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Research article

Association between lipoprotein-associated phospholipase A2 and 25-hydroxy-vitamin D on early stage diabetic kidney disease in patients with type-2 diabetes mellitus

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ABSTRACT

Objective: This study aimed to analyse the association between lipoprotein-associated phospholipase A2 (Lp-PLA2) and 25-hydroxy-vitamin D (25[OH]D) and early diabetic kidney disease (DKD) in patients with type 2 diabetes mellitus (T2DM) and evaluate the potential roles of these two biomarkers in the clinical diagnosis of DKD.

Methods: A total of 422 inpatients with T2DM were retrospectively enrolled between January 2018 and March 2022 at the First Affiliated Hospital of Nanjing Medical University. The baseline clinical parameters of each patient were determined, and their demographic characteristics were extracted from the hospital information system. The patients were separated into groups according to serum Lp-PLA2 and 25(OH)D levels and binary logistic regression analysis was used to determine independent predictors of early DKD incidence.

Results: Levels of Lp-PLA2 significantly increased and those of 25(OH)D significantly decreased with DKD progression (both $P < 0.001$). Lp-PLA2 concentrations were positively correlated with albuminuria levels ($r = 0.37$, $P < 0.001$), whereas 25(OH)D levels were negatively correlation ($r = -0.34$, $P < 0.001$). The incidence of DKD was higher in the Lp-PLA2 elevated and 25(OH)D deficient groups (all $P < 0.001$). Body mass index, systemic immune-inflammatory index, serum uric acid, C-peptide, and triglyceride-glucose indices were positively associated with Lp-PLA2 levels (all $P < 0.001$) and negatively associated with 25(OH)D (all $P < 0.05$). Furthermore, Lp-PLA2 was an independent risk factor ($OR = 1.003$, $P = 0.015$), and 25(OH)D was an independent protective factor ($OR = 0.937$, $P = 0.008$) for early DKD occurrence in binary logistic regression analysis. The area under the curve for the combination of Lp-PLA2 and 25(OH)D for diagnosing DKD was 0.867, with a sensitivity of 70.4 % and a specificity of 89.5 %.

Conclusions: Increased serum Lp-PLA2 and decreased 25(OH)D levels are risk factors for early DKD in patients with T2DM. The combined detection of Lp-PLA2 and 25(OH)D may enhance the diagnostic efficacy of DKD.

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1. Introduction

Diabetic kidney disease (DKD) is one of the most serious and chronic microvascular complications of type 2 diabetes mellitus (T2DM) [1], and has gradually become the leading cause of chronic kidney disease and end-stage renal disease [2]. DKD lacks typical clinical manifestations at onset, until irreversible kidney damage occurs; hence, patients generally reach stage 3 at the time of diagnosis [3]. Therefore, early detection and intervention in DKD are crucial for preventing the progression of renal impairment. The gold standard for DKD diagnosis is renal puncture pathology; however, this is not routinely conducted in clinical practice because of its invasive nature [4]. Moreover, the heterogeneity and subjectivity of histopathological examinations pose challenges in diagnosis [5]; hence, novel biomarkers with high sensitivity and specificity are urgently required.

The pathogenesis of DKD is complex. Besides genetic susceptibility, living environment, and blood glucose levels, recent studies have verified that oxidative stress and inflammation promote the development of DKD and that some novel mediators also participate in this process [6,7]. Lipoprotein-associated phospholipase A2 (Lp-PLA2) is a novel inflammatory marker that plays an indispensable role in various macroscopic cardiovascular events [8]. Moreover, regarding microvascular diseases, Lp-PLA2 is involved in the dysfunction of endothelial cells, which is considered an early event in renal microvascular disease and lays the foundation for the clinical manifestations of subsequent renal disease symptoms [9]. Another study demonstrated that the occurrence of DKD was partially associated with renal microangiopathy and that increased circulating Lp-PLA2 levels were related to disease progression [10]. A newly published controlled trial with a small sample size showed that Lp-PLA2 was positively associated with pro-inflammatory cytokines in a diabetic proteinuria group and could be an independent predictor of DKD [4]. However, few large-scale clinical studies have been conducted on the relationship between Lp-PLA2 and microvascular complications; hence, there is still a lack of reliable data to support its potential relevance as a biomarker for early renal microangiopathy.

In contrast to Lp-PLA2, low 25-hydroxy-vitamin D (25[OH]D) concentrations are associated with an increased risk of cardiovascular events and high levels of circulating C-reactive protein (CRP) and other plasma inflammatory cytokines [11]. 25(OH)D is considered to lower oxidative stress and promote anti-inflammatory actions to improve endothelial function by binding to 25(OH)D receptors (VDRs), which have a nephroprotective effect in patients with DM [12]. Both *in vitro* and *in vivo* investigations have revealed a negative correlation between 25(OH)D and the risk of DKD, sparking interest in the potential role of 25(OH)D in preventing DKD [13]. Although some research has demonstrated that high-dose parenteral 25(OH)D therapy is beneficial for preventing T2DM and reducing albuminuria levels in patients with T2DM, which is widely accepted as the first clinical symptom of DKD [14], other studies have not [15]. Hence, there is no consensus on the predictive value of serum 25(OH)D levels in DKD progression [13].

Considering the stability of Lp-PLA2 and 25(OH)D, and the lack of studies reporting their relationships in early DKD, the present study aimed to explore the association between serum Lp-PLA2 and 25(OH)D levels and DKD status and evaluate the clinical value of these two biomarkers in determining the occurrence of DKD in patients with T2DM.

