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Study of Lipoprotein-Associated Phospholipase A2 as a Potential Biomarker for Diabetic Kidney Disease in Type 2 Diabetes

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ABSTRACT

Background: A common consequence of diabetes is diabetic kidney disease (DKD). Increased Lp-PLA2 plasma levels have been related to higher risk of development of DKD in type 2 diabetes patients.

Objective: To illustrate the significance of using lipoprotein phospholipase A2 as potential early biomarker for detecting diabetic nephropathy among type 2 diabetic patients.

Patients and methods: A case-control study were conducted at the nephrology unit in partnership with Theodor Bilharz Research Institute and the Internal Medicine Department of Zagazig University Hospitals. This study was performed on (33) type 2 diabetic patients and were compared with 11 healthy subjects who were matched age, sex as a control group. Plasma LP-PLA2 was assessed among all subjects.

Results: It is more common in diabetic patients to have elevated plasma LP-PLA2 levels than in healthy people. LP-PLA2 levels were much higher in diabetic patients with microalbuminuria than in those with normoalbuminuria, and higher with macroalbuminuria than with microalbuminuria. Plasma LP-PLA2 levels were correlated to creatinine, urea and UACR reflecting that it is a marker of early nephropathy. The ROC curve for the validity of plasma LP-PLA2 in detecting kidney disease in type2 diabetic patients with micro and macroalbuminuria showed high specificity and sensitivity.

Conclusion: LP-PLA2 could be considered an early indicator of diabetic nephropathy in patients with type 2 diabetes.

Keywords: Diabetic Kidney Disease, Lipoprotein-associated Phospholipase A2, Type 2 Diabetes

INTRODUCTION

One of the most important health issues of our day is type 2 diabetes mellitus (T2DM), which is anticipated to climb by more than half by 2045 on a global scale **(1).** Type 2 diabetes mellitus (T2DM) is one of the most common types of metabolic disease and is caused by a combination of two basic factors: decreased insulin secretion by pancreatic beta cells and impaired insulin responsiveness in insulin-sensitive organs **(2).**

If left unchecked, diabetic kidney damage can lead to chronic renal failure as one of the most common adverse consequences of diabetes poor management **(3).** Patients with diabetic nephropathy who have previously had glomerular damage may display albumin levels that are within the normal range when they first occur, even though microalbuminuria has long been recognized as an early sign of diabetic nephropathy, as a result, improved biomarkers for early diabetic nephropathy have become urgently important **(4).**

Inflammation of the artery wall is indicated by elevated levels of the proinflammatory enzyme lipoprotein-associated phospholipase A2, which has been linked to atherosclerosis independently **(5).** Endothelial dysfunction and plaque inflammation are linked to the hydrolysis of oxidized low-density lipoproteins by Lp-PLA2, which produces proinflammatory molecules from them **(6).** Previous investigations on diabetic retinopathy patients and animals reported higher levels of Lp-PLA2 than expected **(7).**

Diabetes microvascular problems such as diabetic retinopathy and diabetic keratoconus share a common pathological foundation and frequently occur in the same people **(8).**

DKD is more common in patients with type 2 diabetes (T2D) who have raised Lp-PLA2 levels. Because of this, Lp-PLA2 should be taken into account as a biomarker for early diagnosis **(9).** This study aimed to illustrate the significance of using lipoprotein phospholipase A2 as potential early biomarker for detecting diabetic nephropathy among type 2 diabetic patients.

PATIENTS AND METHODS

Patients with type 2 diabetes in Zagazig University Hospitals and Theodor Bilharz Research Institute participated in our case control study from April 2020 to August 2021 in the Nephrology Unit of the Internal Medicine Department. The enrolled number of subjects was 44 participants. The inclusion criteria included patients with type 2 diabetes with their age range from 30 to 70 years old and selected volunteers reflected as healthy control groups.

Ethical consent:

Research Ethics Council at Zagazig University approved the study (ZU-IRB#7692) as long as all participants provided informed consent forms. Ethics guidelines for human experimentation were adhered to by the World Medical Association's Helsinki Declaration.

