of hyperglycemia. Patients with type 1 diabetes or other specific types of diabetes, other causes of chronic kidney disease (interstitial nephritis and nephrosclerosis), acute infections or inflammatory conditions, pregnant or breastfeeding women, or missing essential laboratory data were excluded. The study adhered to the Declaration of Helsinki and received authorization from the Xiangya Hospital of Central South University Ethics Review Committee (No. 2023020085). Appropriate informed consent and assent were obtained from all participants.

• Assessment of DKD and Study Variables

DKD was defined as a history of diabetes mellitus with a urinary albumin creatine ratio (UACR) ≥ 30 mg/g and/or an eGFR < 60 mL/min/1.73 m^2 according to the diagnostic criteria from the Kidney Disease Outcomes Quality Initiative Clinical Practice Guideline. UACR was calculated based on the ratio of urinary microalbumin to urinary creatinine, and eGFR was determined using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation for “Asian origin”.

Demographic characteristics included gender, age, height, weight, systolic blood pressure (SBP), diastolic blood pressure (DBP), hypertension, hyperlipidemia, cardiovascular disease, smoking status, alcohol consumption, course of diabetes, and complications of diabetes. Body mass index (BMI) was computed by dividing the weight (kg) by the square of the height (m). Hypertension was defined as SBP ≥ 140 mmHg and/or DBP ≥ 90 mmHg on repeated examinations or taking antihypertensive medication. Hyperlipidemia was defined as total cholesterol (TC) ≥ 5.2 mmol/L and/or triglycerides (TG) ≥ 1.7 mmol/L, or taking lipid-lowering drugs. Cardiovascular diseases including coronary heart disease, angina pectoris, and myocardial infarction were obtained from participants’ medical history. Smoking status is categorized as never smoker (< 100 cigarettes before the study), former smoker (smoked ≥ 100 cigarettes before the study but quit before the study), and current smoker (smoked ≥ 100 cigarettes before the study and smoked during the study)[44]. Participants who consumed at least 12 drinks of any type of alcoholic beverage within the past year were classified as drinkers[44].

Laboratory investigations were performed in the Laboratory Department of Xiangya Hospital of Central South University. 10 mL of venous blood were drawn from each participant after fasting for more than 8 hours. 2 mL of the blood were placed in a K3 EDTA vacutainer for HbA1c estimation by high-pressure liquid chromatograph (ARKRAY, Kyoto, Japan); another 4 mL were put into a plain tube for serum separation at 3000 rpm for 10 min, used for the determination of FBG, 2-h PG, TG, TC, high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C), blood urea nitrogen (BUN), creatinine, and uric acid (UA) on an AU5800 automatic biochemical analyzer (Beckman Coulter, CA, USA), as well as 25(OH)D determination on a chemiluminescence autoanalyzer; and the last 4 mL were prepared for serum JAML and nesfatin-1 measurement using enzyme-linked immunosorbent assay (ELISA) kits (Jiangsu Meimian Industrial Co., Ltd, Jiangsu, China) with a detection range of 15–550 pg/mL and 30–800 pg/mL, respectively. Simultaneously, morning urine samples were collected and immediately centrifuged upon collection. Urine

microalbumin (UmALB) and creatinine (Ucr) levels were determined by AU680, a fully automated biochemical analyzer (Beckman Coulter, CA, USA), and UACR (mg/g) was calculated.