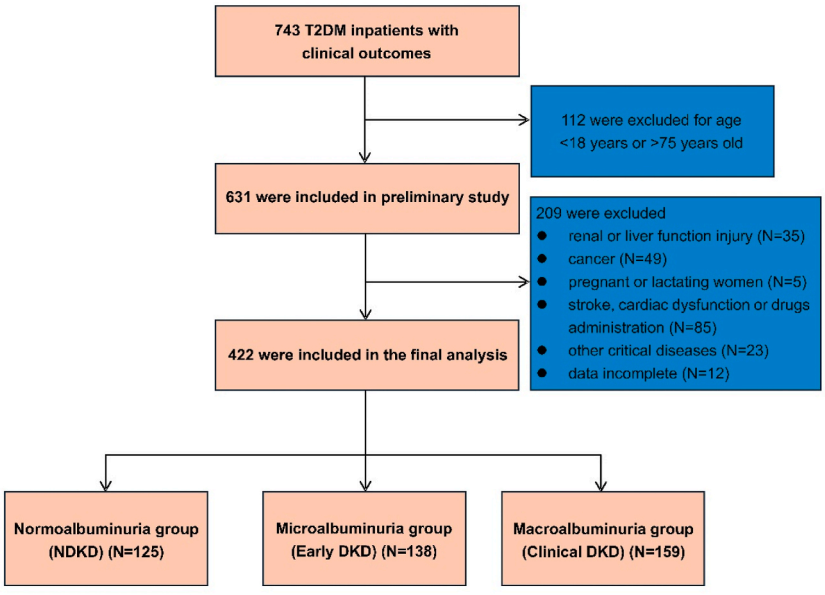


Fig. 1. Flow diagram illustrates exclusion criteria and final study population.

2. Materials and methods

2.1. Participants

A total of 422 inpatients with T2DM, aged between 18 and 75 years, at the First Affiliated Hospital of Nanjing Medical University between January 2018 and March 2022 were enrolled in this study (Fig. 1). All participants were diagnosed with T2DM according to the 1999 World Health Organization Diagnostic criteria for DM [16]. The assessment of kidney involvement for DKD was based on the persistent urinary albumin excretion rate (UAER) of 30 mg/24h or more [17]. Patients with T2DM were grouped into three subgroups according to UAER: normoalbuminuria group (UAER<30 mg/24h, viz NDKD), microalbuminuria group (30 mg/24h ≤ UAER<300 mg/24h, viz early DKD stage) and macroalbuminuria group (UAER≥300 mg/24h, viz clinical DKD stage).

Patient exclusion criteria were: (1) renal function injury or liver insufficiency caused by non-diabetic factors; (2) tumors, acute or chronic infections, acute complications of diabetes, and disorders of the immune system or blood system; (3) long-term usage of active vitamin D and its analogues, calcium agents, Lp-PLA2 inhibitors, insulin and drugs affecting urinary albumin excretion; (4) pregnant or lactating women; (5) patients with severe trauma, surgery, or chemotherapy in the past 5 years; (6) stroke or cardiac dysfunction in the past 6 months; and (7) patients with clinical data defects. The study was approved by the Research Ethics Committee of the First Affiliated Hospital of Nanjing Medical University (approval number: 2022-SR-692).

2.2. Data collection

Demographic statistics, including sex, age, hypertension, family history, smoking history, duration of diabetes, body mass index (BMI), systolic blood pressure (SBP), and diastolic blood pressure (DBP) were extracted from the admission case system. Clinical biochemical parameters such as white blood cell (WBC) count, serum urea nitrogen (sUREA), serum creatinine (sCREA), serum uric acid (sUA), retinol-binding protein (RBP), glycosylated haemoglobin A1c (HbA1c), C-peptide 0 min, insulin 0 min, fasting plasma glucose (FPG), total cholesterol (TC), triacylglycerol (TG), high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C), UAER, Lp-PLA2, and 25(OH)D levels were measured in our laboratory. Additionally, neutrophil-to-lymphocyte ratio (NLR), monocyte-to-lymphocyte ratio (MLR), platelet-to-lymphocyte ratio (PLR), systemic immune-inflammatory index (SII = platelet × neutrophil/lymphocyte), estimated glomerular filtration rate (eGFR [mL/min/1.73 m^2] = $194 \times SCr^{-1.094} \times age^{-0.287} \times 0.739$ [if female]) [13], arteriosclerosis index (AI = [TC-HDL]/HDL), and triglyceride-glucose (TyG = Ln[fasting triacylglycerol {mg/dL} × fasting glucose {mg/dL}/2]) index were calculated for analysis.

2.3. Measurement of UAER, Lp-PLA2, and 25(OH)D levels

The urine of each patient was collected over 24 h and stored at 2–8 °C until analysis to determine the UAER. A colorimetric determination of pyrogallol red was used to detect urine albumin levels (Respons® 920, Diasys, Germany).

Serum detection was based on venous blood samples collected after fasting overnight for at least 8 h. Serum Lp-PLA2 concentrations were measured using a commercially available turbidimetric immunoassay kit (NORMAN-1, Norman, China) and classified into two subgroups according to their levels: normal (<200 ng/mL) and elevated (≥200 ng/mL). Serum 25(OH)D levels were measured by electrochemiluminescence (Roche, Germany, COBAS e601) and segmented into three subgroups based on their levels: normal (≥30 ng/mL), insufficiency (20.0–29.9 ng/mL), and deficiency (<20 ng/mL). All procedures were performed in strict accordance with the manufacturer's instructions.

