Urinary Laminin an Early Marker of Renal Function Decline in Type 2 Diabetic Patients

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ABSTRACT

Diabetes mellitus is a chronic disease that affects millions of people worldwide. Diabetes results in both microvascular and macrovascular complications. Among the microvascular complications, diabetic kidney disease is one of the most serious, with significant impact on morbidity, mortality, and quality of life. Urinary laminin excretion is higher in diabetic patients compared to healthy controls, even before the development of microalbuminuria. determine the value of measurement of urinary laminin as an early marker for prediction of renal function impairment in patients with type-2 diabetes. This is a case-controlled study conducted on 60 patients (40 diabetic patients, 20 non-diabetic CKD patients) admitted to the Department of Internal Medicine, Menoufia University Hospitals. 20 healthy individuals were also included as a control group during the period of study. Informed consent was obtained from all participants who were be fully informed about the study according to ethical medical committee of Menoufia University Hospitals. Routine investigations were done then measurement of urinary laminin level by ELISA as a specific investigation. The urinary laminin was significantly higher in patients with proteinuria (both diabetic and non-diabetic) than control group and diabetics without proteinuria. It showed higher specificity with proteinuria in diabetic patients (80%) and non-diabetic CKD patients (100%) than in diabetic patients without proteinuria (70%). we concluded that Urinary laminin could be used as an early indicator for the presence of nephropathy in type 2-diabetic patients even before the appearance of proteinuria and instead of Microalbuminuria.

1. INTRODUCTION

Diabetes Mellitus (DM) is a growing global health problem. In 2000, diabetes affected an estimated 171 million people worldwide; in 2011 it increased to more than 366 million and numbers are expected to exceed 552 million by 2030 [1]. DM is a metabolic disease of multiple etiologies, characterized by

hyperglycemia resulting from defects in insulin secretion, insulin action or both, and associated with abnormal carbohydrate, fat, and protein metabolism [2] People with diabetes require at least two to three times the health-care resources compared to people who do not have diabetes, and diabetes care may account for up to 15% of national health care budgets [3], [4]. Diabetes results in both microvascular and macrovascular complications. Among the microvascular complications, diabetic kidney disease is one of the most serious, with significant impact on morbidity, mortality, and quality of life [5]. Diabetic nephropathy (DN) is defined as albuminuria (albumin excretion rate > 300 mg/24 h) and declining renal function in a patient with known diabetes in the absence of urinary tract infection or any other renal disease. It is the leading cause of end stage renal disease in the Western world [6]. Laminin is a 900-kDa glycoprotein that is a normal component of basement membranes. It is considered that serum laminin cannot be filtered in the normal glomerulus, and the urinary laminin is derived from the kidneys [7], [8]. It has been shown by immunohistochemistry that laminin is located in the mesangial expansion and thickened capillary basement membranes characteristic of diabetic nephropathy [9]. As expected, urinary laminin excretion correlates with the urinary excretion of type IV collagen, the main glomerular basement membrane (GBM) constituent [10], [11]. Because laminin is also found in the tubular basement membrane, it could be expected to find a relationship between urinary excretion of laminin and markers of tubular injury (i.e., NAG, alfa 1 macroglobulin, beta 2 macroglobulin, and kappa light chains), but conflicting results have been published regarding this correlation [12]. Urinary laminin excretion is higher in diabetic patients compared to healthy controls, even before the development of microalbuminuria. However, there are conflicting results regarding the correlation of urinary laminin excretion with UAE [13].

2. Patients and Methods

2.1 Study design
This is a case-controlled study conducted on 60 patients (40 diabetic patients, 20 non-diabetic CKD patients) admitted to the outpatients and inpatients clinics of the Department of Internal Medicine, Menoufia University Hospitals. 20 healthy individuals were also included as a control group during the period of the study. Informed consent was obtained from all participants who were be fully informed about the study according to ethical medical committee of Menoufia University Hospital.

2.2 Inclusion criteria
1-   Patients diagnosed as type-2 diabetes with proteinuria, diabetes without proteinuria and non-diabetic chronic kidney disease patients with proteinuria.
2-   Healthy subjects as a control group.

2.3 Exclusion criteria
Patients with chronic liver disease, heart failure or history of heart diseases and urinary tract infections and Subject's refusal.
Subjects were divided into 4 groups: Group I: 20 Diabetic patients without proteinuria, Group II: 20 Diabetic patients with proteinuria, Group III: 20 Non-diabetic patients with a history of chronic kidney disease and proteinuria and Group IV: 20 Healthy individuals selected as a control group.

2.4 All subjects were subjected to
A.   Clinical history taking: Personal history and disease history and medications used for diabetes control or for chronic kidney disease.
B.   Full clinical examination
C.   Investigations, which include:

- Complete Urine analysis, Complete blood count, Blood glucose level (Fasting and post- prandial blood sugar), Glycated hemoglobin (Hb A1 C%), Serum levels of albumin, Lipid profile (cholesterol and triglycerides), Kidney function test (Urea and creatinine) and Estimated Glomerular filtration rate (eGFR) by MDRD.

MDRD equation: (ml/min/1.73 m2) Developed from data in the Modification of Diet in Renal Disease (MDRD) study.