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Inclusion Criteria: 30 to 70 years old patients with type 2 diabetes mellitus of duration ≥ 5 years. Males and females. Type 2 diabetes mellitus with normoalbuminuria, microalbuminuria and macroalbuminuria. Patient written consent to share in the study.

Exclusion criteria: Acute metabolic disturbance of diabetes mellitus including ketoacidosis, hyperglycemia, hyperosmolar status. Current infection and inflammation, End stage renal disease patients or hemodialysis patients. Autoimmune diseases. Malignancy. Use of systemic steroidal anti-inflammatory drugs. Cerebrovascular accidents or cardiovascular diseases.

Based on the presence of Diabetes and the Urinary Albumin Creatinine Ratio (UACR), participants were divided into four groups: Control group: apparently healthy subjects, reflected as (Group I), cases with diabetes with normoalbuminuria (Urinary albumin / creatinine < 30 mg/g), as (group II), cases with diabetes with microalbuminuria (Urinary albumin / creatinine = 30 - 300 mg/g), as (group III), cases with diabetes with macroalbuminuria (Urinary albumin / creatinine > 300 mg/g), as (group IV). All patients were submitted to a comprehensive clinical examination and history taking.

Lab investigations: Fasting laboratory investigation were done for fasting as well as 2 hours post prandial blood sugar, HbA1c, CBC, ESR and CRP, lipid profile and uric acid, renal function tests (urea-creatinine-estimated GFR), urinary albumin/creatinine ratio, ECG, echocardiography**.** Microscopic albuminuria has been linked to the early identification of diabetic kidney disease and was defined as the excretion of 30-300 mg of albumin in 24 hours on 2-3 collections of urine or the commonly used 30-300 (g albumin/mg creatinine) spot urine collection. Three months later, all those who had tested positive were reexamined to make sure the diagnosis was correct and to rule out any possibility of temporary albuminuria.

Serum LP-PLA2 (The results were obtained by an ELISA kit commercially available from Shanghai Sunred Biological Technology Co., Ltd., China).

Statistical analysis

In order to analyze the data acquired, Statistical Package for the Social Sciences version 20 was used to execute it on a computer (SPSS). In order to convey the findings, tables and graphs were employed. The quantitative data were presented in the form of the mean, median, standard deviation, and interquartile range. The qualitative data were presented as frequency and percentage. Pearson Chi-Square was used to assess qualitatively independent data. The significance of a P value of 0.05 or less was determined.

RESULTS

Gender, age, BMI as well as smoking did not differ significantly among groups. The difference between the control group and all of the normoalbuminuric, micro and macroalbuminuria groups was significant when compared using Tukey HSD. When it comes to comorbid hypertension, no comorbidity hypertension was seen in the control group (Table 1).

Table (1): Demographics of the studied subjects

Parameters	**Groups**				**Tests**	
	Group I	**Group II**	**Group III**	**Group IV**	**F/χ^2**	**p**
	N=11 (%)	**N=11 (%)**	**N=11 (%)**	**N=11 (%)**		
Gender:						
Female	7 (63.6%)	6 (54.5%)	3 (27.3%)	3 (27.3%)	0.494	0.482
Male	4 (36.4%)	5 (45.5%)	8 (72.7%)	8 (72.7%)		
Age (year)						
Mean ± SD	52.82 ± 3.71	58.18 ± 7.03	54.45 ± 9.91	60.64 ± 7.87	2.537	0.07
BMI (kg/m^2) Mean ± SD	21.55 ± 1.86	25.18 ± 2.04	26.18 ± 3.79	27.18 ± 2.86	8.182	<0.001**
Tukey	P_4 0.333	P_3 0.018*	P_2 0.828	P_1 0.828	P_5<0.001**	$P_6$0.002*
Smoking:						
No	11 (100%)	8 (72.7%)	5 (45.5%)	9 (81.8%)	MC	0.068
Yes	0 (0%)	3 (27.3%)	6 (54.5%)	2 (18.8%)		
Hypertension:						
No	11 (100%)	2 (18.2%)	3 (27.3%)	2 (18.2%)	17.818	<0.001**
Yes	0 (0%)	9 (81.8%)	8 (72.7%)	9 (81.8%)		