2.4. Statistical analysis

All statistical analyses were performed using SPSS version 26.0 (IBM, Armonk, NY, United States) and GraphPad Prism (version 8.0; GraphPad, San Diego, CA, United States). Categorical variables are presented as percentages (%), and comparisons between two groups were performed using the chi-squared test. It is necessary to adjust the testing level α (0.05) when further comparing pairwise population rates (or composition ratios) within multiple sample rates (or composition ratios). The calculation formula is as follows (k is the total number of groups):

$$\alpha' = \frac{\alpha}{\frac{k(k-1)}{2} + 1}$$

Continuous variables are presented as medians (25 % quantile, 75 % quantile) for non-normally distributed variables, after verification using the Kolmogorov-Smirnov test. Comparative analysis of demographic characteristics between two groups was analysed using the Mann-Whitney *U* test, and that of more than two groups was analysed using the Kruskal-Wallis *H* test. Binary logistic regression analysis was used to determine independent predictors of early DKD incidence. Albuminuria levels were converted to log base 10 ($\log_{10}$) for further analysis. Spearman correlation analysis was used to analyse the correlation between two indicators and a receiver operating characteristic (ROC) curve was constructed to evaluate the diagnostic performance of Lp-PLA2 and 25(OH)D in DKD. A two-sided *P*-value of less 0.05 was considered statistically significant.

3. Results

3.1. Basic demographic and clinical characteristics stratified across the study population

A total of 422 inpatients with T2DM were enrolled in our clinical investigation. These included 260 males (61.6 %) and 162 females (38.4 %), with median ages of 52 (41, 59) and 57 (49, 63) years, respectively. The numbers of NDKD, early DKD stage and clinical DKD stage groups were 125, 138 and 159, respectively. As shown in Table 1, WBC, SII, Lp-PLA2, sCREA, sUA, RBP, and TyG index levels significantly increased (all $P < 0.001$), while 25(OH)D and eGFR levels significantly decreased (both $P < 0.001$), as DKD progressed. Although increased smoking rates, BMI, SBP, DBP, C-peptide (0 min), FPG levels, and younger age were found in the early DKD stage group (all $P < 0.05$), there were no significant differences between the early and clinical DKD stage groups. Furthermore, male sex, hypertension rate, diabetes duration, NLR, MLR, PLR, sUREA, TC, TG, HDL-C, LDL-C, AI, and insulin (0 min) significantly increased (all $P < 0.05$) only when the clinical DKD stage was reached.

Table 1
Demographic characteristics of participants with T2DM among three study groups.

Objects	NDKD (UAER<30mg/24h) (n = 125)	Early DKD (30mg/24h ≤ UAER<300mg/24h) (n = 138)	Clinical DKD (UAER≥300mg/24h) (n = 159)	*P*-value
General data				
Male (n, %)	65(52.00 %)	87(63.04 %)	108(67.92 %) a$^{\#}$	0.021
Age (year)	57(47,63)	52(41,60) a*	54(41,60)	0.033
Hypertension (n, %)	47(37.60 %)	67(48.55 %)	101(63.52 %) a$^{\#}$b$^{\#}$	<0.001
Family history (n, %)	55(44.00 %)	55(39.86 %)	63(39.62 %)	0.717
Smoking (n, %)	30(24.00 %)	55(39.86 %) a$^{\#}$	78(49.06 %) a$^{\#}$	<0.001
Diabetes duration (year)	5(1,11)	5(1,10)	10(2,15) a*b*	0.006
BMI (kg/m^2)	24.20(22.44,26.79)	25.71(23.41,28.70) a*	26.73(23.80,29.58) a***	<0.001
SBP (mmHg)	125(114,133)	132(119,143) a**	136(126,150) a***	<0.001
DBP (mmHg)	75(68,81)	81(73,89) a***	81(74,92) a***	<0.001
Whole blood test				
WBC (× 10^9/L)	5.69(4.82,6.78)	6.18(5.28,7.66) a**	6.88(5.75,8.52) a***b*	<0.001
NLR	1.58(1.23,2.21)	1.89(1.34,2.44)	2.16(1.58,3.17) a***b**	<0.001
MLR	0.23(0.18,0.27)	0.23(0.19,0.28)	0.26(0.21,0.39) a***b**	<0.001
PLR	100.50(82.27,133.52)	101.72(86.67,132.00)	113.49(89.36,156.76) a*	0.011
SII	310.54(226.54,451.74)	377.51(265.53,529.46) a*	458.70(330.11,742.92) a***b**	<0.001
Lp-PLA2 (ng/mL)	123(100,177)	158(113,264) a**	240(164,346) a***b***	<0.001
Biochemical profile				
25(OH)D (ng/mL)	20.40(16.60,24.20)	18.32(12.88,23.50) a**	14.48(11.04,18.60) a***b**	<0.001
eGFR (mL/min/1.73m^2)	83.48(73.89,97.14)	78.24(67.77,88.47) a*	67.75(51.00,88.20) a***b*	<0.001
sUREA (mmol/L)	5.48(4.53,6.45)	5.51(4.39,6.80)	6.35(4.72,7.80) a**b**	<0.001
sCREA (μmol/L)	58.50(49.55,69.60)	67.10(57.03,77.48) a**	73.60(59.40,96.90) a***b**	<0.001
sUA (μmol/L)	295(244,359)	338(287,405) a***	383(317,460) a***b*	<0.001
RBP (mg/L)	35.10(28.90,41.0)	41.10(33.18,47.93) a***	45.00(35.20,54.80) a***b*	<0.001
Lipid metabolism				
TC (mmol/L)	4.35(3.69,5.09)	4.58(3.77,5.50)	4.88(4.14,5.67) a**	0.002
TG (mmol/L)	1.31(0.91,2.01)	1.51(1.09,2.45)	1.83(1.34,2.80) a***b*	<0.001
HDL-C (mmol/L)	1.04(0.89,1.22)	1.01(0.88,1.23)	0.96(0.83,1.12) a*	0.009
LDL-C (mmol/L)	2.67(2.17,3.17)	2.88(2.23,3.53)	3.04(2.57,3.54) a**	0.002
AI	3.10(2.32,4.06)	3.47(2.78,4.20)	3.98(3.31,4.81) a***b***	<0.001
Glucose metabolism				
HbA1c (%)	8.60(7.35,10.92)	8.90(7.40,10.65)	9.10(7.66,11.14)	0.495
C-peptide (0 min, ng/mL)	396.0 (279.3572.50)	497.5(320.40,714.10) a*	589.1(349.30,865.30) a***	<0.001
Insulin (0 min, pmol/L)	26.60(11.35,56.15)	29.75(13.68,56.38)	34.60(16.40,86.90) a*	0.032
FPG (mmol/L)	5.61(4.93,6.95)	6.28(5.19,7.87) a*	6.31(5.26,7.62) a**	0.005
TyG	8.74(8.30,9.24)	8.98(8.50,9.42) a*	9.14(8.77,9.70) a***b*	<0.001
Complications				
Peripheral neuropathy (n, %)	17(13.60 %)	32(23.19 %)	52(32.70 %) a$^{\#}$b$^{\#}$	0.002
Retinopathy (n, %)	17(13.60 %)	30(21.74 %)	64(40.25 %) a$^{\#}$b$^{\#}$	<0.001
Atherosclerosis (n, %)	47(37.60 %)	61(44.20 %)	73(45.91 %)	0.329