GFR = 175 x standardized scr-1.154(mg/dl) x age-0.203(years) x 0.742 (if female) (National Kidney Foundation, 2002)

- Urinary albumin/creatinine ratio

- Abdominal and pelvic ultrasonography: To assess the kidney size, echogenicity, liver, spleen and presence or absence of ascites.

- Specific investigations: Urinary laminin level using ELISA.

2.5 Methods

Blood sampling: Six ml of blood were be collected from each patient as follows:-

- Two ml of blood collected in EDTA tube for Complete blood count: WBCs, RBCs, Platelets count, HB.

- Four ml of blood collected in sterile plane tube for:- Blood glucose level (Fasting and post- prandial blood sugar), Glycated hemoglobin (Hb A1 C%), Serum levels of albumin, Lipid profile (cholesterol and triglycerides) and Kidney function test (Urea and creatinine).

2.6 Urinary laminin: Principle of the assay

The microtiter plate provided in this kit has been pre-coated with an antibody specific to Laminin. Standards or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated antibody preparation specific for Laminin and Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. Then a TMB (3,3',5,5'tetramethyl-benzidine) substrate solution is added to each well. Only those wells that contain Laminin, biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm $\pm$ 2 nm. The concentration of Laminin in the samples is then determined by comparing the O.D. of the samples to the standard curve.

- Assay procedure: using Human Laminin(LN) ELISA Kit (GSCIENCE, USA).
- Step 1: Standard: Bring all reagents to room temperature.

Dilute the standard: Pipette 50 p1 standard dilution in each tube. Pipette 1000 standard (540 pg/ml) in the first tube. And take out 100µI from the first tube into the second. Pipette 50µI from the second tube to the third tube and produce dilution series. Repeat each of the concentration to get the mean value of each well. Pipette standard 50µI to testing standard well.

- Step 2: Prepare sample:

Set blank wells separately (blank comparison wells don't add sample and HRP-Conjugate reagent; other each step operation is same). Pipette Sample dilution 40p1 to testing sample well, then add testing sample 10111 (sample final dilution is 5-fold), Pipette sample to wells, do not touch the well wall as far as possible, and mix gently.

- Step 3: Incubate: Cover with the adhesive strip provided, incubate for 30 min at 37'C.
- Step 4: Configurate liquid: Dilute wash solution 30-fold (or 20-fold) with distilled water.
- Step 5: Washing: Uncover the adhesive strip, discard liquid, Pipette washing buffer to every well, still for 30s then drain, repeat 5 times.

- Step 6: Add enzyme: Pipette HRP-Conjugate reagent 50µI to each well, except blank well.
- Step 7: Incubate: Cover with the adhesive strip provided, incubate for 30 min at 37'C.
- Step 8: Washing: Uncover the adhesive strip, discard liquid, Pipette washing buffer to every well, still for 30s then drain, repeat 5 times.
- Step 9: Color: Pipette Chromtogen Solution A 50u1 and Chromogen Solution B to each well, avoid the light preservation for 15 min at 37°C.
- Step 10: Stop the reaction: Pipette Stop Solution 50µI to each well, Stop the reaction (the blue change to yellow).
- Step 11: Assay: take blank well as zero, Read absorbance at 450nm after pipetting Stop Solution within 15min.

- Judgment of assay result

Take the standard concentration as the horizontal, the OD value for the vertical ,draw the standard curve on graph paper, Find out the corresponding concentration according to the sample OD value by the Sample curve, multiplied by the dilution multiple, or calculate the straight line regression equation of the standard curve with the standard concentration and the OD value, with the sample OD value in the equation, calculate the sample concentration, multiplied by the dilution factor, the result is the sample actual concentration.

2.7 Assessment of albumin creatinine ratio

Untimed ('spot') urine samples can be used to detect and monitor proteinuria. ACR corrects for variations in urinary concentration (caused by changes in hydration) and correlates well with measurements obtained from timed collections.

A first morning urine specimen is preferable. Urinary excretion of creatinine generally remains constant (< 30 mg/g).

* Interpretation
. ACR: < 30 mg/g (normal)
. ACR: 30 – 300 mg/g (microalbuminuria)
. ACR: > 300 mg/g (macroalbuminuria) (National Kidney Foundation, 2002)

2.8 Statistical Analysis

- Categorical variables were summarized as n (%), and continuous variables were expressed as mean $\pm$ SD for normally distributed data or median with range for not normally distributed data (normally of data was tested by Kolmogorov-Smirnov), Independent T test was used for normally distributed variables, Mann-Whitney U test was used for not normally distributed continuous variables, and chi-square test ($\chi 2$) test or Fisher's exact test for categorical variables We excluded variables if the number of events was too small to estimate odds ratios. No imputation was made for missing data. Categorization was performed for continuous variables, as it is easier to interpret and also for the simplicity of reporting results. Mann-Whitney test (non-parametric test): is a test of significance used or comparison between two groups not normally distributed having quantitative variables.

- ANOVA (f) used for comparison between three or more groups having quantitative variables. Kruskal-Wallis test used for comparison between three or more groups not normally distributed having quantitative variables.

