Neutrophil–Gelatinase Associated Lipocalin as Biomarker for Diabetic Kidney Disease

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Abstract

Background: Neutrophil–Gelatinase associated Lipocalin is a 25-kDa lipocalin glycoprotein. It's expressed in low amounts in numerous human tissues and induced in the kidney after ischemia or nephrotoxic injury. It predicted diabetics' early renal impairment.
Aim: To evaluate the accuracy of Neutrophil–Gelatinase associated Lipocalin in diagnosis of diabetic kidney disease in Type 2 diabetes mellitus.
Subject and Methods: This cross-sectional study was conducted on 160 participants at internal medicine outpatient clinic of Aswan University hospital.
Results: There was statistically significant difference between the three studied groups regarding disease duration, per glucose profile, fasting blood sugar, 2 hour-post prandial sugar and HbA1c. Also, there was significant difference between the three studied groups regarding per lipid profile parameters. As well, there was significant difference between groups regarding per renal function tests. It was found that neutrophil–gelatinase associated lipocalin can significantly predict diabetic kidney disease at a cutoff of 56 ng/ml.
Conclusion: Tubular injury may precede glomerular injury in diabetic patients and as neutrophil–gelatinase associated lipocalin is a tubular marker it is superior to albumin/Creatinine ratio as an early predictor of diabetic kidney disease in Type 2 diabetic patients as there was significant difference between non- albuminuria diabetic patient and non-albuminuria healthy individuals.

Keywords: Neutrophil – gelatinase associated lipocalin, Diabetes Mellitus, diabetic kidney disease

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1. INTRODUCTION

Type 2 diabetes mellitus (DM) is a public health concern worldwide and an important cause of morbidity and mortality (1). Type 2 DM is a progressive disease with its prevalence also increases with age, thus exposing the patients to an increased risk of long-term diabetic complications, including diabetic kidney disease (2).
Diabetic kidney disease (DKD) has become the single most frequent cause of end-stage renal disease (ESRD) at daunting rates over the past years, in both developed and developing countries (3). The presence of micro-albuminuria is a sign of the presence of diabetic kidney disease and marks the need for more intense glucose and blood pressure control (4).
Neutrophil gelatinase-associated lipocalin (NGAL) is a 25 kDa glycoprotein of the lipocalin super family. It is normally expressed in low concentrations in various human tissues, and it is induced in the kidney early after ischemic or nephrotoxic damage (5). It was used to predict early-stage renal dysfunction in diabetic patients (4). An ideal tubular marker of DKD should possess several properties in addition to good classification ability: (a) good correlations with albuminuria and renal function deterioration; (b) a progressive increase from micro- to macroalbuminuria; and (c) presence in normoalbuminuric patients and usefulness for early detection of diabetic renal impairment (6).
Two studies had found that NGAL is positively correlated with albuminuria and negatively correlated with estimated glomerular filtration rate (eGFR), suggesting that NGAL is correlated with renal involvement and can be used as a marker for DKD grading (7). In a study on Type 2 DM patients, it was found that NGAL level increased significantly in the four groups from healthy controls to diabetic patients with normo-albuminuria, microalbuminuria, and macroalbuminuria (8).
The current study aimed to evaluate the accuracy of neutrophil gelatinase-associated lipocalin (NGAL) in diagnosis of diabetic kidney disease (DKD) in Type 2 DM.

2. PATIENTS AND METHODS

This cross-sectional study was conducted on 160 participants recruited from the outpatient clinic of the Internal Medicine Department, Aswan University Hospital.

Sample size was calculated using G*Power 3 software (9), with a power of 80% and type I error of 5% (α=0.05 and β=80%) on two tailed test, the minimum required sample was 155 participants divided into four equal groups (Group A; 40 patients with normal albumin excretion (Albumin /creatinine ratio (ACR) < 30mg/g creatinine), Group B; 40 patients with moderately increased albuminuria (ACR=30-300 mg/g creatinine), Group C; 40 patients with severely increased albuminuria, ACR>300 mg/ gm creatinine and Group D; 40 control to detect an effect size of 0.33 in level of Serum NGAL between the four groups.

