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Urinary Neutrophil Gelatinase-Associated Lipocalin as an early marker for diagnosis of diabetic nephropathy in T2DM patients and its correlation to albumin creatinine ratio

Khaled Ahmed Elbana[1], Hoda Gouda Bakr[2],AbeerAbdAlla Fekry[3], Mahmoud Samir Elkot[4]

[1]Assistant Professor of Internal Medicine, Faculty of Medicine – Zagazig University,[2]Professor of Internal medicine, Faculty of Medicine – Zagazig University,[3]Lecturer of clinical pathology, Faculty of Medicine – Zagazig University, [4]M.B.B.CH.
Corresponding Author Name: Mahmoud Samir Elkot
Doctorelkot1988@gmail.com

Abstract

Background: The presence of micro albuminuria is considered as the earliest indicator or warning signal to renal and cardiovascular disease in patients with T2DM, associated with significant glomerular damage. In addition, the early structure damage in both tubular structure and glomerular may be present in normal albuminuria. So there is a need to find biomarkers that help in identification the patients at risk of the disease. The appearance of NGAL in the urine of patients may indicate early glomerular injury, and this has been demonstrated at earlier stage than the appearance of microalbuminuria.Objectives were to evaluate Urinary Neutrophil Gelatinase-Associated Lipocalin (NGAL) as an early marker for diagnosis of diabetic nephropathy in T2DM patients and its correlation to albumin creatinine ratio. **Methods:**The study was conducted on 84 subjects, from (18- 80 years old), 45 (53.5%) were males and 39 (46.4%) were females (table 3), with mean age (58.18 ± 13.98) They were collected from Endocrinology and nephrology clinic after a written consent was taken from the patients at Zagazig University Hospital. The subjects were divided into 4 groups according to KDIGO guide lines, Classification of albuminuria (2012): **Group (1):** 21 healthy control subjects of comperative group and sex. Group (2): 21 T2DM patient with normoalbuminuria (albuminuria<30 mg/g). Group (3): 21 T2DM patient with microalbuminuria (albuminuria 30-300 mg/g). Group (4): 21 T2DM patient with macroalbuminuria (albuminuria >300 mg/g). All subjects of this study were be subjected to the following: Estimation of GFR, Albumin, creatinine ratio in urine and Measurement of urinary neutrophil gelatinase associated lipocalin in urine. **Results:**Our study showed that that urinary NGAL is higher in diadetic patients compared with non-diabetic controls with high significant difference ($p < 0.0000$). Also, there was high significant difference between diabetic groups on comaparison as regades urinary NGAL level between Gr III (microalbuminuria) (180.86 ±38.16 ng/ml) and Gr IV (macroalbuminuria) (354.24 ±99.12ng/ml) than Gr II (normoalbuminuria) (20.01±1.89 ng/ml) with high significant difference ($r = -5.668$, $P = 0.000$). Our study showed that Gr IV (macroalbuminuria) had higher NGAL level (354.24 ±99.12ng/ml) in comparison to Gr II (normoalbuminuria) (20.01±1.89ng/ml), Gr III (microalbuminuria) (180.86 ±38.16ng/ml), and Gr I (control) (3.18±1.64ng/ml). We found GFR was high in G1 (normoalbuminuric patients) (115.42 ± 12.52) than control group (107.53 ± 6.59) ($p =0.055$). NGAL was high in G1 (normoalbuminuric patients) (20.01±1.89) than control group (3.18±1.64). So, Urinary NGAL can be considered as a promising early marker for DN as we found a high level of NGAL in normoalbuminuric patients, especially those with long-standing DM, uncontrolled diabetes, and dyslipidemia. **Conclusion:**urinary NGAL was higher in cases with macroalbuminuria than microalbuminuria and normoalbuminuria. Urine NGAL can be used as an early biomarker for DN as we found a

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high level of NGAL in normoalbuminuric patients, especially those with long-standing DM, uncontrolled diabetes, and dyslipidemia. These results suggest a possible role of urinary NGAL to be promising new biomarker with the ability to reflect renal damage and impairment.
Key words: albuminuria-Urinary NGAL T2DM-Biomarker.