• Statistical analysis

Normality was tested using the Kolmogorov-Smirnov test. Continuous variables with a normal distribution were presented as the mean ± standard deviation, non-normally distributed continuous variables were expressed as the median and interquartile range (IQR), and categorical variables were expressed as frequency and percentage (%). The t-test, Mann-Whitney U-test, and chi-square test were utilized to assess parameter differences between the patients with DKD and non-DKD. Spearman correlation analysis was employed to assess the correlation of serum JAML, nesfatin-1, and 25(OH)D with DKD risk indicators. Participants were categorized into tertiles based on the levels of JAML (T1 < 240, T2 240–318, T3 > 318), nesfatin-1 (T1 < 551, T2 551–682, T3 > 682) or 25(OH)D (T1 < 18.9, T2 18.9–25, T3 > 25). Multivariate logistic regression models with three progressive stages of adjustment were used to evaluate the associations between JAML, nesfatin-1 or 25(OH)D tertiles and the DKD incidence. Model 1 was unadjusted for any covariates; model 2 was adjusted for age and sex; and model 3 was adjusted for variables in Model 2 plus BMI, SBP, hypertension, hyperlipidemia, and history of cardiovascular disease. Trend tests were conducted by introducing the JAML, nesfatin-1, and 25(OH)D tertiles in the model as ordered categorical variables and calculating the Wald statistic. Additionally, restricted cubic splines (RCSs) analyses were performed to examine the nonlinear relationship of JAML, nesfatin-1 or 25(OH)D with the DKD incidence. The knots between 3 and 7 were tested respectively, and the model with the lowest Akaike information criterion value was selected for RCS. Finally, we used five knots at the 5th, 27.5th, 50th, 72.5th, and 95th percentiles for RCS analysis between JAML and the risk of developing DKD. Three knots were placed at the 10th, 50th, and 90th percentiles for RCS analysis between nesfatin-1 or 25(OH)D and the risk of developing DKD. The diagnostic performance of serum JAML, nesfatin-1 and 25(OH)D for DKD was assessed by the receiver operating characteristic (ROC) curve. The areas under the curve (AUC) of the three ROC curves were compared using DeLong's method, and Youden's index identified the optimal cutoff values of the index. Finally, the dataset of this study was randomly divided into training and validation cohorts. In the training cohort, the least absolute shrinkage and selection operator (LASSO) logistic regression analysis was operated for multivariate analysis to screen the independent risk factors and build a prediction nomogram to assess the risk of DKD in the T2DM population. Furthermore, the performance and clinical benefit of the nomogram were assessed using the ROC curve, calibration curve, and decision curve analysis (DCA) in the training and validation cohorts, respectively. All statistical analyses were performed using the R software (version 4.2.2). Results with $P < 0.05$ (two-tailed) were considered statistically significant.

Declarations

Competing interests

The authors declare no competing interests.

Ethics approval and consent to participate

The study adhered to the Declaration of Helsinki and received authorization from the Xiangya Hospital of Central South University Ethics Review Committee (No. 2023020085). Appropriate informed consent and assent were obtained from all participants.

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Author Contribution

QH and KH performed the majority of the experiments and wrote the main manuscript draft, YL and WY analyzed the data, LL prepared the figures, WK prepared the tables, and BY amended the first draft, supervised the whole study and approved the final version. All authors reviewed the manuscript.

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Data Availability

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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Figures