Male and female Type 2 DM patients aged 18 to 65 years; with eGFR>60 mL/min/1.73m² were included. Contrarily, patients with altered leukocytic count, sever liver dysfunction, renal transplant, congestive heart failure, pregnancy, malignancies, infectious, rheumatologic diseases, and those on glucocorticoids were excluded from this study.

All patients were subject to:

Full medical history included age, sex, DM history and duration, CKD, risk factors of AKI (sepsis, infections, blood/fluid loss, nephrotoxic drugs, liver failure and heart disease

Clinical examination included body mass index (BMI) (kg/m²), waist circumference (WC) (in centimeters)

Investigations included serum fasting blood sugar (FBS) and 2-h post-prandial (2HPP) glucose was determined by the glucose oxidase method (Trinder, 1969). For the assessment of glycated hemoglobin A1c (HbA1c), high-performance liquid chromatography will be performed according to method (10). Plasma lipid profile involving serum total cholesterol, triglyceride, high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) was determined by using enzymatic method with commercially available kits (11). Measurement of serum Creatinine was performed by enzymatic method. Urinary albumin excretion was measured by calorimetric methods using commercial kits. NGAL was measured using Enzyme-Linked Immunosorbent Assay kit. GFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) method (12). Based on the kidney disease: Improving Global Outcomes (KDIGO), CKD was defined as kidney damage or GFR<60ml/min/1.73m² for ≥3 months, irrespective of cause. Liver function tests and complete blood picture (CBC).

STATISTICAL ANALYSIS:

Analysis of data was carried out using Statistical Package for Social Science (SPSS) version 24 (13). Quantitative variables were described as mean and standard deviation (SD). Qualitative variables were described as frequency and percent. Shapiro-Wilk test was used to test for normality. To compare parametric quantitative variables between groups, ANOVA/Kruskal Wallis test was performed as appropriate and post-hoc test was calculated using Tukey's corrections for pairwise comparisons between the study groups. Qualitative variables were compared using chi-square (X^2) test. Pearson's correlation coefficient/Spearman Ranked correlation coefficient was used to assess for the association between two variables as appropriate. A p-value ≤ 0.05 was considered significant.

ETHICAL CONSIDERATION:

IRB approval was obtained from the Medical Ethic Committee, Faculty of Medicine, Aswan University. Trial registration was prospectively undertaken in clinical trial.gov (**NCT03883958**). The study was carried out in accordance with the Helsinki Declaration guidelines (14). An official written administrative permission letter was obtained from dean of faculty of medicine, Aswan university hospital, and head of internal medicine department. The title and objectives of the study were explained to them to ensure their cooperation. A written informed consent was obtained from the patient before the participation in the study. All collected data was confidential and was used for the purpose of scientific research only. Every research participant had the complete right and freedom to withdraw at any time from the study without any consequences on the medical service provided.

3. RESULTS

This cross-sectional study included 160 participants. The study was conducted at outpatient clinic of Internal Medicine Department, Aswan University hospital. The study cohort was divided into four equal groups in the period from November 2021 to March 2022.

The two groups were comparable with respect to the age, sex, **(Table 1)** and main anthropometric measurements (weight, height, BMI and WC) (p > 0.05). It was found that the mean DM duration in group A was 1.85± 1.10 years, 6.93± 1.16 years in group B, and 12.05± 0.96 years in group C.

Table (1): Comparison between the studied groups as regard demographic data

	Group A (n = 40)	Group B (n = 40)	Group C (n = 40)	Group D (n = 40)	P-value
Age/year					= 0.833* **NS**
• **Mean ± SD**	55.90 ± 10.3	56.35 ± 9.4	54.25 ± 8.2	55.70 ± 7.5	
• **Median (R)**	55 (35-80)	55 (40-75)	54 (34-70)	56 (43-72)	
Sex					= 0.918** **NS**
• **Female**	22 (55%)	21 (52.5%)	23 (57.5%)	20 (50%)	
• **Male**	18 (45%)	19 (47.5%)	17 (42.5%)	20 (50%)	