Introduction:

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action. or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction. and failure of various organs. especially the eyes, kidneys. nerves, heart. and blood vessels **(1).**

Several pathogenic processes are involved in the development of diabetes. These range from autoimmune destruction of the 13-cells of the pancreas with consequent insulin deficiency to abnormalities that result in resistance to insulin action. The basis of the abnormalities in carbohydrate.fat. and protein metabolism in diabetes is due to deficient action of insulin on target tissues. which results from inadequate insulin secretion andlor diminished tissue responses to insulin at one or more points in the complex pathways of hormone action **(2).**

Diabetes has become the primary cause of end-stage renal disease (ESRD). Early diagnosis of diabetes and early intervention are critical in preventing the normal progression to renal failure seen in many patients with type 1 and a significant percentage of T2DM **(3).**

Diabetic nephropathy is associated with an increased riskof all-cause mortality, cardiovascular disease and progressionto end stage renal disease (ESRD). requiring costly renalreplacement therapy in the form of dialysis or transplantation.

Diagnostic marker to detect diabetic nephropathy at earlystage is important as early intervention can slow the loss ofkidney function and reduce adverse outcomes. The appearance ofsmall amount of protein albumin in urine, calledmicroalbuminuria has been accepted as the earliest marker fordevelopment of diabetic nephropathy. However, it has beenreported that a large proportion of renal impairment occurs evenbefore appearance of microalbuminuria**(4).**

It is necessary to implement different strategies fordetecting early diabetic nephropathy in patients with T2DMaiming to delay its progression and improve outcomes .

Increased levels of urinary biomarkers can be detected inT2DM patients before the onset of significant albtuninuria .

There are several glomerular and tubular biomarkers predictingonset or progression of nephropathy in patient with diabetesand may be used as an early marker of renal injury in diabeticnephropathy, this would play a significant role in clinicaldiagnosis and treatment approaches in diabetic care **(5).**

Neutrophil gelatinase-associated lipocalin (NGAL) is a small (25-kd) protein that belongs to the lipocalin protein family. NGAL is produced in epithelial cells and neutrophils inmost tissues. It was found in activated neutrophils, inaccordance with its role as an innate antibacterial factor**(6).**

Urinary NGAL (u-NGAL) levels havebeen demonstrated to be a very promising marker. especially inacute kidney disease. Also plasma NGAL (p-NGAL) has beenshown to be a promising marker of tubular damage in bothacute and chronic kidney damage **(7).**

Neutrophil gelatinase associated lipocalin (NGAL), is produced and secreted into the urine in response to ischemic kidney damage and is therefore a promising early and sensitive biomarker of diabetic nephropathy. The appearance of Neutrophil gelatinase-associated lipocalin (NGAL) in the urine of patients may indicate early glomerular injury, and this has been demonstrated at earlier stage than the appearance of microalbuminuria**(8).**

Evidence also suggests that NGAL somehow may be involved in the pathophysiological process of chronic renal diseases, such as polycystic kidney disease and glomerulonephritis. Neutrophil gelatinase associated lipocain (NGAL) levels clearly correlate with severity of renal impairment.

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probably expressing the degree of active damage underlying the chronic condition. For all these reasons.

Neutropilgelatinase associated lipocain (NGAL) may becomeone of the most promising next-generation biomarkers inclinical nephrology and beyond [6].

The study aimed tomeasure Urinary Neutrophil Gelatinase-Associated Lipocalin (NGAL) as an early marker for diagnosis of diabetic nephropathy in T2DM patients.

Patients and Methods

The study was conducted on 84 subjects, from (18- 80 years old), 45 (53.5%) were males and 39 (46.4%) were females (table 3), with mean age (58.18 ± 13.98) They were collected from Endocrinology and nephrology clinic after a written consent was taken from the patients at Zagazig University Hospital. The subjects were divided into 4 groups according to KDIGO guide lines, Classification of albuminuria (2012):

- **Group (1):** 21 healthy control subjects of comperative group and sex.Their mean age was 57.52± 13.45 years. It included 13 males and 8 females.
- **Group (2):** 21 T2DM patient with normoalbuminuria (albuminuria<30 mg/g). Their mean age was 58.95 ± 7.12 years. It included 12 males and 9 females.
- **Group (3):** 21 T2DM patient with microalbuminuria (albuminuria 30-300 mg/g). Their mean age was 51.33 ± 10.35 years. It included 9 males and 12 females.
- **Group (4):** 21 T2DM patient with macroalbuminuria (albuminuria >300 mg/g). Their mean age was 57.05 ± 15.89 years. It included 13 males and 8 females.