*: Significant, **: Highly significant, BMI; body mass index

Serum creatinine, urea levels as well as eGFR differed significantly between the groups. The macroalbuminuric group differed significantly from the other groups on using Tukey HSD analysis, as did the microalbuminuric group. The difference between the control group and the normoalbuminuric group was insignificant. While the differences between macroalbuminuric and the other groups were significant, the differences between microalbuminuric and the other groups were also remarkable. Study groups differed significantly when it comes to UACR. Control and normoalbuminuric groups did not vary statistically. While the difference between macroalbuminurics and the other groups was large, the difference between microalbuminurics and the other groups was also significant (Table 2).

Table (2): Renal function tests among studied groups

Parameter	**Groups**				**Test**	
	Group I	**Group II**	**Group III**	**Group IV**	**F/χ^2**	**p**
	N=11 (%)	**N=11 (%)**	**N=11 (%)**	**N=11 (%)**		
Creatinine(mg/dL) Mean ± SD	0.74 ± 0.09	0.78 ± 0.1	0.97 ± 0.2	1.92± 0.26	36.38	0.059
Tukey	P_4 <0.001**	P_3 0.985	P_2 0.459	P_1 <0.001**	P_5<0.001**	P_6<0.001**
Urea (mg/dl) Mean ± SD	41.09 ± 4.95	44.27 ± 5.95	74.55 ± 16.2	117.55 ± 8.45	24.402	<0.001**
Tukey	P_4 <0.001**	P_3 0.989	P_2 0.024*	P_1 <0.001**	P_5<0.001**	$P_6$0.011*
UACR Mean ± SD	23.45 ± 5.34	25.09 ± 2.91	115.45 ± 44.85	885.64 ± 195.45	KW 36.416	<0.001**
Pairwise	P4 <0.001**	P3 0.778	P2 0.004*	P10.045*	P5<0.001**	P60.002*
eGFR Mean ± SD	118.85 ± 8.35	109.08 ± 8.35	99.81 ± 9.82	59.26 ± 10.45	76.811	<0.001**
Tukey	P_4 <0.001**	P_3 0.112	P_2 ,0.142	P_1 <0.001**	P_5<0.001**	P_6<0.001**

*: Significant, **: Highly significant, UACR; urinary albumin-creatinine ratio eGFR; estimated glomerular filtration rate

Total, HDL and serum triglyceride levels were significantly different amongst the study groups. Both the micro- and macroalbuminuric groups differed significantly from the control in a Tukey HSD comparison. In terms of LDL cholesterol, there was no statistically significant difference between the two groups that were evaluated. Normo and macro and micro albuminuric groups differed significantly from the control group, as did normo from the macro and micro albuminuric groups (Table 3).

Table (3): lipid profile among studied groups

Parameter	**Groups**				**Test**	
	Group I	**Group II**	**Group III**	**Group IV**	**F/χ^2**	**p**
	N=11 (%)	**N=11 (%)**	**N=11 (%)**	**N=11 (%)**		
Cholesterol (mg/dl) Mean ± SD	102.55 ± 7.85	141.55 ± 30.53	158.73 ± 27.5	173.73 ± 6.86	7.268	<0.001**
Tukey	P_4 0.205	P_3 0.089	P_2 0.711	P_1 0.788	P_5 0.007*	P_6 0.006*
HDL(mg/dl) Mean ± SD	49.0 ± 5.68	41.69 ± 7.31	35.0 ± 7.48	38.91 ± 9.36	6.696	<0.001**
Tukey	P_4 0.833	P_3 0.12	P_2 0.185	P_1 0.624	P_5 0.017*	P_6 <0.001**
LDL(mg/dl) Mean ± SD	78.18 ± 6.26	81.18 ± 14.59	92.82 ± 14.59	90.27 ± 21.04	1.517	0.225
Triglycerides Mean ± SD	77.73 ± 10.56	112.55 ± 23.87	171.82 ± 40.13	191.45 ± 46.41	26.226	<0.001**
Tukey	P_4 <0.001**	P_3 0.093	P_2 0.482	P_1 0.535	P5<0.001**	P_6 <0.001**

*: Significant, **: Highly significant, HDL; high density lipoprotein LDL; low density lipoprotein

Lipoprotein-associated phospholipase A2 differed significantly between the tested groups (Table 4).