a: compare with NDKD group; b: compare with Early DKD group.
*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, $^{\#}P < 0.0125$.
Abbreviations: BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; WBC, white blood cell count; NLR, neutrophil-to-lymphocyte ratio; MLR, monocyte-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; SII, systemic immune-inflammatory index; Lp-PLA2, lipoprotein-associated phospholipase A2; 25(OH)D, 25-hydroxy-vitamin D; eGFR, estimate glomerular filtration rate; sUREA, serum urea nitrogen; sCREA, serum creatinine; sUA, serum uric acid; RBP, retinol-binding protein; TC, total cholesterol; TG, triacylglycerol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; FPG, fasting plasma glucose; TyG, triglyceride-glucose.

3.2. Status of different Lp-PLA2 and 25(OH)D circulating levels

Patients were classified into Lp-PLA2 normal and elevated groups and 25(OH)D normal, insufficient, and deficient groups based on the clinical determination of serum Lp-PLA2 and 25(OH)D levels, respectively. There was a difference in albuminuria levels between patients with elevated and normal Lp-PLA2 levels ($P < 0.001$) (Fig. 2A and Supplementary Table 1). Additionally, albuminuria levels in both patients with normal and insufficient 25(OH)D levels differed from those with deficient 25(OH)D levels (both $P < 0.001$) (Fig. 2B and Supplementary Table 2). The number of patients with T2DM and high Lp-PLA2 levels accounted for 43.13 % (182/422) of the study population, among which 85.16 % (155/182) had DKD, which was significantly higher than the number of DKD cases in the Lp-PLA2 normal group (59.17 %, 142/240) ($\chi^2 = 33.56$, $P < 0.001$). Additionally, we found that 62.09 % (262/422) of patients in our study had a 25(OH)D deficient status and the incidence rate of DKD in this population reached 78.63 % (206/262), which was much higher than that in patients with 25(OH)D normal (50.00 %, 19/38) ($\chi^2 = 16.02$, $P < 0.001$) and insufficient status (59.02 %, 72/122) ($\chi^2 = 14.51$, $P < 0.001$).

3.3. Association of Lp-PLA2 and 25(OH)D levels with albuminuria and other clinical indicators

We analysed the correlation between albuminuria ($\log_{10}$), Lp-PLA2, and 25(OH)D levels using Spearman correlation analysis. A positive correlation between Lp-PLA2 and albuminuria ($r = 0.37$, $P < 0.001$) (Fig. 3A) and a negative correlation between 25(OH)D and albuminuria ($r = -0.34$, $P < 0.001$) (Fig. 3B) were found. Moreover, the Lp-PLA2 and 25(OH)D levels showed a significant negative correction with each other ($r = -0.23$, $P < 0.001$) (Fig. 3C). We further comparatively analysed in patients with T2DM the association between Lp-PLA2 and 25(OH)D levels and other clinical indicators, which increased or decreased in the early stages of DKD. The results showed that BMI, SII, sUA, C-peptide (0 min), and TyG index were positively correlated with Lp-PLA2 levels (all $P < 0.001$), but negatively correlated with 25(OH)D levels (all $P < 0.05$) (Table 2). SBP, DBP, WBC count, and FPG showed the same upward trend as Lp-PLA2 circulating concentrations (all $P < 0.05$); however, they seemed to be unrelated to 25(OH)D levels (all $P > 0.05$).

3.4. Association of Lp-PLA2 and 25(OH)D levels with risk of early DKD

We created a binary logistic regression analysis model with the occurrence of early DKD as the dependent variable and Lp-PLA2 and 25(OH)D levels as independent variables. The indexes with *P*-values less than 0.05 at the early DKD stage, such as: age, smoking history, BMI, SBP, DBP, WBC count, SII, eGFR, sCREA, sUA, RBP, C-peptide (0 min), FPG, and TyG, were adjusted as covariates. Given that sex and hypertension rates generally play important roles in various diseases, although these two variables did not increase in the early stages of DKD, they were included in the model. After adjusting for confounders associated with the occurrence of early stage DKD, smoking history ($P = 0.032$), Lp-PLA2 ($P = 0.015$), and FPG ($P = 0.013$) were found to be independent risk factors, while 25(OH)D ($P = 0.008$) was found to be an independent protective factor for DKD (Table 3).