• Pearson correlation (r): is a test used to measure the association between two quantitative variables.

• The ROC (receiver operating characteristic) curves: This procedure used to evaluate the performance of classification schemes in which there is one variable of two categories by which subjects are classified. They were constructed by calculating the sensitivities and specificities of the variable. The cutoff value with the highest accuracy was selected as the diagnostic cutoff points. For common laboratory values, we used the cutoff points which were widely recognized and adopted in clinical practice. Results were collected, tabulated, statistically analyzed by IBM personal computer and statistical package SPSS version 22.0, with statistical significance set at 2-sided p < 0.05.

3. Results

Comparison of mean values of different Sociodemographic characteristics and clinical results of the studied patients and controls show a high significant difference between different groups of patients and controls regarding age, mean systolic and diastolic blood pressure values with P value (<0.001). (Table 1,2). Comparison of mean values of different laboratory characteristics of the studied patients and controls show a high significant difference between different groups regarding Mean Hb level, Mean Fasting blood glucose, HbA1c, 2HPP, Mean Serum albumin with P value (<0.001). (Table 3a).

Table 3b shows that mean Cholesterol Level in the studied group was in group I 182.90 mg/dl, group II 248.40 mg/dl, group III 229.25 mg/dl and control group 119.45 mg/dl and it was significant with P value (<0.001). Mean Triglycerides level in group I was 154.25 mg/dl, group II was 235.0 mg/dl, group III was 222.0 mg/dl and control group were 98.70 mg/dl and it was significant with P value (<0.001).

Mean blood urea was in group I 37.80 mg/dl, group II 66.85 mg/dl, group III 99.40 mg/dl and control group 32.20 mg/dl and it was significant with P value (<0.001).

Mean Serum creatinine was in group I 1.16 mg/dl, group II 2.12 mg/dl, group III 3.70 mg/dl and control group 0.65 mg/dl and it was significant with P value (<0.001).

Mean eGFR (MDRD) in group I was 76.25, group II was 40.15, group III was 22.79 and control group was 95.60 and it was significant with P value (<0.001).

Mean Urinary albumin creatinine ratio in group I was 21.75, group II was 199.0, group III was 550.50 and control group was 15.20 and it was significant with P value (<0.001).

Table 4 shows the mean urinary laminin level in the studied groups, in group I was 98.53, group II was 535.75, group III was 434.25 and control group was 54.35 with P value (<0.001). The table demonstrated that the urinary laminin is significantly increased in patients with proteinuria (both diabetic and non-diabetic) with much increase in diabetic patients with proteinuria.

Table 5 shows the Correlation between Ur. laminin and different parameters in each group, there was a Significant correlation between Hb. A1.C% and Ur. Laminin level in group I (P value 0.020), but insignificant correlation in group II P value 0.113, group III (P value 0.659) and in control group (P value 0.394).

It shows also Significant correlation between Serum albumin and laminin level in group I (P value 0.034) but insignificant correlation in group II (P value 0.995), group III (P value 0.536) and in control group (P

value 0.821).

However Insignificant correlation between Ur. Laminin level and HB, fasting blood glucose, post prandial blood glucose, serum albumin, cholesterol, triglycerides, blood urea, serum creatinine, eGFR and Urinary albumin creatinine ratio levels in the different studied groups.

Table 6 shows that in GI, Diabetic patients with +ve proteinuria, the Sensitivity of Laminin was 95.0. While Specificity was 80.0. The AUC for Laminin was 0.934. P value < 0.001. Youden index was 0.750. Cutoff was >375. PPV was 61.3, NPV was 98.0 for Laminin. However, it shows that AUC of Laminin is 1.000 with P value <0.001, Youden index1.000, Cutoff >135, Sensitivity100.0, Specificity100.0, PPV 100.0 and NPV100.0 in CKD with proteinuria and it shows that AUC of urinary Laminin was 0.883with P value <0.001, Youden index 0.700, Cutoff >70, Sensitivity100.0, Specificity 70.0, PPV 76.9 and NPV100.0 in DM, - ve proteinuria.

Figure (1) shows the ROC curve for urinary laminin to predict proteinuria in diabetic patients, Figure (2) shows ROC curve for urinary laminin in CKD, with proteinuria, it shows that the Specificity and Sensitivity of urinary laminin in CKD with proteinuria was 100%. Figure (3) shows ROC curve for urinary laminin in DM without proteinuria.