*Kruskal Wallis test was used to compare the median difference between groups
**Chi-square test was used to compare the proportion difference between groups

There was significant difference between the three studied groups regarding DM duration (p<0.001) as the duration was increasing steadily from group A (1.9± 1.1) to group B (6.9 ± 1.2) to group C (12.1 ± 0.9) **(Fig. 1).**

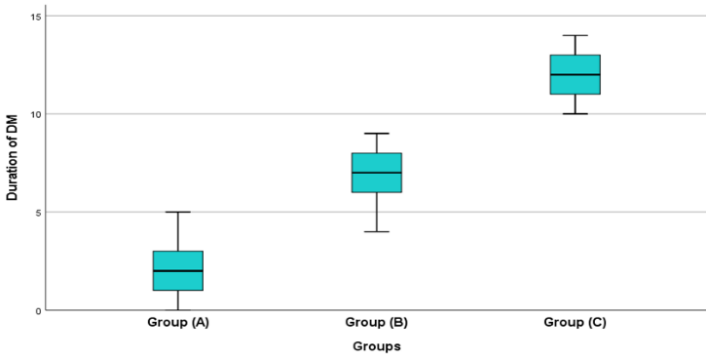

Fig. 1: Boxplot showing difference between the study groups regarding DM duration.

Table 2 showed the difference in glucose profile between groups. The mean level of FBG was significantly (p<0.001) lower in the control (93.6 ± 6.1 mg/dl) compared with the three patient groups (Group A (140.6 ± 24.7 mg/dl), Group B (158.6 ±41.5 mg/dl), and Group C (165.3 ± 129.9 mg/dl). Unlikely, the patient groups were comparable for the FBG (p>0.05). Likewise, the mean level of 2 hrs. post prandial sugar was significantly (p<0.001) lower in the control (102.6 ± 4.5 mg/dl) compared with the three patient groups (Group A (211.5 ± 57.1 mg/dl), Group B (239.2 ± 66.9 mg/dl), and Group C (226.1 ± 73.9 mg/dl). Unlikely, the patient groups were comparable for the FBG (p>0.05). Moreover, the mean HbA1c level was significantly (p<0.001) lower in the control (4.1 ± 0.5 mmol/l) compared with the three patient groups (Group A (7.3 ± 1.3 mmol/l), Group B (8.2 ± 0.9 mmol/l), and Group C (9.9 ± 1.3 mmol/l). Likely, the mean level in Group C was significantly higher than Groups A and B (p<0.001). on the contrast, insignificant difference was found between Groups A and B (p=0.063).

Table (2): Comparison between the studied groups regarding Blood Glucose Profile

	Group A (n = 40)	Group B (n = 40)	Group C (n = 40)	Group D (n = 40)	P-value
FBG (mg/dl)					**< 0.001***
• **Mean ± SD**	140.63 ± 24.7	158.60 ± 41.5	165.25 ± 129.9	93.60 ± 6.1	
• **Median (R)**	136.5 (91-187)	153.5 (73-265)	142.5 (65-905)	93.3 (82-105)	
P-value**	A vs. B=0.162	B vs. C=0.178	**C vs. D<0.001**	**A vs. D<0.001**	
	A vs. C=0.960	**B vs. D<0.001**			
2HPP (mg/dl)					**< 0.001***
• **Mean ± SD**	211.50 ± 57.1	239.17 ± 66.9	226.10 ± 73.9	102.60 ± 4.5	
• **Median (R)**	211 (112-382)	236 (90-358)	220 (90-447)	102 (93-113)	
P-value**	A vs. B=0.174	B vs. C=0.624	**C vs. D<0.001**	**A vs. D<0.001**	
	A vs. C=0.573	**B vs. D<0.001**			
HbA1c (mmol/l)					**< 0.001***
• **Mean ± SD**	7.43 ± 1.3	8.18 ± 0.9	9.93 ± 1.3	4.11 ± 0.5	
• **Median (R)**	7.1 (5.2-9.9)	8.2 (6.6-10.6)	10 (7.1-12.6)	4.1 (2.9-5.7)	
P-value**	A vs. B=0.063	**B vs. C<0.001**	**C vs. D<0.001**	**A vs. D<0.001**	
	A vs. C<0.001	**B vs. D<0.001**			

