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Original article

LIPOPROTEIN-ASSOCIATED PHOSPHOLIPASE A2 (Lp-PLA2) AS A PREDICTOR OF END-STAGE RENAL DISEASE IN PATIENTS WITH DIABETIC NEPHROPATHY

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Abstract

Lipoprotein-associated phospholipase A2 (Lp-PLA2) is a specific biomarker for vascular inflammation. It is associated with microvascular complications such as diabetic nephropathy (DN) in patients with type 2 diabetes mellitus. To determine the predictive value of Lp-PLA2 concentration/activity for end-stage renal disease (ESRD) in patients with DN.

A total of 94 patients included in this cross-sectional study with DN were divided into four stages of chronic kidney disease (CKD) according to CKD-EPI: Stage II (n=20), Stage IIIa (n=29), Stage IIIb (n=38), and Stage IV (n=7). Forty-four healthy subjects were used as a control group. In addition to anamnestic data (age, gender, body weight, height, glycemic control), we measured the concentration of glucose, total cholesterol, triacylglycerols, blood urea, and creatinine in the blood serum using standard photometric methods. Lp-PLA2 concentration/activity was measured by chemiluminescence immunoassay-CLIA. Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) serum creatinine was used to determine the GFR and CKD stages.

We found significant differences among subgroups of patients with DN divided according to CKD stage and healthy subjects regarding age, body mass index, duration of disease, blood glucose, glycated hemoglobin, total cholesterol, triacylglycerols, blood urea, serum creatinine, GFR, and Lp-PLA2. A significant negative correlation was found between Lp-PLA2 and GFR. ROC analysis showed that Lp-PLA2 had a positive predictive value of 98.3% in patients with DN. Lp-PLA2 gradually increased in stages of DN.

Lp-PLA2 could be considered as a potential predictive biomarker for the progression of DN to ESRD.

Keywords: Lipoprotein-associated phospholipase A2 (Lp-PLA2), end-stage renal disease, diabetic nephropathy

Introduction

The prevalence of type 2 diabetes mellitus (T2DM), a chronic metabolic condition caused by an absolute or relative absence of insulin in the body, has rapidly risen in the past decade. T2DM leads to abnormalities in protein, lipid, and glucose metabolism[1]. One of diabetic patients'

most frequent microvascular complications and a significant cause of mortality is diabetic nephropathy (DN)[2]. A particular theory regarding the pathophysiology of DN is that inflammation plays an essential role[3]. DN generally starts with non-typical clinical symptoms that increase over time and are irreversible, ultimately leading to kidney failure. The most effective method for diagnosing DN is renal puncture biopsy. However, this invasive test does not satisfy clinical practice requirements. The most common biomarker for diagnosing DN is still microalbuminuria[4]. By regulating blood lipid metabolism, lipoprotein-associated phospholipase A2 (Lp-PLA2) leads to vascular inflammation. Consequently, an extensive investigation has been done to determine its significance in conditions associated with vascular inflammation.

Lysophosphatidylcholine (LPC) and free fatty acids (FFA), two lipid pro-inflammatory substances that contribute to the inflammatory response in the body, are produced by hydrolysis of phospholipid components of low-density lipoprotein (LDL) by lipoprotein-associated phospholipase A2 (Lp-PLA2), a member of the phospholipase superfamily [5]. Currently, a considerable amount of research has shown that Lp-PLA2 is a reliable predictor of cardiovascular events. Abnormal vasoactive and inflammatory molecules produced by Lp-PLA2 include oxidized FFA and LPC, which promote vascular wall lesions and damage. Lp-PLA2 significance in diabetic patients is still unknown, and its reliability as a cardiovascular risk marker in diabetes remains controversial despite its significant correlation with oxidative stress and cardiovascular events [6]. It is generally accepted that Lp-PLA2 is a crucial marker of primary vessel disease, but its association with microvascular disease has not been thoroughly investigated. It has been established that Lp-PLA2 is associated with a higher risk of diabetic retinopathy in people, while in animal models, Lp-PLA2 suppression effectively prevented retinal vascular permeability [7]. However, reliable data regarding Lp-PLA2 potential use as a biomarker of early microvascular renal change is currently lacking.

Considering the above, this study aimed to determine Lp-PLA2 predictive value for ESRD in patients with DN.