Exclusion Criteria:

1) Type 1 D.M.
2) Urinary tract infection.
3) Fever
4) Glomerulonephritis and proteinuria due other causes than diabetes mellitus.
5) Drugs causing proteinuria for example (amlodipine, amoxicillin and azithromycin).
6) Pregnancy.
7) Patient with hepatic disease.
8) Malignancy.
9) Hypertension

All subjects of this study were be subjected to the following:

A) Medical history taking.
B) Thorough clinical examination.
C) Anthropometric measurements including: Weight, height, Body mass index (weight in kilograms divided by square of height in meters).
D) **Laboratory investigations**:
   1) Complete blood picture.
   2) Liver enzymes test (ALT, AST) (IU/L) (serum albumine) (mg/dl) using enzymeticcolorimetry methods.
   3) Estimation of GFR using Cockcroft Gault equation [ml/min]; e CoCr = (140-age (in years)) ×weight (in kilograms) / 72× serum creatinine, multiply by 0.85 if female (in mg/dl) (Cockcroft et al., 1976).
   4) Fasting plasma glucose (after fasting 8 hours), two hours post prandial blood glucose were measured using an automated glucose oxidase method using Behring Diagnostics Reagents (SVR Glucose Test; Behring, La Jolla, CA).

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5) HbA1c % by Quantitative colorimetric determination of Glycohemoglobin in blood.
6) Lipid profile (after fasting 12 hours) (serum cholesterol, triglycerides, LDL by friedewald equation (LDL cholesterol = total cholesterol - HDL cholesterol –Triglyceride / 2.2), HDL), by enzyme colorimetric assay using commercially available Kit (Boehringer Mannheim, Germany) after an overnight 12 hours fasting.
7) Albumin creatinine ratio in urine by enzyme immunoassay (mg/g) .
8) Measurement of urinary neutrophil gelatinase associated lipocalin in urine samples of T2DM patients with different grades of albuminuria by ELISA. (The kit assay Human NGAL level in the sample, use purified Human NGAL antibody to coat microtitrer plate wells, make solid –phase antibody, then add NGAL to wells, combined NGAL which with HRR labeled, become antibody-antigen-enzyme - antibody complex, after washing completely, add TMB substrate solution, TMB substrate becomes blue color at HRR enzyme –catalyzed, reaction is terminated by the addition of asulphuric acid solution and the color change is measured spectrophotometrically at awavelength of 450 nm. The concentration of NGAL in the samples is then determined by comparing the O.D of the samples to the standard curve) (0.2- 5 ng/dl) (Glory science co., ltd, 2015).
9) Pelviabdominal u/s (to exclude ESRD and polycystic kidney disease).
10) Fundus examination.