*Kruskal Wallis test was used to compare the median difference between groups
**Post-hoc test was used for pairwise comparison using Tukey's Correction

Regarding lipid profile, the mean level of triglycerides, cholesterol, LDL showed significant (p<0.01) steady increase from control (145.6 ± 36, 156.7 ± 28.3, 63.9 ± 23.3 mg/dl), Group A (162.6 ± 19.2, 180.6 ± 36, 91.5 ± 23 mg/dl), Group B (192 ± 48.5, 195 ± 28.2, 92.8 ± 21.5 mg/dl,) and Group C (242.2 ± 75.9, 196.2 ± 33.5, 101.8 ± 25 mg/dl). On the other hand, there was insignificant difference in the mean HDL between the studied groups (p=0.556) **(Table 3).**

Table (3): Comparison between the studied groups regarding Lipid Profile

	Group A (n = 40)	**Group B (n = 40)**	**Group C (n = 40)**	**Group D (n = 40)**	**P-value**
S. TGD (mg/dl)					
• **Mean ± SD**	162.35 ± 19.2	192.01 ± 48.5	242.15 ± 75.9	145.59 ± 36.1	
• **Median (R)**	160 (109-211)	190 (107-283)	277 (120-450)	156 (79-217)	**< 0.001***
P-value**	**A vs. B=0.003**	**B vs. C=0.011**	**C vs. D<0.001**	A vs. D=0.220	
	A vs. C<0.001	**B vs. D<0.001**			
S. Cholesterol (mg/dl)					
• **Mean ± SD**	180.63 ± 36.1	195.02 ± 28.2	196.19 ± 33.5	156.74 ± 28.3	
• **Median (R)**	176 (123-270)	195 (135-393)	201 (138-270)	157 (89.5-226)	**< 0.001***
P-value**	**A vs. B=0.033**	B vs. C=0.923	**C vs. D<0.001**	**A vs. D=0.003**	
	A vs. C=0.042	**B vs. D<0.001**			
LDL (mg/dl)					
• **Mean ± SD**	91.49 ± 23.1	92.80 ± 21.5	101.76 ± 25.1	93.92 ± 23.3	
• **Median (R)**	91 (45-134)	90 (43.5-138)	101 (49-145)	56.5 (30-124)	**< 0.001***
P-value**	A vs. B=0.902	B vs. C=0.136	**C vs. D<0.001**	**A vs. D<0.001**	
	A vs. C=0.106	**B vs. D<0.001**			
HDL (mg/dl)					
• **Mean ± SD**	45.15 ± 10.6	48.01 ± 8.9	48.76 ± 17.9	45.80 ± 10.3	= 0.556*
• **Median (R)**	46 (19-74)	49 (29-68)	49 (12-86)	47 (20-66)	

*Kruskal Wallis test was used to compare the median difference between groups
**Post-hoc test was used for pairwise comparison using Tukey's Correction

Furthermore, **Table 4** showed the difference in the levels of renal function parameters and urinary NGAL. The mean level of creatinine and ACR found significant (p<0.001) steady increase from control (0.71 ± 0.1 and 9.9 ± 5.1), Group A (0.73 ± 0.1 and 22.1 ± 5.2), Group B (0.91 ± 0.2 and 101.3 ± 61.4) and Group C (0.98 ± 0.1 and 407.4 ± 110.6). Additionally, the mean level of GFR found significant (p<0.001) steady decrease from control (89.2 ± 4.5), Group A (83.6 ± 4.9), Group B (73.7 ± 5.1) and Group C (71.7 ± 5.2 ml/min/1.73 m2). The mean level of urinary NGAL was significantly (p<0.001) lower in the control (33.2 ± 16.7) compared with the three patient groups (Group A (55.8 ± 22.4), Group B (89.9 ± 33.7), and Group C (144 ± 50 ng/ml). Likely, in the patient groups, U. NGAL showed gradual increase according to group from Group A to Group C (p<0.05) **(Table 4).**