4. Discussion

The primary microvascular complications of diabetes include damage kidneys known as diabetic nephropathy (DN), is the most common complication of diabetes [13]. Diabetic nephropathy is the leading cause of kidney disease in patients starting renal replacement therapy and affects 40% of type 1 and type 2 diabetic patients [14]. Laminin is a 900-kDa glycoprotein that is a normal component of basement membranes. It is considered that serum laminin cannot be filtered in the normal glomerulus, and the urinary laminin is derived from the kidneys [8]. It has been shown by immunohistochemistry that laminin located in the mesangial expansion and thickened capillary basement membranes characteristic of diabetic nephropathy [8]. Urinary laminin excretion is higher in diabetic patients compared to healthy controls, even before the development of microalbuminuria. However, there are conflicting results regarding the correlation of urinary laminin excretion with UAE [12]. Thus, this study was conducted to determine the values of measurement of urinary laminin as a marker for early diagnosis of diabetic nephropathy in patients with type II diabetes. In the present study, urine albumin/creatinine ratio was significantly higher in proteinuria patients either having diabetes or not, but the CKD non-diabetic patients were higher than diabetic proteinuria patients followed by diabetic group in comparison with controls. In accordance, urine albumin/creatinine ratio was increased in diabetic patients as compared to controls, and it was highly significant (p<0.01) [15]. Also, in a study by [16]., 95 patients with type 2 diabetes found an equally high correlation between 24-hour urine albumin excretion and the albumin/creatinine ratio in the first morning urine specially in case of nephropathy.

Our study demonstrated that the urinary laminin is significantly higher in patients with proteinuria (both diabetic and non-diabetic) than control group and diabetics without proteinuria. Also, the urinary laminin showed more specificity with proteinuria in diabetic patients (80%) and non-diabetic CKD patients (100%) than diabetic patients with no proteinuria (70%), this could suggest that urinary laminin can be a specific indicator for nephropathy in diabetic and non-diabetic proteinuric patients. Moreover, it can be used to differentiate between diabetic proteinuric patients from non-proteinuric diabetic patients. Thus, urinary laminin can be an early indicator for nephropathy in type-2 diabetic patients even before onset of albuminuria. In accordance, urinary laminin excretion was found to be higher in diabetic patients when

compared with healthy controls before the onset of microalbuminuria [17]. Urinary L-P1 was similarly found to be higher in both IDDM and NIDDM patients when compared with nondiabetics, even in the absence of nephropathy but significantly higher in case of nephropathy [18].

Furthermore, laminin excretion was found to be increased with increasing grade of diabetic nephropathy, as well as when compared to patients with nondiabetic renal disease who had a similar degree of urinary albumin excretion as the urinary laminin excretion was higher in nondiabetic chronic nephropathy compared to controls. Also, type 2 diabetic patients with evidence of nephropathy had significantly higher laminin/albumin ratio compared to patients with nondiabetic nephropathy, suggesting that urinary laminin excretion could help differentiate diabetic versus nondiabetic nephropathy [10]. Moreover, a significantly higher urinary laminin-to-albumin ratio was seen in type 2 diabetic patients with evidence of nephropathy compared with subjects with nephropathy of nondiabetic origin, which suggested that this marker may be more specific for DN than for other kidney diseases [19]. In another agreement with our results, found that urinary LN in diabetic nephropathy was significantly higher than that in diabetes without nephrosis group (P<0.05) and control group (P<0.01), and higher in diabetics than non-diabetic group thus it may be used as an important indicator in the diagnosis of the early diabetic nephropathy [20]. These findings, however, conflicted with an earlier study by Nakajima et al. who were not able to demonstrate any specification between diabetic patients (IDDM or NIDDM) with and without proteinuria [21].

Another study was also in contrast with our results found that laminin P1 concentrations in type I diabetic patients without nephropathy or with microalbuminuria were not significantly different from those of control subjects [22]. This study showed a significant correlation between urinary laminin and albumin level in diabetic patients without proteinuria while there was no significant correlation between urinary laminin and albumin level in proteinuria patients. In accordance urinary laminin was significantly correlated with urinary albumin in diabetic patients [10]. Also, there were significant correlations between urinary laminin P1 levels and urinary albumin levels in children with diabetes [17]. In contrast, no correlation was found between urinary laminin and urinary albumin in a group of 30 diabetic patients [23]. We also found a significant correlation between Hb. A1.C% and laminin level in diabetic patients in group 1 (P value 0.020), but insignificant correlation in group II P value 0.113, group III (P value 0.659) and in control group (P value 0.394). In consistence with these results, a significant correlation was also shown between urinary laminin P1 and HbA1c concentrations (p < 0.01) in diabetic patients [16]. During the study, insignificant correlation between eGFR and laminin level in all studied groups of patients was found. This was in contrast with another study conducted to observe the relationship of serum and urinary laminin and glomerular filtration rate (GFR) in diabetes mellitus where they found that the level of serum and urinary LN had significantly negative correlation with GFR (P<0.01) suggesting that LN may accelerate the alteration of GFR and causes microangiopathy of diabetic nephropathy [23].

5. Conclusion and Recommendation
Urinary laminin was significantly higher in diabetic patients with proteinuria in comparison to diabetic patients without proteinuria. Thus, urinary laminin could be used as an early indicator for the presence of nephropathy in type 2-diabetic patients before the appearance of proteinuria and instead of Microalbuminuria. Long term follow-up studies in large number of patients to evaluate the diagnostic relevance of urinary laminin as compared for other tubular markers for future predictive value of clinical diabetic nephropathy.

6. Compliance with Ethical Standards
All procedures followed were in accordance with the ethical standards of the responsible committee on

human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008.

Institutional Review Board Statement: The Institutional Review Board (IRB) of Faculty of Medicine, Menoufia University Hospitals approved the study.

Informed Consent Statement: Informed consent was obtained from all participants for being included in the study.