Materials and methods

The study included 94 T2DM and DN patients (50 males and 44 females) who had been admitted to the Department of Endocrinology, Diabetes and Metabolic Diseases, City General Hospital 8th September in Skopje, Republic of North Macedonia between January 2023 and December 2023. Meanwhile, 44 healthy subjects (22 men and 22 women) were randomly selected as the control group. Each subject signed a statement of informed consent. The Ethics Committee of the Faculty of Medicine in Skopje, Republic of North Macedonia, approved this study, which had been carried out according to the Declaration of Helsinki (No: 03-5602/11 from16.12.2022). The 2021 Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equationwas utilized to classify the patients into six CKD stages: ≥90(Stage I), 60-89 (Stage II), 45-59 (Stage IIIa), 30-44 (Stage IIIb), 15-29 (Stage IV), and < 15 (StageV) mL/min/1.73 m2. In our study, we have patients with DN categorized into four subgroups according to CKD stages based on the serum creatinine equation (CKD-EPI): Stage II (n = 20), Stage IIIa (n = 29), Stage IIIb (n = 38), and Stage IV (n = 7) [8]. Two control group subjects showed reduced GFR (Stage II), while the remaining forty-two healthy participants had normal GFR. Patients with T2DM and diagnosed DN between 40 and 70 met the inclusion criteria. T1DM,additional primary or secondary renal disorders, pregnant women, and lactation were the exclusioncriteria. Gender, age, glycemic control (HbA1c), and medical history were collected during admission or physical examination. Furthermore, height, weight, and body mass index (BMI) measurements were conducted. Venous blood samples were obtained

during a twelve-hour fastingto measure serum creatinine, blood urea, total cholesterol, triglycerides, blood glucose, and Lp- PLA2 concentration/activity. Blood glucose, total cholesterol, triglycerides, urea, and serum creatinine were all measured using the Roche completely automatic biochemical analyzer (Cobas 600, Roche, Basel, Switzerland). Using a fully automated analyzer, a chemiluminescence immunoassay kit measured the Snibe Maglumi 800 (Snibe Diagnostic, Shenzen New Industries Biomedical Engineering Co. Ltd., Shenzen, China) and Lp-PLA2. The reference value supplied by the manufacturer is less than 250 ng/ml (95^{th} percentile).

Statistical analyses

Statistical analysis was performed using the IBM SPSS Statistics for Windows, version XX (IBM Corp., Armonk, N.Y., USA) and MedCalc for Windows, version 15.0 (MedCalc Software, Ostend, Belgium). We performed statistical analysis using the Kolmogorov-Smirnov test, Mann-Whitney U test, one-way analysis of variance (ANOVA), Kruskal-Wallis test, non-parametric Kendall's tau-b (τb) correlation. Receiver Operating Characteristic (ROC) analysis was used to determine the diagnostic value of Lp-PLA2 in patients with DN. P-value < 0.05 was considered to be statistically significant.

Results

Comparison of clinical and laboratory data among subgroups divided according to CKD stage and healthy subjects and among all patients with DN and healthy subjects

Our results showed a significant difference among subgroups of patients with DN divided according to CKD stage and healthy subjects regarding all examined clinical and laboratory data: age, body mass index (BMI), duration of disease, blood glucose, glycated hemoglobin (HbA1c), total cholesterol, triacylglycerols, blood urea, serum creatinine, GFR, and Lp-PLA2. Table 1 illustrates the comparison of clinical and laboratory data among subgroups of patients with DN divided according to CKD stage and healthy subjects.

Table 1. Comparison of clinical and laboratory data among patients divided according to CKD stage and healthy subjects

	Stage II n=20	**Stage IIIa n=29**	**Stage IIIb n=38**	**Stage IV n=7**	**Healthy subjects n=44**	**p-value**
Age (years)	52.3±5.6	56.3±6.7	59.5±5.05	58.8±4.1	40.5±8.1	<0.05
BMI (kg/m^2)	28.4±4.2	29.1±6.6	29.5±5.1	32.4±5.04	22.3±2.4	<0.05
Duration of T2DM (years)	6.5±2.8	5.7±1.9	6.7±1.6	8.8±1.5	/	<0.05
Blood glucose (mmol/L)	6.4±0.6	7.5±0.9	7.5±1.2	7.8±0.5	4.7±0.4	<0.05
HbA1c (%)	6.3±1.2	6.1±1.3	6.7±1.6	8.8±1.5	/	<0.05
Total cholesterol (mmol/L)	6.8±0.9	6.9±0.9	7.01±0.9	7.5±1.1	4.9±0.6	<0.05
Triacylglycerols (mmol/L)	4.4±1.4	5.1±1.5	4.9±1.9	7.5±2.8	1.07±0.4	<0.05
Blood urea (mmol/L)	7.7±1.6	7.8±2.1	9.1±1.6	9.7±1.3	4.3±1.3	<0.05
Serum creatinine (μmol/L)	99.3±12.6	130.1±19.2	152.2±20.1	207.7±26.8	66.7±13.8	<0.05
GFR (ml/min per 1.73 m2)	67.2±7.7	51.3±4.6	39.3±3.7	26.6±2.4	132.6±22.3	<0.05
Lp-PLA2 (ng/ml)	228.7±56.4	280±61.6	290.2±87.6	505.4±80.6	147.6±47	<0.05