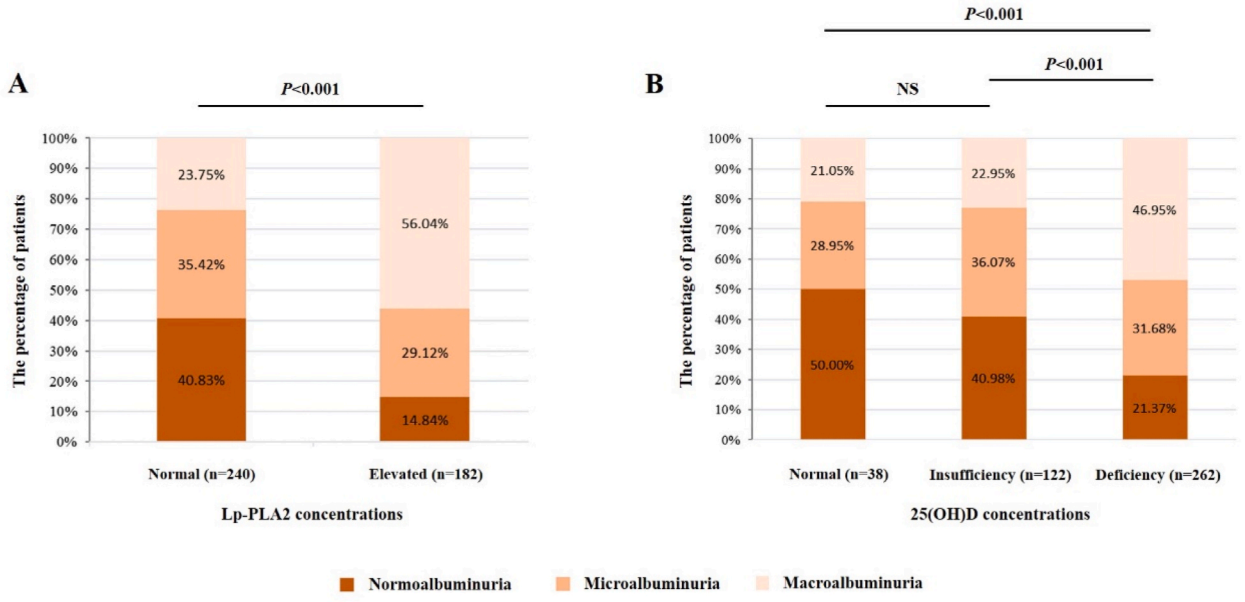


Fig. 2. Status of different Lp-PLA2 (A) and 25(OH)D (B) circulating levels in patients with T2DM.
Abbreviations: Lp-PLA2, lipoprotein-associated phospholipase A2; 25(OH)D, 25-hydroxy-vitamin D; T2DM, type 2 diabetes mellitus; NS, not significant.

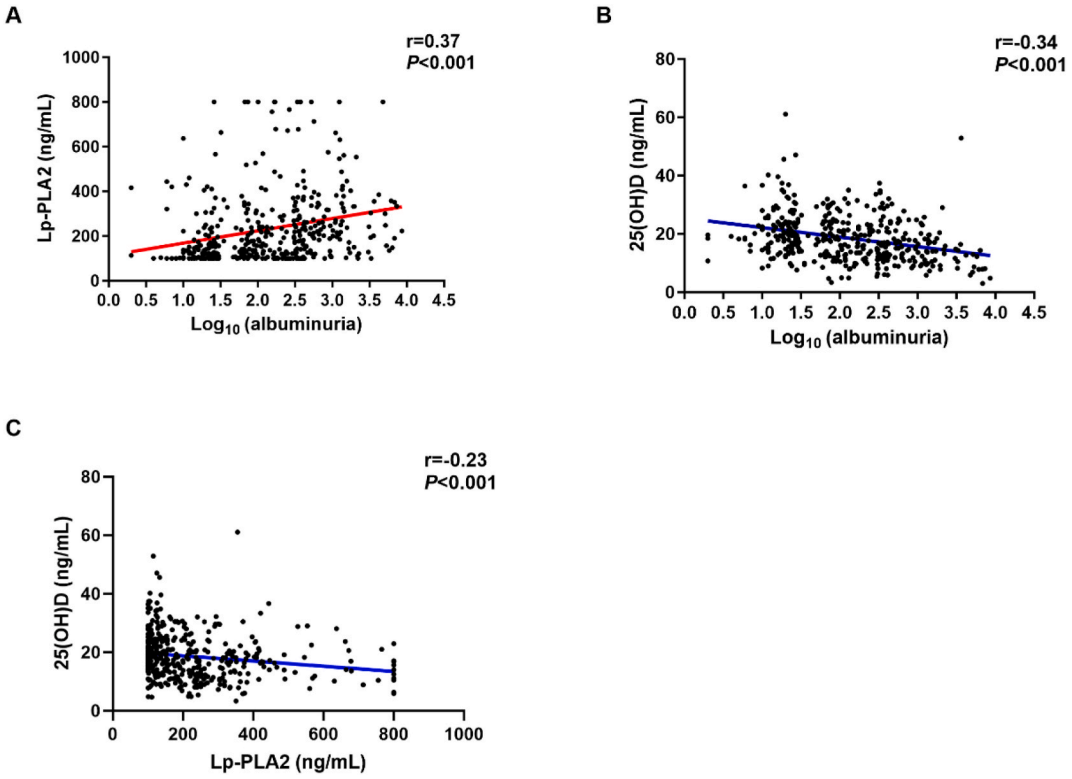


Fig. 3. Correlation analysis of Lp-PLA2 (A) and 25(OH)D (B) with albuminuria, and between Lp-PLA2 and 25(OH)D (C).
Abbreviations: Lp-PLA2, lipoprotein-associated phospholipase A2; 25(OH)D, 25-hydroxy-vitamin D.

Table 2
Spearman correlation analysis of Lp-PLA2 and 25(OH)D with clinical indicators.