Table (4): Comparison between the studied groups regarding KFT and Urinary NGAL

	Group A (n = 40)	**Group B (n = 40)**	**Group C (n = 40)**	**Group D (n = 40)**	**P-value**
S. Creatinine (mg/dl)					
• **Mean ± SD**	0.73 ± 0.1	0.91 ± 0.2	0.98 ± 0.1	0.71 ± 0.1	
• **Median (R)**	0.7 (0.7-0.9)	0.9 (0.7-1.2)	0.9 (0.7-1.2)	0.7 (0.6-1.0)	**< 0.001***
P-value**	**A vs. B<0.001**	**B vs. C=0.039**	**C vs. D<0.001**	A vs. D=0.348	
	A vs. C<0.001	**B vs. D<0.001**			
ACR					
• **Mean ± SD**	22.13 ± 5.2	101.26 ± 61.5	407.40 ± 110.6	9.86 ± 5.1	
• **Median (R)**	23 (10.5-30)	63 (38-266)	362 (312-780)	9 (2-22)	**< 0.001***
P-value**	**A vs. B<0.001**	**B vs. C<0.001**	**C vs. D<0.001**	**A vs. D=0.001**	
	A vs. C<0.001	**B vs. D<0.001**			
GFR (ml/min/1.73 m^2)					
• **Mean ± SD**	83.53 ± 4.9	73.70 ± 5.1	71.70 ± 5.1	89.20 ± 4.3	
• **Median (R)**	83 (71-94)	74 (62-88)	71.5 (60-86)	89.5 (75-102)	**< 0.001***
P-value**	**A vs. B<0.001**	B vs. C=0.290	**C vs. D<0.001**	**A vs. D=0.008**	
	A vs. C<0.001	**B vs. D<0.001**			
NGAL (ng/ml)					**< 0.001***

• **Mean ± SD**	55.81 ± 22.4	89.85 ± 33.7	143.98 ± 50.1	33.15 ± 16.7	
• **Median (R)**	62.5 (17-86.5)	82.5 (45-166)	136.5 (66-260)	34 (9-70)	
P-value**	**A vs. B=0.001**	**B vs. C=0.001**	**C vs. D<0.001**	**A vs. D=0.003**	
	A vs. C<0.001	**B vs. D<0.001**			

*Kruskal Wallis test was used to compare the median difference between groups
**Post-hoc test was used for pairwise comparison using Tukey's Correction

Regarding the correlation between U. NGAL and other parameters, in group A, no significant correlation was found (p >0.05). Conversely, in group B, there was significant positive very high correlation between U. NGAL and ACR (r=0.880, p<0.001) (Fig. 2). Also, in group C, there was significant positive moderate correlation between U. NGAL and ACR (r=0.508, p<0.001) (Fig. 3). Similarly, in control group, there was significant positive moderate correlation between U. NGAL and ACR (r=0.447, p=0.004) **(Fig. 4).**

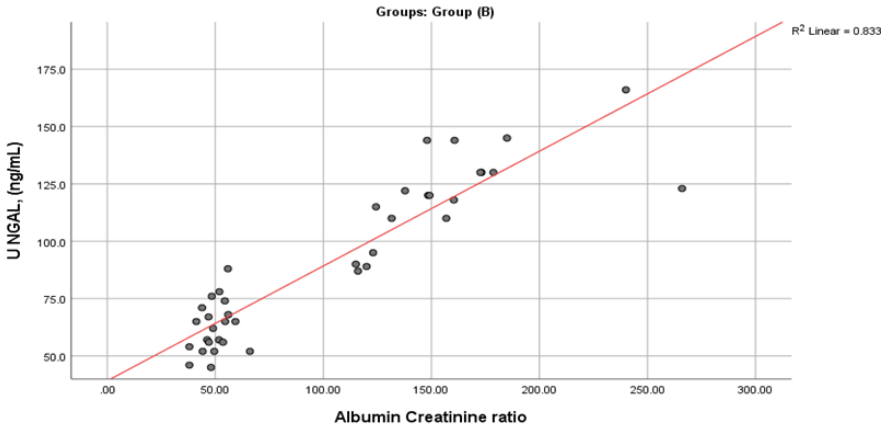

Fig. 2: Correlation between U. NGAL with ACR in group A.