Results are presented as mean ±SD. Abbreviations: T2DM – type 2 diabetes mellitus, HbA1c - glycated hemoglobin, BMI - body mass index, GFR - Glomerular Filtration Rate, Lp-

PLA2 - Lipoprotein-associated phospholipase A2

A comparison of clinical and laboratory data between all patients with DN and healthy subjects showed significant differences regarding age, duration of disease, blood glucose, glycated hemoglobin (HbA1c), total cholesterol, triacylglycerols, blood urea, and Lp-PLA2. The results are shown in Table 2.

Table 2. Comparison of clinical and laboratory data between all patients with DN and healthy subjects

	Patients with DN n=84	**Healthy subjects n=44**	**p-value**
Age (years)	56.9±6.2	40.5±8.1	0.007
BMI (kg/m^2)	29.3±4.6	22.3±2.4	0.211
Duration of T2DM (years)	6.9±2.8	/	<0.001
Blood glucose (mmol/L)	7.3±1.1	4.7±0.4	0.003
HbA1c (%)	6.6±1.6	4.5±1.0	<0.001
Total cholesterol (mmol/L)	7.0±0.9	4.9±0.6	0.01
Triacylglycerols (mmol/L)	5.04±1.9	1.07±0.4	<0.001
Blood urea (mmol/L)	8.4±1.9	4.3±1.3	<0.001
Serum creatinine (μmol/L)	138.3±33.8	66.7±13.8	0.199
GFR (ml/min per 1.73 m2)	47.9±13.1	132.6±22.3	0.021
Lp-PLA2 (ng/ml)	290±98	147.6±47	<0.001

Results are presented as mean ±SD. Abbreviations: T2DM - type 2 diabetes mellitus, BMI - body mass index, GFR - Glomerular Filtration Rate, Lp-PLA2 - Lipoprotein-associated phospholipase A2.

Correlation between Lp-PLA2 levels and clinical and laboratory data of subjects

A nonparametric Kendall's tau-b (τb) correlation test was performed to determine the correlation coefficient between Lp-PLA2 levels and laboratory tests. There was a significant negative correlation between Lp-PLA2 and GFR (r=-0.307, p=<0.001) and a weak positive but statistically significant correlation between Lp-PLA2 and serum creatinine (r=0.204, p==<0.001). Furthermore, the same correlation test was used to calculate the correlation between Lp-PLA2 activity, other laboratory tests, and the patient's clinical data.

Table 3. Correlation between Lp-PLA2 activity and clinical and laboratory data of subjects

Clinical and laboratory data	**Lp-PLA2**	
	r	**p**
Age (years)	0.058	0.209
Duration of disease (years)	0.028	0.354
BMI (kg/m^2)	0.023	0.372
Glucose (mmol/L)	0.153	0.017
HbA1c (%)	0.065	0.197
Total cholesterol (mmol/L)	0.159	0.013
Triglycerides (mmol/L)	0.153	0.016
Blood urea (mmol/L)	0.110	0.063
Serum creatinine (μmol/L)	0.241	<0.001
GFR (ml/min per 1.73 m2)	-0.307	<0.001

BMI - body mass index, HbA1c - glycated hemoglobin A1c, GFR - Glomerular Filtration Rate

A weak positive correlation was found between Lp-PLA2 and blood glucose levels, total cholesterol, and triglycerides. There was no correlation between Lp-PLA2 and age, BMI, HbA1c, blood urea, and disease duration. The correlation coefficients between Lp-PLA2 activity and clinical and laboratory data of patients are presented in Table 3.