Clinical indicators	Lp-PLA2 (ng/mL)		25(OH)D (ng/mL)	
	r	*P*-value	r	*P*-value
BMI (kg/m^2)	0.28	<0.001	−0.12	0.023
SBP (mmHg)	0.18	<0.001	−0.07	0.172
DBP (mmHg)	0.14	0.004	−0.07	0.138
WBC (× 10^9/L)	0.23	<0.001	−0.08	0.097
SII	0.26	<0.001	−0.15	0.002
eGFR (mL/min/1.73m^2)	−0.04	0.373	−0.01	0.775
sCREA (μmol/L)	0.05	0.325	0.05	0.323
sUA (μmol/L)	0.22	<0.001	−0.14	0.005
RBP (mg/L)	−0.05	0.283	−0.06	0.195
C-peptide (0 min, ng/mL)	0.24	<0.001	−0.16	0.002
FPG (mmol/L)	0.12	0.020	−0.08	0.084
TyG	0.21	<0.001	−0.20	<0.001

Abbreviations: Lp-PLA2, lipoprotein-associated phospholipase A2; 25(OH)D, 25-hydroxy-vitamin D; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; WBC, white blood cell count; SII, systemic immune-inflammatory index; eGFR, estimate glomerular filtration rate; sCREA, serum creatinine; sUA, serum uric acid; RBP, retinol-binding protein; FPG, fasting plasma glucose; TyG, triglyceride-glucose.

It is typically accepted that an eGFR of less than 60 mL/min/1.73 m^2 indicates renal dysfunction; however, we noted that eGFR was not a risk factor for early DKD in this study ($P = 0.746$). By comparing and analysing Lp-PLA2 and 25(OH)D levels in the normal and reduced eGFR (<60 mL/min/1.73 m^2) groups, it was found that Lp-PLA2 levels in the reduced eGFR group were higher ($P < 0.001$) (Fig. 4A) and 25(OH)D levels were lower ($P = 0.021$) (Fig. 4B) than those in the normal eGFR group.

3.5. Diagnostic performance of Lp-PLA2 and 25(OH)D in patients with DKD

In our ROC analysis (Fig. 5), the area under the curve (AUC) of Lp-PLA2 detection for the identification of DKD was 0.813, with a sensitivity of 80.3 % and specificity of 78.9 %, and that of 25(OH)D was 0.705, with a sensitivity of 67.6 % and specificity of 68.4 %.

Table 3
Binary logistic regression analysis of early DKD incidence.

Variables	*B*	*OR* (95 % *CI*)	*P*-value
Gender	−0.864	0.422 (0.090–1.980)	0.274
Age	−0.045	0.956 (0.912–1.001)	0.057
Hypertension	0.412	1.510 (0.730–3.124)	0.267
Smoking	0.917	2.502 (1.081–5.790)	0.032
BMI	−0.046	0.955 (0.864–1.055)	0.362
SBP	0.016	1.016 (0.993–1.041)	0.180
DBP	0.033	1.033 (0.994–1.074)	0.098
WBC	0.113	1.119 (0.876–1.430)	0.369
SII	0.001	1.001 (1.000–1.002)	0.857
Lp-PLA2	0.003	1.003 (1.001–1.005)	0.015
25(OH)D	−0.065	0.937 (0.894–0.983)	0.008
eGFR	−0.008	0.992 (0.945–1.041)	0.746
sCREA	0.049	1.050 (0.972–1.136)	0.214
sUA	0.001	1.001 (0.997–1.006)	0.542
RBP	0.026	1.027 (0.989–1.066)	0.168
C-peptide	0.000	1.000 (0.999–1.001)	0.927
FPG	0.259	1.296 (1.056–1.590)	0.013
TyG	−0.158	0.854 (0.453–1.609)	0.625

Abbreviations: OR, odds ratio; CI, confidence interval; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; WBC, white blood cell count; SII, systemic immune-inflammatory index; Lp-PLA2, lipoprotein-associated phospholipase A2; 25(OH)D, 25-hydroxy-vitamin D; eGFR, estimate glomerular filtration rate; sCREA, serum creatinine; sUA, serum uric acid; RBP, retinol-binding protein; FPG, fasting plasma glucose; TyG, triglyceride-glucose.

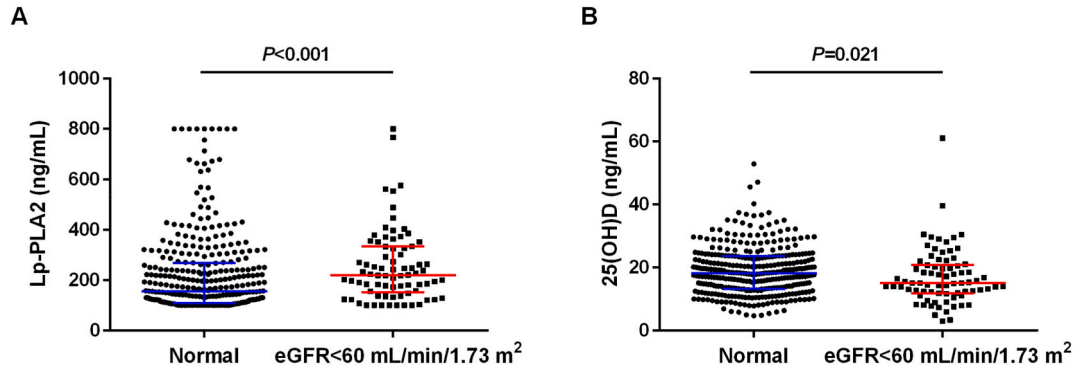


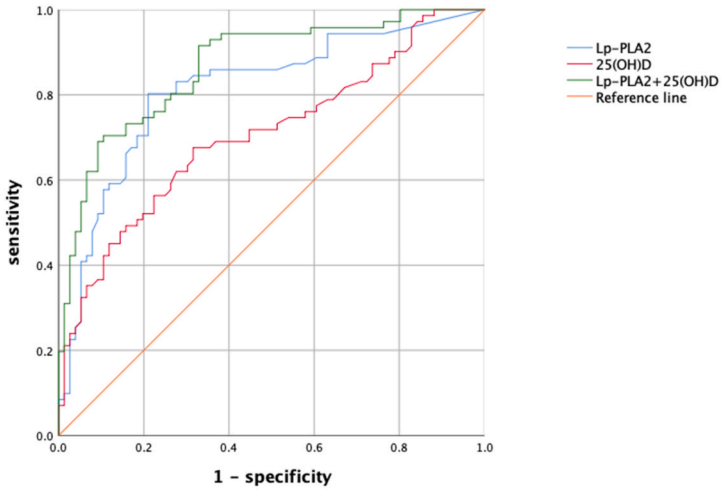
Fig. 4. Lp-PLA2 (A) and 25(OH)D (B) levels in different eGFR subgroups.
Abbreviations: Lp-PLA2, lipoprotein-associated phospholipase A2; 25(OH)D, 25-hydroxy-vitamin D; eGFR, estimated glomerular filtration rate.