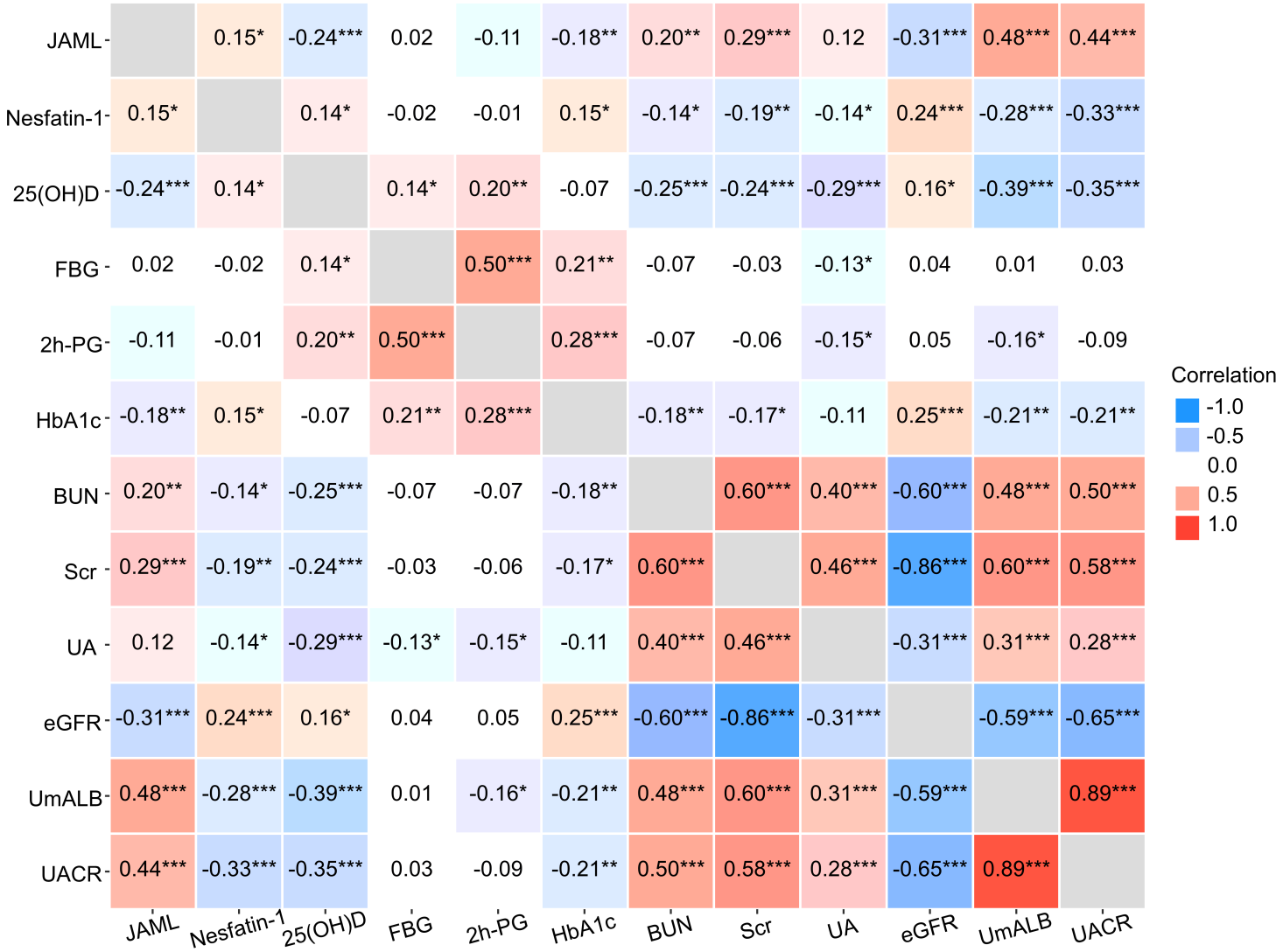

Figure 1

Correlations of serum JAML, nesfatin-1, and 25(OH)D levels with DKD. Spearman correlation analysis was performed to disclose the correlation of serum JAML, nesfatin-1, and 25(OH)D levels with DKD. FBG, fasting blood glucose; 2h-PG, 2 hours plasma glucose; HbA1c, glycosylated hemoglobin; BUN, blood urea nitrogen; Scr, serum creatinine; UA, uric acid; eGFR, estimated glomerular filtration rate; UmALB, urine albumin; UACR, urine albumin creatine ratio; JAML, junctional adhesion molecule-like protein; 25(OH)D, 25-hydroxy vitamin D. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