Assessment of diagnostic accuracy of Lp-PLA2 in patients with DN

Non-parametric ROC analysis was used to assess diagnostic accuracy of Lp-PLA2 in patients with DN. The manufacturer determined the optimal cut-off value for Lp-PLA2- < 250 ng/ml (95th percentile). The area under the ROC curve was 0.997, CI 95% [0.991-1.0] for Lp-PLA2. The diagnostic accuracy data for Lp-PLA2 in patients with DN are shown in Table 4 and Figure 1.

Table 4. Diagnostic accuracy data for Lp-PLA2 in patients with DN

Area under the ROC curve (AUC)	0.997
95% Confidence interval (95% CI)	0.991-1.0
Significance level p (Area=0.5)	<0.0001
Youden index J	0.983
Optimal cut-off	250 ng/ml
Sensitivity	62.7%
Specificity	97.7%
NPV - negative predictive value	55.1%
PPV - positive predictive value	98.3%
Diagnostic effectiveness (accuracy)	73.9%

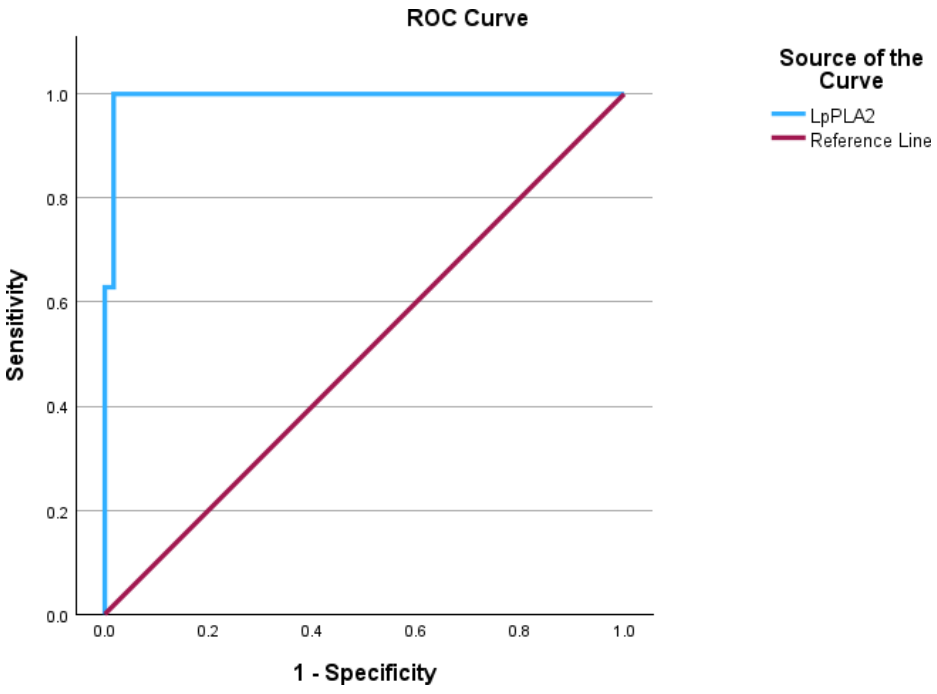

Fig. 1. Diagnostic accuracy data for Lp-PLA2 in patients with DN

Elevated Lp-PLA2 activity in patients with DN divided into subgroups according to CKD stage and healthy subjects

Lp-PLA2 was higher than the cut-off value in 35% of patients in Stage II, 62% in Stage IIIa, 68.4% in Stage IIIb, and 100% in Stage IV. Only one healthy subject had elevated Lp-PLA2 activity. Lp-PLA2 gradually increased in stages of DN. The results are shown in Figure 2.