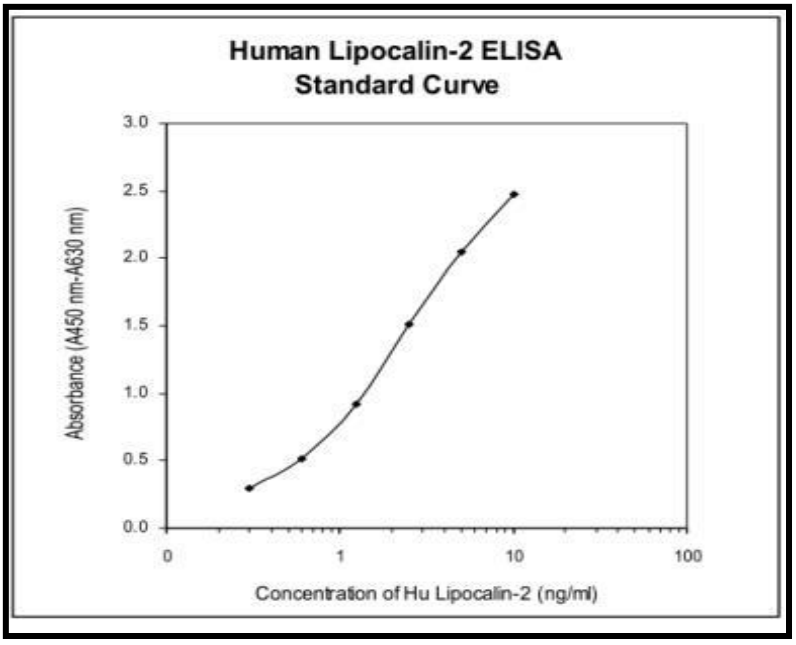

Fig. (1): (Human lipocalin-2 ELISA standard curve).

Statistical Analysis: Data were collected, revised, coded and entered to the Statistical Package for Social Science (IBM SPSS) version 20. The quantitative data were presented as mean, standard deviations and ranges when their distribution found parametric while qualitative data were presented as number and percentages. The comparison between two independent groups with qualitative data was done by using Chi-square test. The comparison between two independent groups with quantitative data and parametric distribution was done by using Independent t-test. Spearman correlation coefficients were used to assess the correlation between two quantitative parameters in the same group. The confidence interval was set to 95% and the margin of error accepted was set to 5%. So, the p-value was

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considered significant as the following: P > 0.05: Non significant. P < 0.05: Significant. P < 0.01: Highly significant.

Results:

Table (1) showedno statistical significant difference as regard age, gender, weight, BMI on comparing the studied and control groups and statistical significant difference was found as regardsystolic and diastolic blood pressure.

In comparison between control and patients groups as regard NGAL we found a high statistical significant difference (P=0.000) (**figure 1).**

Table (2) On comparing the three patient's groups there was no statistical significant difference as regard age, gender, weight, BMI and blood pressure, but on the other hand there was a high statistical significant difference as regard duration of D.M (p=0.001).

On comparing group, II and group III there was statistical significant difference was found as regard, HDL-C, TG, LDL-C and high statistical significant difference as regard NGAL, GFR and Alb/Cr .On comparing group, II and group IV there was high statistical significant difference was found as regard FPG, 2hpppG, NGAL, Total cholesterol, HDL-C, TG, LDL-C, HbA1C, GFR and Alb/Cr.On comparing group III and group IV there was high statistical significant difference was found as regard FPG, Alb/Cr and 2hpppG, NGAL, LDL-C, HbA1C, TC and GFR**Table (3).**

The above table shows statistical significant difference between all studied groups as regard fundus findings in fundus examination (P=0.022) **Table (4)**.

On comparing the three patients groups with the NGAL there was no statistical significant difference as regard age, gender, weight, BMI and blood pressure, , HDL CHOL, AST and ALT. but on the other hand there was a statistical significant difference as regard duration of D.M , pr/c, GFR, FPG, 2hpppG, HBA1C, TC, TG, LDL cholesterol and Alb/Cr **Table (5)**.

The previous table shows that there was highly statistically significant association between NGAL level and HBA1c and Alb/Cr ratio while no statistically significant association found with the other studied parameters **Table (6)**.

Table (1): Comparison between all studied groups as regard different clinical parameters:

		Control group	Patients group	Independent t-test	
		No. = 21	No. = 63	t / χ^2*	P-value
Gender	Female	8 (38.1%)	31 (49.2%)	0.377*	0.453
	Male	13 (61.9%)	32 (50.8%)		
Age (years)	Mean±SD	57.52± 13.45	55.78 ± 11.96	0.562	0.576
	Range	25 – 78	33--81		
Weight (kg)	Mean±SD	74.29 ± 12.74	75.88 ± 12.09	-0.557	0.579
	Range	59– 98	55 – 99		
BMI (kg/m2)	Mean±SD	24.69 ± 3.21	25.28 ± 5.18	-0.486	0.628
	Range	20.15 – 30.26	18.56 – 41.26		
SBP(mmHg)	Mean±SD	120.19± 6.89	131.67± 10.53	-3.563	0.001*
	Range	100 – 125	100 – 140		
DBP(mmHg)	Mean±SD	82.90 ± 3.35	89.43 ± 8.58	-3.388	0.002*
	Range	70 – 80	85 – 100		
Duration of DM (years)	Mean±SD	–	9.09 ± 4.07	–	–
	Range	–	2--21		