•   Name of institutional or national ethical committee on human experimentation: The Institutional Review Board (IRB) of Faculty of Medicine, Menoufia University Hospitals "Approval Number/Date: not applicable".

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Table (1): Demographic characteristics of the studied groups

	G I (DM, -ve proteinuria) (N= 20)		G II (DM, with +ve proteinuria) (N= 20)		G III CKD Non-Diabetic with proteinuria (N= 20)		Control (N= 20)		f	p
Age (years) **Range** **Mean ± SD.**	40.0 – 54.0 47.15 ± 4.12		49.0 – 74.0 56.25 ± 6.72		25.0 – 64.0 44.20 ± 9.80		21.0 – 33.0 28.90 ± 3.06		61.751*	<0.001*
P cont.	<0.001*		<0.001*		<0.001*					
Sig. bet. grps.	p_1<0.001*, p_2= 0.478, p_3<0.001*									
Sex **Male** **Female**	13 7	65.0 35.0	10 10	50.0 50.0	12 8	60.0 40.0	11 9	55.0 45.0	X^2=1.023	0.796

F: F test (ANOVA), Sig. bet. grps was done using Post Hoc test (Tukey)

P $_{cont.}$: p value for comparing between control and each other group.

p_1: p value for comparing between DM, -ve proteinuria and DM, with +ve proteinuria.

p_2: p value for comparing between DM, -ve proteinuria and CKD, with proteinuria, No DM.

p_3: p value for comparing between DM, with +ve proteinuria and CKD, with proteinuria, No DM.

*: Statistically significant at $p \leq 0.05$.

Table (2): Clinical data of the studied groups.

	G I (DM, -ve proteinuria) (n= 20)	G II (DM, with +ve proteinuria) (n= 20)	G III CKD Non-Diabetic with proteinuria (n= 20)	Control (n= 20)	f	p
Sys Bl Pressure **Range mmHg** **Mean ± SD.**	120.0 – 150.0 134.0 ± 8.21	130.0 – 160.0 137.50 ± 7.86	100.0 – 160.0 133.50 ± 15.99	110.0 – 160.0 117.0 ± 6.37	15.833*	<0.001*
P cont.	<0.001*	<0.001*	<0.001*			
Sig. bet. grps.	p_1=0.999, p_2<0.001* , p_3=0.612					
Dia Bl Pressure **Range mmHg** **Mean ± SD.**	70.0 – 90.0 83.50 ± 5.87	80.0 – 90.0 86.50 ± 4.89	70.0 – 90.0 84.75 ± 5.73	70.0 – 90.0 77.0 ± 5.71	11.096*	<0.001*

P cont.	0.002[*]	<0.001[*]	<0.001[*]			
Sig. bet. grps.	p₁=0.893, p₂=0.002[*] , p₃=0.753					
Weight (kg) Range Mean ± SD.	65.0 – 85.0 74.30 ± 5.90	65.0 – 88.0 76.90 ± 6.32	61.0 – 90.0 76.25 ± 7.47	60.0 – 90.0 74.20 ± 8.87	0.717	0.545
Height (m2) Range Mean ± SD.	1.61 – 1.81 1.71 ± 0.06	1.60 – 1.77 1.68 ± 0.04	1.61 – 1.83 1.72 ± 0.06	1.55 – 1.80 1.69 ± 0.06	1.767	0.161
BMI (kg/m2) Range Mean ± SD.	20.15 – 30.85 25.68 ± 3.03	22.49 – 30.85 27.25 ± 2.33	20.62 – 31.51 26.03 ± 3.33	20.62 – 33.95 26.19 ± 3.59	0.951	0.420

Sys bl pr (Systolic Blood Pressure) (mmHg)   Dia bl pr (Diastolic Blood Pressure) (mmHg) F: F test (ANOVA), Sig. bet. grps was done using Post Hoc test (Tukey)

P cont.: p value for comparing between control and each other group.

p₁: p value for comparing between DM, -ve proteinuria and DM, with +ve proteinuria.

p₂: p value for comparing between DM, -ve proteinuria and CKD, with proteinuria, No DM.

p₃: p value for comparing between DM, with +ve proteinuria and CKD, with proteinuria, No DM. *: Statistically significant at $p \leq 0.05$.

Table (3a): Laboratory characteristics of the studied groups.

	G I (DM, -ve proteinuria) (n= 20)	G II (DM, with +ve proteinuria) (n= 20)	G III CKD Non-Diabetic with proteinuria (n= 20)	Control (n= 20)	Test of sig.	p
HB (gm/dl) Range Mean ± SD.	9.50 – 13.50 11.71 ± 1.07	8.50 – 14.0 11.23 ± 1.35	8.80 – 13.0 10.14 ± 1.04	11.0 – 16.0 13.71 ± 1.43	f=29.308*	<0.001[*]
P cont.	<0.001[*]	<0.001[*]	<0.001[*]			
Sig. bet. grps.	p₁=0.611, p₂=0.001[*] , p₃=0.032[*]					
WBCs (10³) Range Mean ± SD.	5.0 – 8.40 6.74 ± 0.95	4.0 – 63.0 9.94 ± 12.55	4.30 – 9.0 6.57 ± 1.43	4.90 – 72.0 11.96 ± 16.20	KW	0.234
Platelets (10³) Range Mean ± SD.	180.0 – 430.0 273.20 ± 53.70	155.0 – 400.0 275.45 ± 68.27	168.0 – 320.0 244.10 ± 49.97	190.0 – 400.0 288.50 ± 57.52	KW	0.122