Table (4): Lipoprotein-associated phospholipase A2 among studied groups

Parameter	Groups				Test	
	Group I	Group II	Group III	Group IV	F	p
	N=11 (%)	N=11 (%)	N=11 (%)	N=11 (%)		
LP-PLA1 (ng/ml) Mean ± SD	5.09 ± 1.76	8.36 ± 1.01	18.36 ± 4.06	33.91 ± 7.52	56.601	<0.001**
Tukey	P_4 0.01*	P_3 0.041*	P_2 <0.001**	P_1 <0.001**	P_5 <0.001**	P_6 0.045*

*: Significant, **: Highly significant, LP-PLA1; lipoprotein-associated Phospholipase A2

The best cutoff of lipoprotein-associated phospholipase A2 in diagnosis of macroalbuminuria among the studied patients was ≥20.5 ng/ml with area under curve 0.977, sensitivity 90.9%, specificity 93.9%, positive predictive value (PPV) 83.3%, negative predictive value (NPV) 96.9% and accuracy 93.2% (Figure 1).

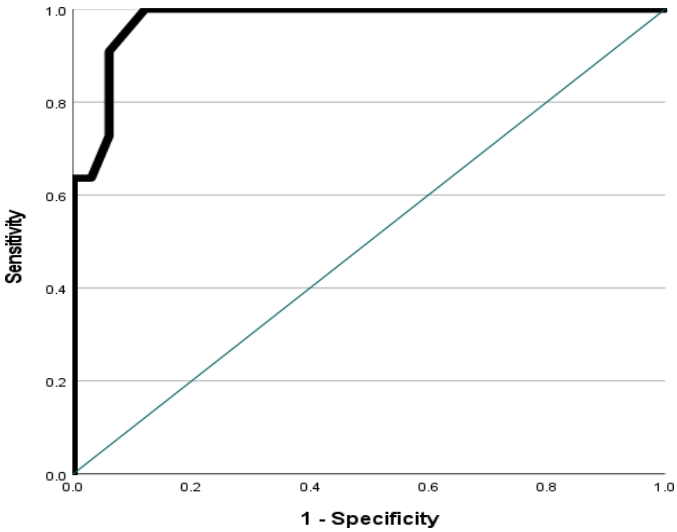

Figure (1): The ROC curve for lipoprotein-associated phospholipase A2 in the diagnosis of macroalbuminuria among the examined patients is shown in this graph.

Lipoprotein-associated phospholipase A2's best cutoff in diagnosing microalbuminuria was found in the patients investigated ≥14.5 to <20.5 ng/ml, with area under curve 0.996, accuracy 97%, positive predictive value (PPV) 91.7%, specificity 95.5%, sensitivity 100%, negative predictive value (NPV) 100% (Figure 2).