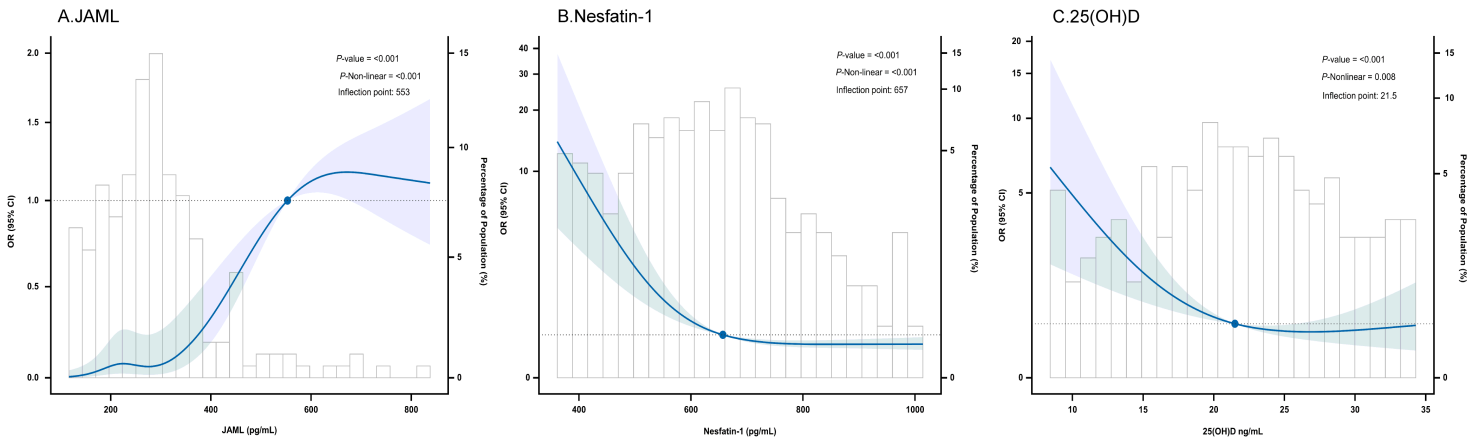

Figure 2

Nonlinear associations of the serum JAML (A), nesfatin-1 (B), and 25(OH)D (C) levels with different DKD in patients with T2DM. JAML, junctional adhesion mole-cule-like protein; 25(OH)D, 25-hydroxy vitamin D; T2DM, type 2 diabetes mellitus; DKD, diabetic kidney disease; OR, odds ratio; CI, confidence interval.

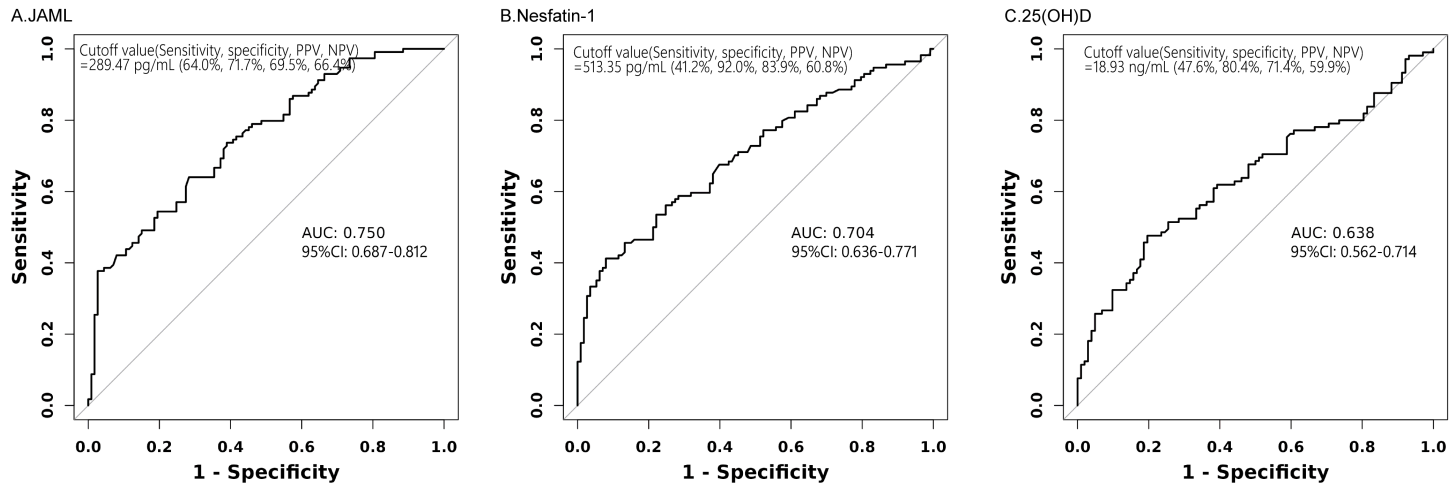

Figure 3

Receiver operating characteristic curves showing the performance of serum JAML (A), nesfatin-1 (B), and 25(OH)D (C) for predicting DKD incidence. JAML, junctional adhesion molecule-like protein; 25(OH)D, 25-hydroxy vitamin D; AUC, area under the curve; CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value.