Fasting blood glucose (mg/dl)						
Min. – Max.	130.0 – 170.0	131.0 – 190.0	75.0 – 105.0	44.0 – 109.0	F=212.288*	<0.001*
Mean ± SD.	148.75 ± 12.13	164.80 ± 11.82	91.35 ± 8.90	89.50 ± 14.26		
P cont.	<0.001*	<0.001*	0.961			
Sig. bet. grps.	p₁<0.001*,p₂<0.001*,p₃<0.001*					
Post prandial blood glucose (mg/dl)						
Min. – Max.	220.0 – 309.0	250.0 – 490.0	120.0 – 150.0	122.0 – 149.0	KW= 62.153*	<0.001*
Mean ± SD.	274.20 ± 27.33	314.40 ± 49.98	135.40 ± 8.26	133.30 ± 7.38		
P cont.	<0.001*	<0.001*	0.417			
Sig. bet. grps.	p₁=0.001*, p₂<0.001*, p₃<0.001*					
HbA1C (%)						
Min. – Max.	6.80 – 8.0	7.70 – 11.0	5.40 – 6.40	4.80 – 6.10	f=133.888*	<0.001*
Mean ± SD.	7.53 ± 0.39	8.97 ± 1.04	5.99 ± 0.28	5.45 ± 0.44		
P cont.	<0.001*	<0.001*	0.036*			
Sig. bet. grps.	p₁<0.001*, p₂<0.001*, p₃<0.001*					
Serum albumin (g/dl)						
Min. – Max.	3.50 – 4.50	2.70 – 3.80	2.80 – 4.0	3.80 – 4.80	f=34.476*	<0.001*
Mean ± SD.	3.85 ± 0.28	3.34 ± 0.27	3.39 ± 0.36	4.16 ± 0.27		
P cont.	0.008*	<0.001*	<0.001*			
Sig. bet. grps.	p₁<0.001*, p₂<0.001*, p₃=0.951					

Table (3b continuation): Laboratory characteristics of the studied groups.

	G I (DM, -ve proteinuria) (n= 20)	G II (DM, with +ve proteinuria) (n= 20)	G III CKD Non-Diabetic with proteinuria (n= 20)	Control (n= 20)	Test of sig.	p
Cholesterol (mg/dl)						
Min. – Max.	160.0 – 205.0	195.0 – 310.0	165.0 – 320.0	90.0 – 150.0	f=79.425*	<0.001*
Mean ± SD.	182.90 ± 11.99	248.40 ± 34.25	229.25 ± 41.50	119.45 ± 16.68		
P cont.	<0.001*	<0.001*	<0.001*			
Sig. bet. grps.	p₁<0.001*, p₂<0.001*, p₃=0.161					
Triglycerides (mg/dl)						

Min. – Max.	110.0 – 190.0	170.0 – 280.0	130.0 – 340.0	75.0 – 130.0	KW=	<0.001*
Mean ± SD.	154.25 ± 20.08	235.0 ± 34.56	222.0 ± 61.25	98.70 ± 13.85	60.517*	
P cont.	<0.001*	<0.001*	<0.001*			
Sig. bet. grps.	p₁<0.001*, p₂<0.001*, p₃=0.356					
Blood Urea (mg/dl)						
Min. – Max.	22.0 – 49.0	20.0 – 130.0	52.0 – 160.0	26.0 – 52.0	KW=	<0.001*
Mean ± SD.	37.80 ± 6.23	66.85 ± 32.29	99.40 ± 26.53	32.20 ± 6.94	53.039*	
P cont.	0.003*	<0.001*	<0.001*			
Sig. bet. grps.	p₁<0.001*, p₂<0.001*, p₃= 0.002*					
Serum creatinine (mg/dl)						
Min. – Max.	0.70 – 1.40	1.20 – 3.80	1.40 – 6.0	0.50 – 0.80	KW=	<0.001*
Mean ± SD.	1.16 ± 0.18	2.12 ± 0.72	3.70 ± 1.31	0.65 ± 0.08	68.679*	
P cont.	<0.001*	<0.001*	<0.001*			
Sig. bet. grps.	p₁<0.001*, p₂<0.001* , p₃<0.001*					
eGFR (MDRD)						
Min. – Max.	44.0 – 125.0	20.0 – 60.0	12.0 – 49.0	44.0 – 110.0	KW=	<0.001*
Mean ± SD.						
P cont.	0.001*	<0.001*	<0.001*			
Sig. bet. grps.	p₁<0.001*, p₂<0.001*, p₃<0.001*					
Urinary albumin creatinine ratio						
Range	15.0 – 28.0	80.0 – 500.0	320.0 – 810.0	7.0 – 23.0	KW=	<0.001*
Mean ± SD.	21.75 ± 3.64	199.0 ± 116.89	550.50 ± 180.14	15.20 ± 4.92	66.578*	
P cont.	<0.001*	<0.001*	<0.001*			
Sig. bet. grps.	p₁<0.001*, p₂<0.001* p₃ <0.001*					

KW: Kruskal Wallis test sig. bet. Grps. Using Mann Whitney test

F: F test (ANOVA), Sig. bet. grps was done using Post Hoc test (Tukey)

P cont.: p value for comparing between control and each other group.

p₁: p value for comparing between DM, -ve proteinuria and DM, with +ve proteinuria.

p₂: p value for comparing between DM, -ve proteinuria and CKD, with proteinuria, No DM.

p₃: p value for comparing between DM, with +ve proteinuria and CKD, with proteinuria, No DM.