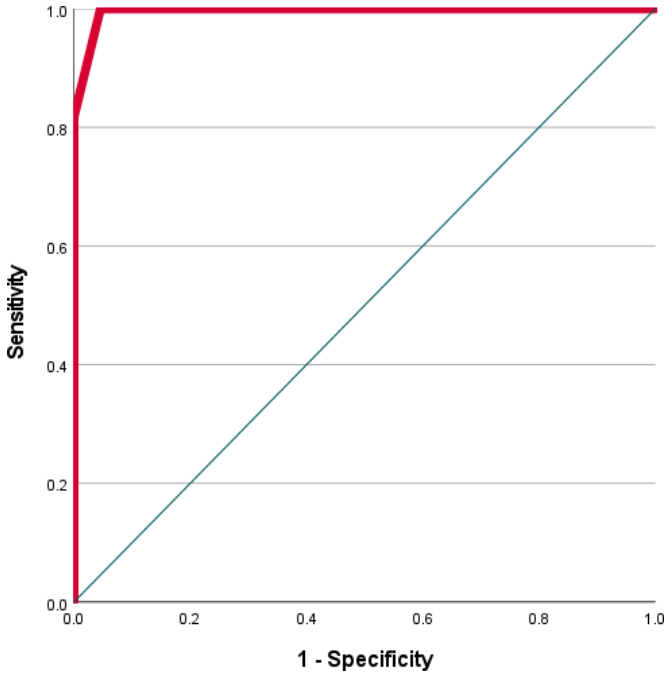

Figure (2): Diagnostic performance of lipoprotein-associated phospholipase A2 in individuals with microalbuminuria, as shown by the ROC curve.

Phospholipase A2 was found to be the best diagnostic marker for normoalbuminuria in the patients investigated with best cutoff ≥8.5 to <14.5 ng/ml, with area under curve 0.975, sensitivity 90.9%, positive predictive value (PPV) 90.9%, negative predictive value (NPV) 90.9%, accuracy 90.9%, and specificity 90.9% (Figure 3)

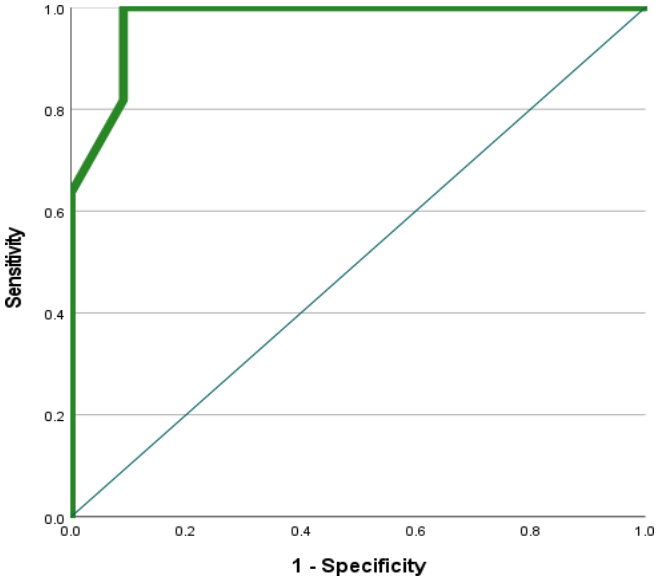

Figure (3): Lipophospholipase A2's diagnostic performance in patients with normoalbuminuria is seen in the ROC curve.

There was statistically significant positive correlation between lipoprotein-associated phospholipase A2 and systolic blood pressure, fasting blood sugar, HbA1c, serum creatinine, urea, UACR, and serum triglycerides. There was statistically significant negative correlation between LP- PLA2) and eGFR. Lipoprotein-associated phospholipase A2 has a statistically non-significant negative connection with other metrics (Table 5).

Table (5): Correlation between lipoprotein-associated phospholipase A2 and studied parameters

Parameter	Lipoprotein-associated phospholipase A2	
	r	p
Age (year)	0.19	0.216
BMI (kg/m^2)	0.028	0.857
SBP (mmHg)	0.315	0.038*
DBP (mmHg)	0.265	0.082
FBS (mg/dL)	0.489	<0.001**
HbA1c (%)	0.553	<0.001**
Serum creatinine (mg/dL)	0.771	<0.001**
Serum urea (mg/dL)	0.696	<0.001**
UACR	0.683	<0.001**
eGFR (ml/min/1.73 m^2)	-0.75	<0.001**
Total cholesterol (mg/dL)	0.28	0.065
HDL cholesterol (mg/dL)	-0.172	0.265
LDL cholesterol (mg/dL)	0.14	0.364
Serum triglycerides (mg/dL)	0.333	0.027*

*: Significant, **: Highly significant

DISCUSSION

Overt DKD in diabetics with persistent microalbuminuria is more likely to emerge 15 years from the commencement of the disease on average, as proteinuria (albuminuria >300 mg/24 hours/day) increases over time, as does blood pressure (BP), and the progress of chronic kidney disease (CKD) [10].