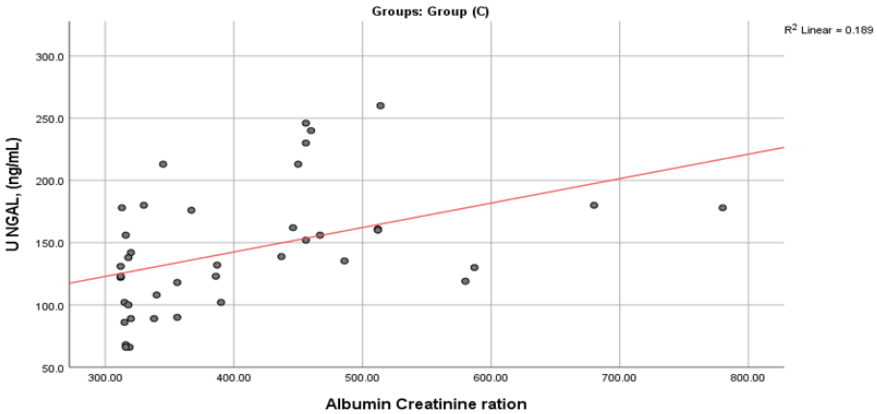

Fig. 3: Correlation between U. NGAL with ACR in group B.

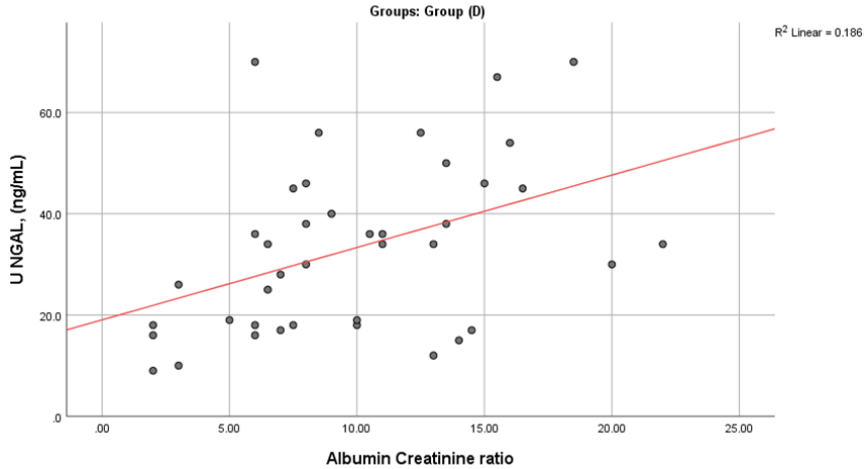

Fig. 4: Correlation between U. NGAL with ACR in group C.

By using ROC-curve analysis, U. NGAL was found to be significant predictor of DKD at a cut-off of 56 ng/ml. It showed excellent predictive power (AUC=0.911, 95% CI: 0.801-0.998) with accuracy, sensitivity, specificity, PPV and NPV of 86%, 78.3%, 92.5%, 91.3% and 81%, respectively (p<0.001) **(Fig. 5).**