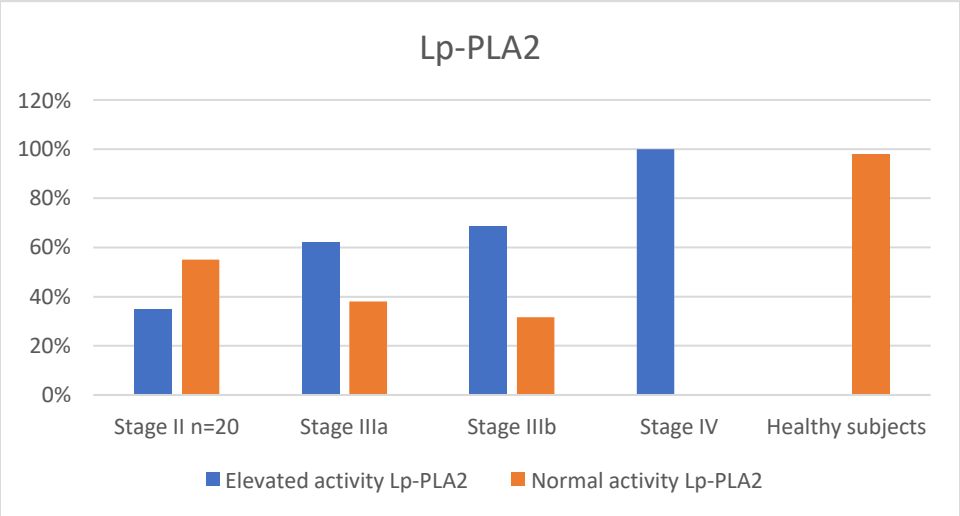

Fig. 2. Elevated Lp-PLA2 in patients with DN divided into subgroups according to CKD stage and healthy subjects

Discussion

The prevalence of DN in T2DM patients has been rising globally in recent years. One of the most frequent complication of type 2 diabetes is DN, which also plays a significant role in the development of CKD and ESRD[9]. Increased glomerular filtration, growing proteinuria, decreased GFR, and the eventual irreversible development of end-stage renal disease (ESRD) are among the pathological alterations in DN. Patient life expectancy and quality of life will decrease significantly as renal damage progresses[10]. Hence, early DN treatment and early diagnosis improve patients' long-term outcomes. Renal biopsy is less common in clinical practice, although being considered the gold standard for confirming kidney damage in diabetic patients. Clinical practice continues to depend on routine UACR and eGFR tests as indirect markers of DN diagnosis. The accepted theory holds that the primary causes of DN are inappropriate glucose and lipid metabolism caused by hyperglycemia and renal hemodynamic abnormalities induced by resistance to insulin. Glomerulosclerosis is triggered by high filtration, high-pressure state, thickening of the glomerular basement membrane, and matrix deposition. However, *in vitro* experiments and renal pathology have demonstrated recently that if inflammatory response occurs, then DN will develop in patients with T2DM[11]. Patients with T2DM suffer from severe insulin accumulation, impairing the body capacity to metabolize lipids and contributing to oxidative stress response. Hyperglycemia also significantly increases body glycosylation products, consequently triggering a cascade of inflammatory signaling in cells[12]. Various inflammatory mediators, such as adhesion factors, chemokines, and cytokines, possess distinct relationships with each other, suggesting that they play a role in the pathophysiology of DN. According to this research, inflammatory factors are now a new focus in investigating DN clinical diagnosis and therapy, as they can predict ESRD in individuals with DN[13]. About 70–80% of the inflammatory marker Lp-

PLA2 links to LDL and acts on phospholipids that have been oxidized, hydrolyzing glycerol phosphate to create LPC and FFA. In addition to increasing vascular endothelial permeability, FFA and LPC may serve as proinflammatory mediators by inducing a discharge of cytokines and adhesion molecules. Furthermore, Lp-PLA2 may induce endothelial death of cells and enhance the cell response to oxidative stress. A specific indicator of vascular inflammation is Lp-PLA2, and numerous studies have demonstrated a high correlation between Lp-PLA2 levels and the increased risk of cardiovascular events. Higher Lp-PLA2 activity was independently related to cardiovascular events in patients with stable coronary atherosclerotic heart disease who were not treated with Lp-PLA2 inhibitors, according to a meta-analysis encompassing 15 studies and more than 30,000 people[5]. Lp-PLA2 is also strongly associated with the onset and progression of DN[4].

This study found that Lp-PLA2 level in DN patients was significantly higher than in the control group. This suggests that Lp-PLA2 has the potential to serve as a predictor of DN progression. Lp-PLA2 levels in DN patients were negatively correlated with GFR (r=-0.307, p=<0.001). These results correspond to Zhai et al. findings[4]. Lp-PLA2 demonstrates excellent specificity and sensitivity for identifying DN, with a total diagnostic accuracy of 74% and PPV of 98.4%, according to diagnostic accuracy tests (ROC analyses). The activity of Lp-PLA2 gradually increased in the stages of DN. According to our results, Lp-PLA2 can be used as an ESRD predictor in patients with DN. Consequently, it should be considered a biomarker for prediction and follow-up of DN in patients with T2DM. Thus, DN progression may be reduced by using a Lp-PLA2 inhibitor. This is an interesting hypothesis that requires further investigation.

Conclusion

Lp-PLA2 can be clinicallyusefull biomarker in prediction of DN and monitoring of DN progression to ESRD.

Conflict of interest. None declared.

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