The combination of Lp-PLA2 and 25(OH)D exhibited increased diagnostic effectiveness, with a higher AUC of 0.867, a sensitivity of 70.4 %, and a specificity of 89.5 %.

4. Discussion

T2DM is a chronic metabolic disease that induces glucose, lipid, and protein metabolism disorders because of a relative or absolute lack of insulin in the body [18]. In this retrospective study targeting newly admitted patients with T2DM in our hospital, patients with DKD manifested worse basic conditions, such as younger age, higher incidence of hypertension and smoking, higher BMI, worse renal function, more severe inflammatory status, and poorer serum lipid and glycaemic control.

DKD is primarily caused by the interaction of oxidative stress and inflammation, mesangial dilation, and hyperfiltration damage of the glomerular filtration barrier, ultimately resulting in glomerular and tubular basement membrane thickening and glomerulosclerosis [19]. Recently, both *in vivo* and *in vitro* studies have indicated that the inflammatory response is an essential mechanism for patients with diabetes to develop DKD and occurs earlier than the appearance of albuminuria [18,20]. As a potent pro-inflammatory product, Lp-PLA2 can stimulate cytokine (such as interleukin-6 [IL-6] and tumor necrosis factor α [TNF-α]) production by participating in systemic or local inflammatory responses, thereby increasing the risk of DKD [21]. This is in line with the present study which found that the proportion of DKD was up to 85.16 % in the elevated Lp-PLA2 group. In contrast, a number of recent studies reported that 25(OH)D deficiency was associated with an increased risk of diabetic microangiopathy [22,23], which could be observed in our study with 62.09 % of patients with T2DM having a 25(OH)D deficient status, among which up to 78.63 % suffered from DKD, suggesting that insufficient intake or conversion disorder of 25(OH)D was a common phenomenon in patients with DKD.



Lp-PLA2: AUC=0.813, Sensitivity=80.3%, Specificity=78.9%

25(OH)D: AUC=0.705, Sensitivity=67.6%, Specificity=68.4%

Lp-PLA2+25(OH)D: AUC=0.867, Sensitivity=70.4%, Specificity=89.5%

Fig. 5. ROC curves of Lp-PLA2 and 25(OH)D in the diagnosis of DKD.
Abbreviations: DKD, diabetic kidney disease; Lp-PLA2, lipoprotein-associated phospholipase A2; 25(OH)D, 25-hydroxy-vitamin D; ROC, receiver operating characteristic.

Owing to its highly vascular-specific characteristics, the role of Lp-PLA2 in cardiovascular events has been demonstrated in several studies, and glomerulosclerosis seems to share a similar pathogenesis with atherosclerosis [8,24,25]. About 80 % of Lp-PLA2 in plasma binds to LDL to catalyse the production of pro-inflammatory substances such as lysophosphatidylcholine and oxidised free fatty acids [26]. These inflammatory mediators promote platelet aggregation and participate in the adhesion of inflammatory cells, thus aggravating atherosclerotic and glomerular injury [27]. Contrary to Lp-PLA2, 25(OH)D exhibits opposite regulatory functions on IL-6 and TNF-α, and contributes to the prevention of DKD progression [28]. Furthermore, previous studies have verified that 25(OH)D could reduce other systemic inflammatory mediators, such as IL-1, IL-1β, transforming growth factor-β, and monocyte chemoattractant protein-1, and facilitate the release of anti-inflammatory cytokines from the immune system [29,30]. 25(OH)D has also been reported to reduce inflammatory factors to protect podocytes and alleviate glomerulosclerosis through the Wnt and nephrin-PI3K-Akt signalling pathways, contributing to its renoprotective role [31]. Data from our study showed that patients with DKD had higher Lp-PLA2 and lower 25(OH)D levels as early as the microalbuminuria stage, and more severe inflammation in the body (WBC count and SII). Spearman analysis suggested a positive relationship between Lp-PLA2 and a negative relationship between 25(OH)D levels and the WBC count or SII, confirming their roles in inflammatory regulation.

Lp-PLA2 in a pro-inflammatory environment releases prostaglandins and leukotrienes, stimulating the production of cytokines and adhesion factors and causing glomerular endothelial cell dysfunction [32]. In addition, elevated Lp-PLA2 levels can strengthen cellular oxidative stress responses and promote endothelial cell apoptosis [33]. In contrast, 25(OH)D may ameliorate metabolism and reduce oxidative stress, resulting in anti-proteinuria in patients with T2DM. Moreover, 25(OH)D deficiency induces endothelial dysfunction via inflammation and upregulation of renin-angiotensin-aldosterone system activity [13]. The glomerular endothelial barrier is a prominent and complex part of the glomerular filtration system, and its injury is widely acknowledged to be closely related to proteinuria and a decline in eGFR [34]. Our data indicated that Lp-PLA2 levels were significantly elevated in the albuminuria group compared with the normoalbuminuria group and showed a positive relationship with albuminuria; in contrast, 25(OH)D manifested the opposite trend. Moreover, eGFR was reduced in both the early and clinical stages of DKD. Serum Lp-PLA2 levels were increased and 25(OH)D levels were decreased significantly in patients with T2DM and decreased eGFR. However, eGFR was not significantly correlated with Lp-PLA2 or 25(OH)D levels, nor was it an independent factor in the presence of DKD. This may be because of the relatively low sensitivity of eGFR to minor changes in renal function [35]. Additionally, a high sUA level has been reported to stimulate oxidative stress and induce the formation of inflammasomes in monocytes and macrophages, leading to inflammation, impaired endothelial function in the vascular system, and accelerated development of microvascular complications [36,37]. Elevated sUA levels in patients with DKD were positively or negatively related to Lp-PLA2 or 25(OH)D levels, respectively, in this study. Therefore, our results indicate that vascular inflammation and endothelial injury participate in the occurrence and progression of DKD, and that Lp-PLA2 and 25(OH)D are closely related to this process.