*: Chi-square testP > 0.05: Non significant P< 0.05: Significant P < 0.001: Highly significant BMI = body mass index

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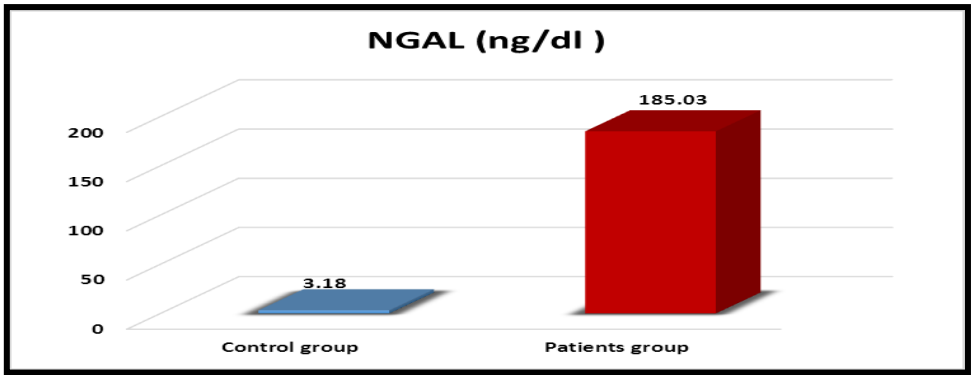

Fig. (1): Comparison between the patients groups as regard NGAL:

Table (2): Comparison between patients groups as regard clinical parameters.

Parameters		Control group Gr(I)	Norm albuminuria Gr (II)	Micro albuminuria Gr (III)	Macro albuminuria Gr (IV)	One Way ANOVA	
		no. = 21	no. = 21	no. = 21	no. = 21	F / χ^2*	P-value
Gender	Female	8 (38.1%)	9(42.9%)	12 (57.1%)	8(38.1%)	2.032*	0.362
	Male	13 (61.9%)	12 (57.1%)	9(42.9%)	13 (61.9%)		
Age(years)	Mean±SD	57.52± 13.45	58.95 ± 7.12	51.33 ± 10.35	57.05 ± 15.89	2.414	0.098
	Range	25 – 78	42--68	40-72	33--81		
Weight(kg)	Mean±SD	74.29 ± 12.74	76.14 ± 11.61	75.95 ± 12.34	75.86 ± 12.27	0.003	0.997
	Range	59– 98	61--96	55 – 99	55—98		
BMI(kg/m2)	Mean±SD	24.69 ± 3.21	24.96 ± 4.63	25.97 ± 5.69	24.92 ± 5.35	0.270	0.764
	Range	20.15 – 30.26	18.98– 37.56	18.56— 41.26	18.56— 37.56		
Syst Bl. Pr(mmHg)	Mean±SD	120.19 ± 6.89	126.76± 19.71	130.86 ± 12.73	137.38 ± 5.23	2.235	0.254
	Range	100 – 125	100 – 136	100 – 140	100 – 140		
Diast Bl. Pr(mmHg)	Mean±SD	82.90 ± 3.35	84.14± 4.52	91.19 ± 8.88	93.19 ± 9.21	1.345	0.117
	Range	70 – 80	85--90	85 – 100	85 – 100		
Duration	Mean±S	–	7.98 ± 3.79	8.19 ± 1.94	11.10 ±	4.229	0.019*

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of DM (years)	D				5.20		
	Range	–	3– 15	4--11	2 – 21		

Table (3): Comparisons between the 3 patients groups as regard to laboratory parameters:

	Control group Gr(I)		Norm albuminuria Gr(II)	Microalbuminuria Gr (III)	Macroalbuminuria Gr (IV)	One Way ANOVA		Post HocAnalysis by LSD test		
	no. = 21		No. = 20	No. = 20	No. = 20	F	P-value	P1	P2	P3
Hb(g/dl)	Mean ±SD	11.19 ± 1.45	11.04 ± 1.32	10.74 ± 0.78	10.35 ± 0.84	2.442	0.096			
	Range	10--15	8--12	9— 12.1	8.9 –12					
KFT(mg/dl)	Mean ±SD	0.88± 0.15	0.85± 0.19	0.91 ± 0.31	1.27 ± 0.38	1.764	0.056			
	Range	0.55 – 1.30	0.50 – 1.30	0.50 – 1.30	1.2 – 2					
AST(Iu/l)	Mean ±SD	22.76 ± 6.89	25.86 ± 7.58	26.15 ± 6.35	23.86 ± 6.36	0.700	0.501			
	Range	15—40	12--38	13 – 38	15 – 38					
ALT(Iu/l)	Mean ±SD	21.90 ± 10.33	27.67 ± 8.08	23.42 ±5.25	28.24 ± 10.55	1.960	0.150			
	Range	7 – 51	18--51	15--36	7--45					
GFR(ml/min/1.73m2)	Mean ±SD	107.53 ± 6.59	115.42 ± 12.52	95.30± 23.87	60.24±12.45	53.924	0.000**	0.000**	0.000**	0.000**
	Range	95 – 110	85 – 125	64 – 105	50– 97					
FPG(mg/dl)	Mean ±SD	82.29 ± 9.32	150.90 ± 32.77	154.81 ±39.91	197.33 ± 39.94	9.805	0.000**	0.940	0.000**	0.000**
	Range	67--99	110--210	95--210	140--300					
2hPPPG(mg/dl)	Mean ±SD	126.14 ± 9.85	185.90±39.21	194.38 ± 47.78	255.33 ± 58.44	12.490	0.000**	0842	0.000**	0.000**
	Range	109 – 140	150 – 275	150 – 310	155 – 300					
HBA1C (%)	Mean ±SD	5.46 ± 0.45	7.56 ± 0.75	8.42 ± 0.97	10.19 ± 0.85	5.065	0.001*	0.051	0.005*	0.001*
	Range	4.80– 5.9	7 – 13	7.00 – 11.50	6.90 – 13.00					
Total CHOL(mg/dl)	Mean ±SD	184.24± 23.15	235.62 ± 37.64	265.05 ± 43.76	289.76 ± 50.09	7.926	0.001*	0.041	0.001*	0.173
	Range	163 – 220	180 – 349	240 – 429	175 – 420					
TAG(mg/dl)	Mean ±SD	153.10± 18.90	157.62 ± 3.69	179.76 ± 17.50	181. ± 44.11	5.003	0.010*	0.030*	0.000**	0.968
	Range	125--	120 –	133 –	133 – 241					

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		185	242	277						
HDL CHOL(mg/dl)	Mean ±SD	41.05 ± 13.47	45.33 ± 11.92	34.52 ± 3.84	31.24 ± 11.56	5.033	0.001*	0.002*	0.001*	0.529
	Range	21 – 79	26--59	29 – 40	13 – 51					
LDL CHOL(mg/dl)	Mean ±SD	91.81 ± 23.73	113.14 ± 22.94	127.95 ± 29.59	150.38 ± 36.50	8.100	0.001*	0.048*	0.001*	0.049*
	Range	49—180	88--152	95--200	98 – 210					
Alb/Cr (mg/g)	Mean ±SD	20.87 ± 4.72	23.12 ± 7.62	218.71 ± 58.38	557.73 ± 119.67	259.923	0.000**	0.000**	0.000**	0.000**
	Range	19– 41	21.12 – 29	46—296.58	320 – 692.85					
NGAL (ng/dl)	Mean ±SD	3.18±1.64	20.01±1.89	180.86 ±38.16	354.24 ±99.12	200.176	0.000**	0.000**	0.000**	0.000**
	Range	1--7	20--28	150--320	250--641					

Parameters in the studied group:

P1: Norm albuminuria Vs Micro albuminuria. P2: Norm albuminuria Vs Macro albuminuria.

P3: Micro albuminuria Vs Macro albuminuria.

Table (4): Comparisons between different studied groups as regard fundus examination.