*: Statistically significant at p ≤ 0.05.

Table (4): Urinary laminin level in the studied groups.

	G I (DM without proteinuria) (n= 20)	G II (DM, with proteinuria) (n= 20)	G III CKD Non-Diabetic with proteinuria (n= 20)	Control (n= 20)	KW	p
Ur. Laminin						
Range	75.0 – 135.0	300.0 – 675.0	305.0 – 900.0	23.0 – 105.0	66.404*	<0.001*
Mean ± SD.	98.53 ± 20.95	535.75 ± 101.70	434.25 ± 128.55	54.35 ± 27.43		
P cont.	<0.001*	<0.001*	<0.001*			
Sig. bet. groups.	p1= <0.001*, p2= <0.001*, p3= 0.001*					

KW: Kruskal Wallis test sig. bet. Groups. Using Mann Whitney test

P cont.: p value for comparing between control and each other group.

p1: p value for comparing between DM, without proteinuria and DM, with proteinuria.

p2: p value for comparing between DM, without proteinuria and CKD, with proteinuria and No DM.

p3: p value for comparing between DM, with +ve proteinuria and CKD, with proteinuria, No DM.

*: Statistically significant at $p \leq 0.05$

Table (5): Correlation between urinary laminin and different parameters in each group

		Ur. laminin			
		DM, -ve proteinuria	CKD Non-Diabetic with proteinuria	CKD Non-Diabetic with proteinuria	Control
HB	r	0.315	-0.173	0.074	-0.078
	p	0.176	0.467	0.757	0.745
Hb.A1.C%	r	0.514	0.366	0.105	-0.202
	p	0.020*	0.113	0.659	0.394
Fasting blood glucose	r	0.092	-0.113	-0.344	-0.304
	P	0.699	0.634	0.138	0.193
Post prandial blood glucose	r	0.258	-0.091	0.188	0.285
	p	0.273	0.701	0.428	0.223
Serum albumin	r	0.476	-0.002	-0.147	-0.054
	P	0.034*	0.995	0.536	0.821
Cholesterol	r	0.005	-0.356	0.292	0.204
	p	0.982	0.124	0.212	0.387
	r	0.180	0.061	-0.208	-0.029

Triglycerides	P	0.446	0.797	0.378	0.904
Urea	r	0.139	0.264	0.140	0.381
	P	0.558	0.261	0.556	0.098
Creatinine	r	0.115	0.068	0.415	-0.216
	P	0.630	0.777	0.069	0.361
eGFR	r	-0.068	0.100	-0.201	0.097
	P	0.775	0.675	0.395	0.685
Urinary albumin creatinine ratio	r	0.186	0.119	0.190	-0.300
	P	0.433	0.617	0.423	0.198

r_s: Spearman coefficient

*: Statistically significant at $p \leq 0.05$

Table (6): Agreement (sensitivity, specificity, and accuracy) for urinary laminin in three studied patients groups.

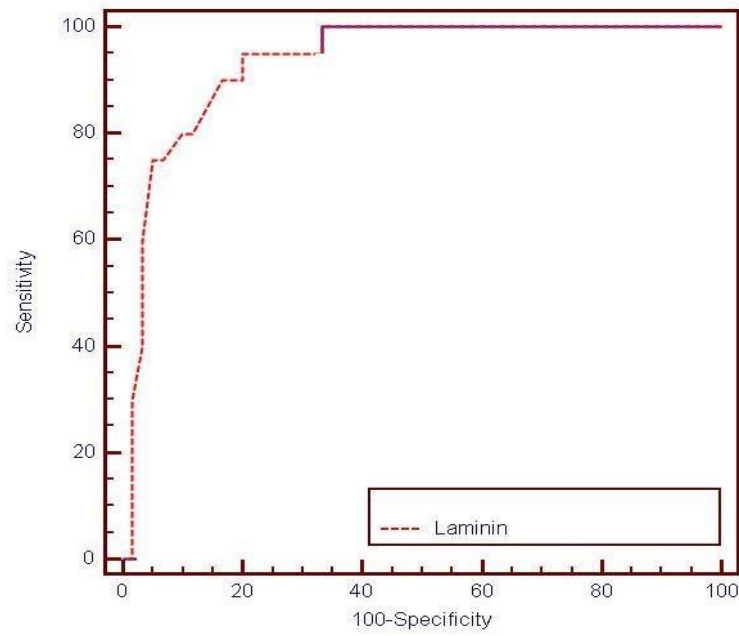| Urinary Laminin	AUC	P Value	Youden index	Best Cut off	Sensitivity %	Specificity %	PPV %	NPV %
GI, Diabetic patients with +ve proteinuria.	0.934*	<0.001*	0.750	>375	95.0	80.0	61.3	98.0
GII ,CKD with proteinuria	1.000*	<0.001*	1.000	>135	100.0	100.0	100.0	100.0
GIII,DM without proteinuria	0.883*	<0.001*	0.700	>70	100.0	70.0	76.9	100.0