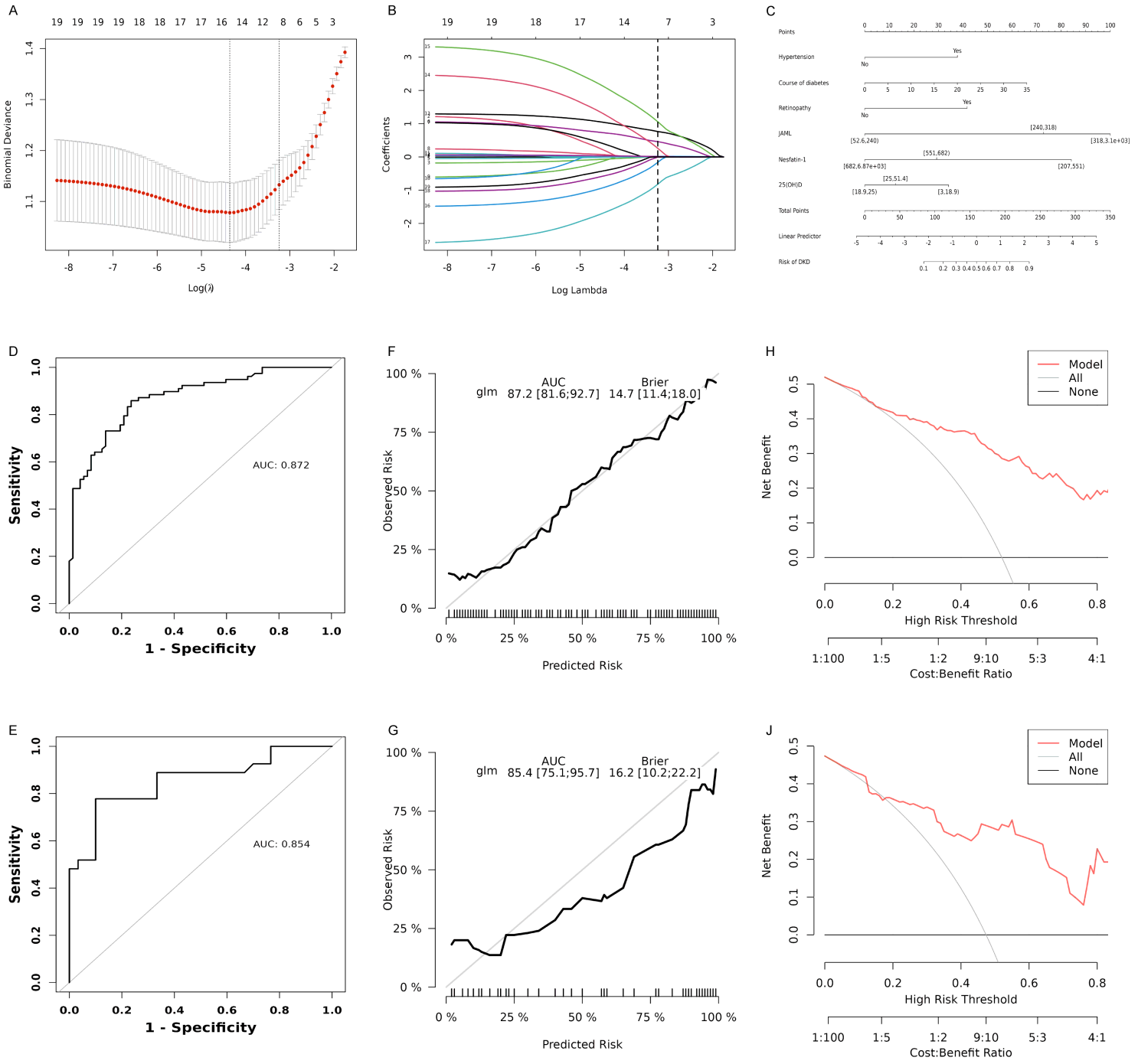

Figure 4

Nomogram model for predicting the risk of DKD in population with T2DM. A: LASSO regression cross-validation plot. B: LASSO regression coefficient path plot. C: Nomogram model for predicting the risk of DKD in the population with T2DM based on serum JAML, nesfatin-1, and 25(OH)D. D-E: Receiver operating characteristic curves of the nomogram prediction model for the training and internal test cohorts. F-G: Calibration curve of the nomogram prediction mode for the training and internal test cohorts. H-J: Decision curve analysis of the nomogram for the training and internal test cohorts. T2DM, type 2 diabetes mellitus; DKD, diabetic kidney disease; JAML, junctional adhesion molecule-like protein; 25(OH)D, 25-hydroxy vitamin D; LASSO, least absolute shrinkage and selection operator; AUC, area under the curve.

Supplementary Files

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- SupplementalTables.docx