Fundus	**Control group**	**Normo albuminuria Gr (I)**	**Micro albuminuria Gr (II)**	**Macro albuminuria Gr (III)**	**Chi-square tests**	
	no. = 21	**no. = 21**	**no. = 21**	**no. = 21**	χ^2	**P-value**
NAD	19(90.5%)	19(90.5%)	10 (47.6%)	9 (42.9%)	21.056	0.012*
NPDR	0 (0.0%)	0 (0.0%)	1 (4.8%)	2 (9.5%)		
PDR	1 (4.8%)	1 (4.8%)	5 (23.8%)	4 (19%)		
Macularoedema	1 (4.8%)	1 (4.8%)	5 (23.8%)	6 (28.6%)		

NAD: No abnormalities detected.

NPDR: Nonproliferative diabetic retinopathy.

PDR: Proliferative diabetic retinopathy.

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Table (5): Correlation between NGAL and all the studied parameters:

	NGAL (ng/dl)							
	All Patient		Norm albuminuria Gr (II)		Micro albuminuria Gr (III)		Macro albuminuria Gr (IV)	
	R	P-value	R	P-value	R	P-value	R	P-value
Age(years)	0.101	0.443	-0.139	0.548	0.115	0.620	0.222	0.333
Weight(kg)	0.048	0.665	-0.042	0.856	-0.108	0.642	0.154	0.504
BMI(kg/m2)	0.100	0.365	-0.122	0.599	-0.036	0.725	0.204	0.374
Syst Bl. Pr(mmHg)	0.025	0.754	-0.165	0.523	0.126	0.651	0.138	0.561
Diast Bl. Pr(mmHg)	0.065	0.548	-0.154	0.392	0.321	0.452	-0.147	0.452
Hb(g/dl)	0.228	0.321	0.031	0.894	-0.263	0.277	0.070	0.762
Creatinine (mg/dl)	0.168	0.126	-0.071	0.758	0.165	0.474	-0.068	0.769
AST(Iu/l)	0.027	0.811	-0.380	0.089	0.102	0.668	0.114	0.631
ALT(Iu/l)	0.062	0.583	-0.059	0.800	0.028	0.911	-0.197	0.392
GFR (ml/min/1.73m^2)	-0.459	0.000**	0.225	0.451	-0.306	0.189	0.392	0.079
FPG(mg/dl)	0.626	0.000**	-0.254	0.098	0.075	0.850	0.321	0.221
2hPPPG(mg/dl)	0.687	0.000**	0.276	0.226	0.475	0.000**	0.721	0.000**
HBA1C (%)	0.778	0.000**	0.436	0.032*	0.541	0.002*	0.658	0.000**
Total CHOL(mg/dl)	0.423	0.001*	-0.541	0.031*	0.082	0.722	-221	0.221
TAG(mg/dl)	0.457	0.005*	-0.345	0.064	-0.293	0.198	-0.075	0.754
HDL CHOL(mg/dl)	0.053	0.487	0.061	0.794	-0.165	0.475	-0.283	0.213
LDL CHOL(mg/dl)	0.128	0.232	-0.562	0.008*	-0.612	0.005*	-0.425	0.280
Alb/cr(mg/g)	0.888	0.000**	0.814	0.000**	0.895	0.000**	0.918	0.000**
Duration of Dm (years)	0.279	0.027*	0.483	0.026*	0.615	0.015*	0.471	0.012*

Table (6): Multivariate linear regression analysis for predictors of NGAL in all the studied patients:

NGAL (ng/dl)					
	Unstandardized Coefficients		Standardized Coefficients	t	Sig.
	B	Std. Error	Beta		
(Constant)	-220.591	144.268		-1.529	0.132
GFR (ml/min/1.73m^2)	-0.747	0.366	-0.142	-2.040	0.051
FBG (mg/dl)	-0.211	0.236	-0.059	-0.896	0.374
2Hpppg (mg/dl)	0.322	0.182	0.122	1.766	0.083
HBA1C (%)	18.220	9.517	0.167	3.874	0.001
TC (mg/dl)	0.219	0.215	0.070	01.021	0.312
TG (mg/dl)	-0.384	0.328	-0.074	1.172	0.246

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HDL- C (mg/dl)	-0.393	0.897	-0.030	-0.438	0.663
Alb/Cr (mg/g)	-0.442	0.073	-0.684	-6.045	0.000

Discussion

Our study showed that that urinary NGAL is higher in diadetic patients compared with non-diabetic controls with high significant difference ($p < 0.0000$). Also, there was high significant difference between diabetic groups on comaparison as regades urinary NGAL level between Gr III (microalbuminuria) (180.86 ±38.16 ng/ml) and Gr IV (macroalbuminuria) (354.24 ±99.12ng/ml) than Gr II (normoalbuminuria) (20.01±1.89 ng/ml) with high significant difference ($r = -5.668$, $P = 0.000$).