Figure (1): ROC curve for urinary laminin to predict proteinuria in diabetic patients.
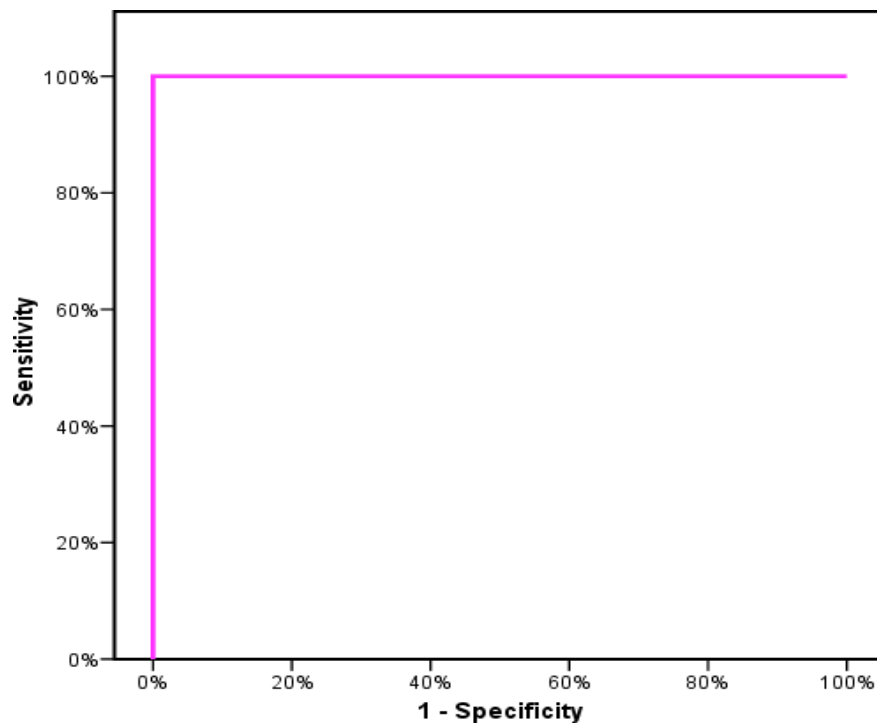

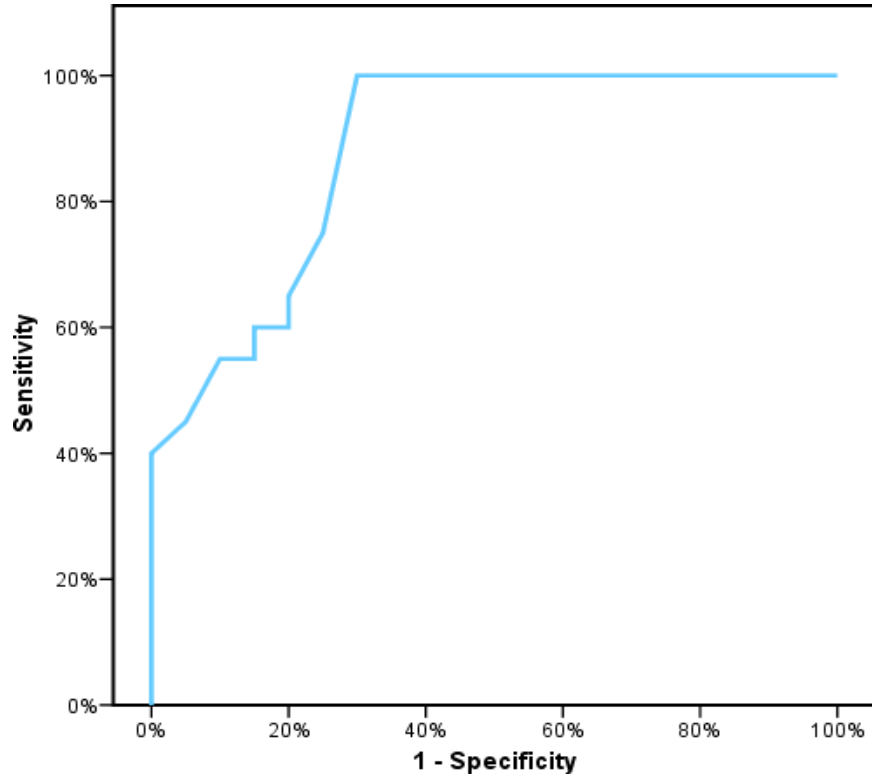**Figure (2):** ROC curve for urinary laminin in CKD, with proteinuria

Figure (3): ROC curve for urinary laminin in DM without proteinuria.