Patients and rats with diabetic retinopathy have higher levels of Lp-PLA2, according to previous research [7]. New research shows that plasma Lp-PLA2 levels are much greater in individuals who have DKD, and this higher Lp-PLA2 level is linked to an increased chance of DKD in those with type 2 diabetes [9].

In our study, we found no statistically significant difference among different groups as regards most of clinical characteristics, such as, age, sex, smoking and BMI. But the difference was significant between control group, and all of normoalbuminuric, micro and macroalbuminuria group regarding BMI. Statistically significant differences were found between the research groups and the non-hypertensive control group. This is matched with **Wang *et al.*** [11] who reported that the role of hypertension in the development of persistent microalbuminuria supports the idea that glomerular hypertension is vital in the onset and progression of diabetic kidney disease. Each of the examined groups had a statistically significant difference in blood pressure between the macroalbuminuric and non-macroalbuminuric groups, respectively.

As regard urinary albumin to creatinine ratio (UACR), our study showed that the difference was non-significant between control group and normoalbuminuric groups. According to **Wang *et al.*** [11], there is a considerable difference between macroalbuminuric and microalbuminuric. Our research on kidney function tests found a statistically significant difference in serum creatinine levels between the groups we examined. There was a significant difference between the macro-albuminuric and microalbuminuric groups, as well as between the two. When it comes to estimating GFR, the difference between macroalbuminurics and the other groups was statistically significant. According to the research, this is in agreement with recent study [9].

In terms of serum urea, statistically significant difference existed between the groups tested. The differences between macroalbuminuric and microalbuminuric patients are enormous. That being said, there isn't much of a difference between the normal albuminuric group and the control group.

Dyslipidemia linked with diabetes and insulin resistance is known as diabetic dyslipidemia because of the traditional triad of high triglyceride, low HDL and small dense LDL [12].

In our study we found that LP-PLA2 was starting to grow early in diabetic patients with normoalbuminuria and gradual increase with the progression of diabetic nephropathy. Findings were

consistent with those of **Hu *et al.* [9],** who found that elevated Lp-PLA2 levels in T2D patients were related with an increased incidence and development of DKD. This suggests an inflammatory reaction mechanism. A biomarker for DKD identification and follow-up should therefore be Lp-PLA2.

Increased Lp-PLA2 concentrations in people with DKD are related with an increased risk of developing DKD in those who have type 2 diabetes **[9].** To start DKD, a dysregulated metabolic environment must be present (including high blood sugar, high cholesterol, elevated lipid levels, and insulin resistance) **[13].**

Our study demonstrated that lipoprotein-associated phospholipase A2 has a positive link with systolic blood pressure, fasting blood sugar, HbA1c, serum creatinine, urea, UACR, and serum triglycerides; this correlation is statistically significant. This is consistent with the study **Hu *et al.*[9]** where it was shown that patients with DKD had more co-morbid conditions including diabetes, such as higher HbA1c, higher blood pressure, higher uric acid levels, as well as higher TG levels than individuals without DKD who had high Lp-PLA2 levels.

In diabetic nephropathy patients, eGFR, creatinine, and BUN are considered the traditional biomarkers reflecting changes in renal function. In practice, eGFR was the best overall kidney function parameter, and BUN and creatinine were conventional biomarkers representing changes in renal function in CKD and DN patients [14].