Our results similar to the results of **Nielsen et al., [9]** who studied 69 T2DM patients and 75 control subjects and they found that NGAL was higher (279 ± 58 ng/ml vs 263 ± 38 ng/ml) in T2DM patients than control patients respectively with high significant difference ($p < 0.001$).

Our study showed that Gr IV (macroalbuminuria) had higher NGAL level (354.24 ±99.12ng/ml) in comparison to Gr II (normoalbuminuria) (20.01±1.89ng/ml), Gr III (microalbuminuria) (180.86 ±38.16ng/ml), and Gr I (control) (3.18±1.64ng/ml).

Our results were in harmony with those reported by **Abeer et al., [10].** Their study included 46 T2DM patients on either oral anti diabetic or insulin therapy. In addition, 15 apparently healthy, non-diabetic control subjects, without CKD were included in the study. It showed that there was high significant difference among the studied groups regarding uNGAL ($P = 0.0001$).

This was not in line with results reported by **Wong et al., [11]** which included 63 non-diabetic controls and 201 patients with T2DM. The patients with diabetes were subsequently followed-up for 28 months, with routine measurements of creatinine and urinary albumin excretion (UAE). **Wong et al., [11]** revealed that Urine NGAL levels were significantly increased in the macroalbuminuria group, whereas no significant differences were observed between the normoalbuminuria, microalbuminuria and control groups and this might be due to ethnic differences.

We found GFR was high in G1 (normoalbuminuric patients) (115.42 ± 12.52) than control group (107.53 ± 6.59) ($p =0.055$).

NGAL was high in G1 (normoalbuminuric patients) (20.01±1.89) than control group (3.18±1.64).

So, Urinary NGAL can be considered as a promising early marker for DN as we found a high level of NGAL in normoalbuminuric patients, especially those with long-standing DM, uncontrolled diabetes, and dyslipidemia.

Our study found a negative significant correlation between NGAL and GFR ($r = -0.459$, $P= 0.000$).

This result was in aggrement with **Mahfouz et al., [12]** as they found a highly significant negative correlation with the GFR ($r = −0.415$, $P= 0.0001$).

Similar to our study, **Nielsen et al., [9],** found a highly significant negative correlation with GFR ($r = -0.358$, $P= 0.001$).

In union to our results, **Fu et al., [13]**found a negative significant correlation between uNGAL with eGFR ($r = -0.215$, $P = 0.02$) when he studied a group of 101 short-term T2DM patients and 28 control subjects.

Patients with T2DM are older and, therefore, have greater likelihood of developing atherosclerotic vascular changes that influence GFR and glomerular size [14].

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In our study, there was a positive correlation between NGAL level and Alb/Cr with significant difference (r =0.888, p =0.000) in patients groups.
This also in line with **Yang et al., [(15)]**, they found a positive correlation between NGAL and Alb/Cr (r=0. 932, p = 0.000).
This proves that uNGAL levels have been demonstrated to be a very promising early and sensitive biomarker for diagnosis diabetic nephropathy and can help in estimating its progression. The appearance of Neutrophil gelatinase-associated lipocalin (NGAL) in the urine of patients may indicate early glomerular injury, and this has been demonstrated at earlier stage than the appearance of microalbuminuria.

Conclusion:
Our study showed that there was a high significant difference in the level of urinary NGAL in diabetic patients than control subjects. In addition, it was higher in cases with macroalbuminuria than microalbuminuria and normoalbuminuria. Urine NGAL can be used as an early biomarker for DN as we found a high level of NGAL in normoalbuminuric patients, especially those with long-standing DM, uncontrolled diabetes, and dyslipidemia. These results suggest a possible role of urinary NGAL to be promising new biomarker with the ability to reflect renal damage and impairment.

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