Insulin resistance (IR) caused by hyperglycaemia can also mediate the production of Lp-PLA2 and is involved in renal haemodynamic disorders [38]. Lipid and glucose metabolism disorders, coupled with Lp-PLA2-induced inflammatory activity, further aggravate IR [39]; thereby, synergistically facilitating the progression of DKD. As a positive regulator of endothelial function, studies have demonstrated the role of 25(OH)D on IR [40,41]. In a recent investigation, 25(OH)D was confirmed to be positively associated with

insulin sensitivity, and its deficiency negatively regulated β-cell function. In addition, 25(OH)D could act directly on β-cells to promote insulin secretion via binding to its nuclear VDRs [42]. The TyG index is a new, reliable, and accessible surrogate indicator of IR, and its increase is strongly associated with increased diabetic retinopathy prevalence [43]. In this study, we found that serum C-peptide (0 min), FPG, and TyG indices were significantly increased in patients with T2DM and micro- or macroalbuminuria, indicating increased IR in the early stage of DKD. In addition, C-peptide (0 min) and the TyG index showed positive correlations with Lp-PLA2 levels and negative correlations with 25(OH)D levels in patients with T2DM. Long-term unsatisfactory glycaemic control in patients with T2DM can lead to microvascular complications that impair the kidney and develop into DKD [44], which support our findings. Although FPG concentrations in the DKD group exceeded those in the NDKD group and showed a positive correlation with Lp-PLA2 levels, there was no correlation with 25(OH)D levels, which may be partially due to the bias caused by emergency insulin pump therapy in some patients with critical hyperglycaemia on first admission. Higher IL-6 levels were found in overweight/obese postmenopausal women, implying a tendentious inflammatory state in obese individuals [45], which is consistent with the higher BMI in the DKD group in our results. Consistently, BMI was also positively associated with Lp-PLA2 and negatively correlated with 25(OH)D levels. Because lipid metabolism biomarker levels, such as TC, TG, HDL-C, LDL-C, and AI, showed no difference in the early DKD stage compared with the NDKD stage, we did not include them in the correlation analysis, possibly because some patients had already received lipid-lowering therapy and did not have extremely high serum lipid levels.

Further binary logistic regression analysis showed that after adjusting for confounders, Lp-PLA2 was an independent risk factor and 25(OH)D was an independent protective factor for early DKD occurrence. In the ROC curve analysis, serum Lp-PLA2 and 25(OH)D levels showed satisfactory diagnostic performance in the detection of DKD, and their combination displayed better diagnostic efficacy, with an AUC of 0.867 (sensitivity: 70.4 %, specificity: 89.5 %). As common clinical serological indicators, Lp-PLA2 and 25(OH)D are promising because of their low biological variability [18,41]. In addition to eGFR, traditional renal function indicators, including sCREA and albumin, are susceptible to changes in metabolic status and lack sensitivity [35]. Moreover, the detection of serum Lp-PLA2 and 25(OH)D levels is non-invasive, rapid, and stable, which can partly help avoid the risks associated with renal puncture.

Among the studies on DKD, our research investigated the correlation between Lp-PLA2 and 25(OH)D and the significance of their detection in early DKD for the first time. Additionally, it firstly analysed the role of the two biomarkers' combination in the diagnosis of DKD, and concluded that it had a higher diagnostic efficacy than a single one. Moreover, compared to other studies [4,42], the research subjects we included were based on the latest clinical practice, and the number of participants had further expanded (N = 422), making our results and conclusions more convincing. This study had a few limitations. First, considering the influence of COVID-19, many patients with T2DM could only accept non-specialist diabetes care in community hospitals, which may have had equal impacts on the NDKD, early DKD, and clinical DKD subgroups. Second, we did not obtain a complete list of the use of antihypertensive and lipid-lowering drugs, which depended on the medical history of each patient; therefore, they were not included in our analysis. Finally, this was a retrospective cross-sectional study. Well-designed prospective studies with larger sample sizes are warranted to explore the causal relationship between Lp-PLA2 and 25(OH)D and DKD.

5. Conclusion

Our findings suggest that serum Lp-PLA2 and 25(OH)D levels may play prominent roles in microvascular inflammation and endothelial function, which are some of the pathological mechanisms involved in the development of DKD in patients with T2DM. Furthermore, Lp-PLA2 and 25(OH)D levels are associated with the occurrence and development of DKD, with significant changes occurring in the early stages, suggesting the potential roles of these two biomarkers in the early detection and follow-up of DKD.

Ethics approval statement

The study obtained consent waiver and was approved by the Research Ethics Committee of the First Affiliated Hospital of Nanjing Medical University (approval number: 2022-SR-692).

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Data availability statement

The datasets are available from the corresponding author on reasonable request.

Conflict of interest disclosure

No authors declared any potential conflicts of interest.

CRediT authorship contribution statement

Zheng Zhang: Writing – original draft, Investigation, Formal analysis. **Xiang Qian:** Writing – original draft, Visualization. **Ziwei Sun:** Software, Methodology. **Chen Cheng:** Software, Methodology. **Min Gu:** Writing – review & editing, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e35635.

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