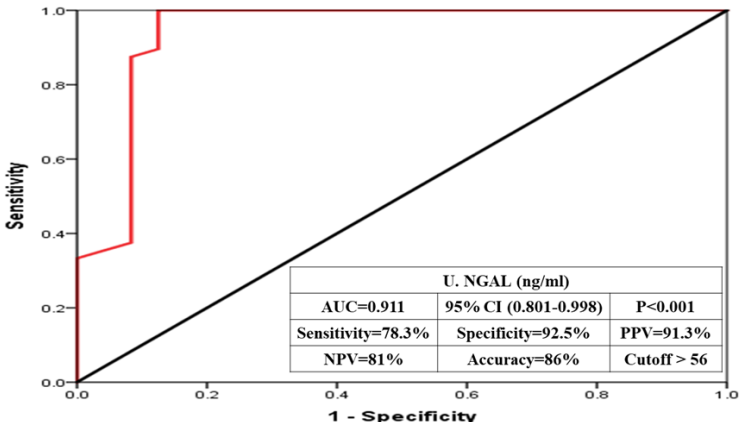

Fig. 5: ROC curve of U. NGAL in diagnosis of diabetic kidney disease

4. DISCUSSION

A major microvascular complication of DM is diabetic nephropathy (DN), which leads to ESRD and is associated with increased cardiovascular mortality. Moderately increased albuminuria (formerly called microalbuminuria) can be described as an increase in the level of albumin in urine below the clinical albuminuria levels and is considered an early sign of DN. At the stage of moderately increased albuminuria with euglycemic control, DN can be reversible. For this reason, it is important to detect nephropathy at or prior to this stage (15).

Numerous biomarkers have been investigated for risk stratification of DKD. One promising marker is NGAL, elevated s-NGAL levels have been reported in several clinical conditions such as AKI, CKD, sepsis, and neoplasm (16). Along with urine NGAL, s-NGAL has recently been regarded as a sensitive early marker of AKI (17). The mechanism underlying elevated s-NGAL levels in AKI is due to NGAL overexpression in distal nephrons as well as impaired tubular reabsorption (18). In T2DM patients, several studies have shown that the s-NGAL level is significantly higher in T2DM patients than in healthy controls (19). Some T2DM studies have also shown that s-NGAL levels were higher in patients with microalbuminuria than in those with normoalbuminuric (20).

The present study showed that there were no significant differences between the four studied groups as regard age and sex. In agreement with the current study **Abd El Kader et al.**(21) reported that there was no statistically significant difference between diabetic normo-albuminuria, microalbuminuria, macroalbuminuria and healthy control groups as regard age and sex. Also, in agreement with our results **Kaul et al.**(22) found that there was no significant difference between the groups for age and sex. As well, **Motawi et al.**(20) compared T2DM patients with normoalbuminuric, with microalbuminuria and healthy controls and found that there was no significant difference between groups as regard age and sex.

Regarding DM duration, it we found that the mean duration was increasing steadily with the progression of albuminuria. In accordance with this, **Kaul et al.**(22) revealed that there was significant difference between normo-albuminuria, microalbuminuria, and macroalbuminuria groups as regard DM duration, as the duration increased with the progression of albuminuria. Also, **Vijay et al.**(23) revealed that the duration of diabetes was significantly different in patients with and without microalbuminuria. As well, **Motawi et al.**(20) reported that the duration of diabetes was significantly longer in patients with microalbuminuria than those with normo-albuminuria. Furthermore, our results were supported by **Al-Hazmi et al.**(24) reported that there was statistically significant association between DM duration with the severity of albuminuria.

Regarding glycemic profile, the current study showed that there was statistically significant difference between the studied groups as regard FBG, 2HPP and HbA1c and pairwise comparison showed that the progression of albuminuria was associated with glycemic profile worsening. **Kaul et al.**(22) found similar results, revealed that there was significant difference between groups as regard HbA1c and it increased with the progression of albuminuria. Also, **Motawi et al.**(20) reported comparable results. As well, **Al-Hazmi et al.**(24) reported that the mean levels of FBG and HA1c were significantly higher in all the 3 diabetic groups as compared to the control

($p<0.001$). Our results were also supported by **Vijay et al.** (23).

Respecting lipid profile, the current study declared that there was significant difference between the groups per triglyceride, cholesterol, and LDL, but there was insignificant difference for HDL. Pairwise comparison showed that the progression of albuminuria was associated with the increase of triglyceride, cholesterol, and LDL. The current results were supported by **Kaul et al.** (22), **Motawi et al.** (20),

Al-Hazmi et al. (24). Furthermore, **Siddiqi et al.** (25) revealed that TG and LDL were significantly higher in cases with albuminuria and HDL was significantly lower in cases with albuminuria.