In our study we found that there was statistically significant negative correlation between lipoprotein-associated phospholipase A2 and eGFR. These findings suggested that serum LP-PLA2 levels in diabetic nephropathy patients were strongly related to renal function. Furthermore, clinical indicators of diabetic nephropathy such as urine albumin creatinine ratio (UACR) and serum creatinine were positively correlated with serum LP-PLA2, whereas eGFR was negatively correlated with serum LPLA2. This is consistent with other study, which has found that LP-PLA2 is positively correlated with creatinine and BUN, and negatively correlated with eGFR **[9].**

In our study, according to our findings, patients with DN who were either macro or microalbuminuric had significantly higher blood lp-pla2 levels than either the normoalbuminuric or control groups. The study's findings that LP-PLA2 is linked to diabetic kidney damage are thus confirmed [9] in this study, which reported that DKD and Lp-PLA2 were found to be closely linked.

CONCLUSION

According to our findings of increased plasma levels of Lp-PLA2. T2D patients' DKD progression was linked to it. Hyperglycemia-induced inflammation and LP-PLA2 have been linked to atherosclerosis and endothelial dysfunction, suggesting that inflammatory mechanisms may be to blame for DKD.

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Author contribution: Authors contributed equally in the study.

REFERENCES

1. **Perreault L, Skyler J, Rosenstock J (2021):** Novel therapies with precision mechanisms for type 2 diabetes mellitus. Nature reviews. Endocrinology, 17(6): 364–377.
2. **Galicia-Garcia U, Benito-Vicente A, Jebari S *et al.* (2020):** Pathophysiology of type 2 diabetes mellitus. International Journal of Molecular Sciences, 21(17): 6275-79.
3. **Tuttle K, Bakris G, Bilous R *et al.* (2014)**: Diabetic kidney disease: a report from an ADA Consensus Conference. Diabetes Care, 37(10):2864–83.
4. **Caramori M, Kim Y, Huang C *et al.* (2002):** Cellular basis of diabetic nephropathy: 1. Study design and renal structural-functional relationships in patients with long-standing type 1 diabetes. Diabetes, 51(2): 506–513.
5. **Zalewski A, Macphee C, Nelson J (2005):** Lipoprotein- associated phospholipase A2: a potential therapeutic target for atherosclerosis. Curr Drug Targets Cardiovasc Haematol Disord., 5(6):527–32.
6. **Karabina S, Liapikos T, Grekas G *et al.* (1994):** Distribution of PAF-acetylhydrolase activity in human plasma low-density subfractions. Biochim Biophysics Acta., 1213:34–38.
7. **Gong Y, Jin X, Wang Q *et al.* (2014):** The involvement of high mobility group 1 cytokine and phospholipases A2 in diabetic retinopathy. Lipids Health Dis., 13:156-69.
8. **Romero-Aroca P, Mendez-Marin I, Baget-Bernaldiz M *et al.* (2010):** Review of the relationship between renal and retinal microangiopathy in diabetes mellitus patients. Curr Diabetes Rev., 6(2):88– 101.
9. **Hu Y, Li T, Zhou W *et al.* (2019):** Lipoprotein-associated phospholipase A2 is a risk factor for diabetic kidney disease. Diabetes Research and Clinical Practice, 150: 194–201.
10. **Krolewski A (2015):** Progressive renal decline: The new paradigm of diabetic nephropathy in type 1 diabetes. Diabetes Care, 38 (6): 954–62.
11. **Wang S, Wang y, Zheng R *et al.* (2015):** Osteoinductive factor is a novel biomarker for the diagnosis of early diabetic nephropathy. International Journal of Clinical and Experimental Pathology, 8(3): 3110-15.
12. **Ormazabal V, Nair S, Elfeky O *et al.* (2018):** Association between insulin resistance and the development of cardiovascular disease. Cardiovascular Diabetology, 17(1): 1–14.
13. **Reidy K, Kang H, Hostetter T *et al.* (2014):** Molecular mechanisms of diabetic kidney disease. The Journal of Clinical Investigation, 124(6): 2333–2340.
14. **Coresh J, Turin T, Matsushita K *et al.* (2014):** Decline in estimated glomerular filtration rate and subsequent risk of end-stage renal disease and mortality. JAMA., 311: 2518– 2531.