Additionally, the current study showed that there was significant difference between the studied groups as regard s. creatinine, ACR and GFR. Pairwise comparison showed that the mean renal function parameters increased steadily as diabetic nephropathy progressed. **Abd El Kader et al.** (21), **Kaul et al.** (22), **Al-Hazmi et al.** (24) and **Siddiqi et al.** (25) concluded that there was statistically significant difference between the studied groups as regard S. creatinine, Albumin Creatinine ratio and GFR. The renal functions test results were worsened with the progression of albuminuria.

Regarding NGAL, the current study showed that the mean level of NGAL was 55.8 ± 22.4, 89.9 ± 33.7, 144 ± 50 and 33.2 ± 16.7 ng/mL, in group A, group B, group C and group D, respectively. It was found that there was significant difference between the four studied groups regarding level of NGAL (p-value was <0.001) and pairwise comparison showed that the mean level of NGAL increased steadily as diabetic nephropathy progressed, with statistical difference among four groups.

In agreement with the current study **Abd El Kader et al.** (21) showed that there was significant difference between control, normo-, micro- and macro-albuminuria diabetic patients regarding s-NGAL and u-NGAL), where their levels incremented parallel to the degree of albuminuria. Also, this study results were supported by **Kaul et al.** (22), **Vijay et al.** (23), **Siddiqi et al.** (25) and **Al-Hazmi et al.** (24) revealing that compared with healthy control, diabetic patients with normal albuminuria excreted significantly higher levels of u-NGAL ($p<0.001$). In addition, significantly elevated u-NGAL, was observed in moderately increased albuminuria and severely increased albuminuria groups when compared to the control and normoalbuminuric groups ($p<0.001$).

Regarding the Correlation between U. NGAL and different variables, the current results showed that in diabetic groups there was significant positive moderate to very high correlation between U. NGAL and ACR. Similar results were found by **Abd ElKader et al.**(21), **Al-Hazmi et al.**(24), **Kaul et al.** (22) and **Motawi et al.** (20).

Further, U. NGAL was found to have excellent predictive power for DKD with sensitivity, specificity, PPV and NPV was 78%, 92.5%, 91% and 81%, respectively.

This was supported by **Abd El Kader et al.** (21) who revealed that Cutoff values of normal NGAL: s-NGAL (142 ng/ml) and u-NGAL (78ng/ml). s-NGAL with a cutoff value of ≥197.55 (ng/dl) can diagnose early non-albuminuria DN with sensitivity of 100%, and specificity of 88%, PPV of 89.3%, and NPV of 100%. U-NGAL with a cutoff value of ≥ 681.095 (ng/dl) can diagnose early non-albuminuria DN with sensitivity of 100%, and specificity of 92 %, 93% PPV and 100%NPV. Also, **Kaul et al.** (22), **Vijay et al.** (23) found resembling results.

A systematic review and meta-analysis by **He et al.** (26) included 19 studies aimed to evaluate the diagnostic value of NGAL for DKD. It was found that the s-NGAL had a pooled sensitivity and specificity of 0.79 (95% CI: 0.60–0.91) and 0.87 (95% CI: 0.75–0.93). For u-NGAL, the pooled sensitivity, specificity was 0.85 (0.74–0.91) and 0.74 (0.57–0.86).

5. Conclusionand Recommendations

In conclusion, tubular injury may precede glomerular injury in diabetic patients and as NGAL is a tubular marker NGAL is superior to ACR as an early predictor of DKD among T2DM patients as there was significant difference between non-albuminuria diabetics and non-albuminuria healthy individuals. U-NGAL can be used to predict and follow up progression of DKD as they correlate with DKD severity. Poor glycemic control has a significant correlation with progression of DKD, proven by presence of significant difference between the diabetic groups with different stages of albuminuria with HbA1c.

Further studies with larger sample size, longer follow-up, multicenter are needed to confirm our results and to identify risk factors of adverse events. Also, NGAL either alone or in combination with other diagnostic modalities is recommended to be used as biomarker for DKD for follow up for improvement of patients.

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Zein El-Abdeen A. Said (ZAS); design, literature search, manuscript preparation and review,
Omaima M. Ali (OMS); clinical studies, manuscript editing and final draft,
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