The Egyptian Journal of Hospital Medicine (April 2019) Vol. 75 (2), Page 2276-2284

Role of Kidney Injury Molecule-1 and β2-Microglobulin in Early Diagnosis of Diabetic Nephropathy

Glonar Otiefy Ahmed, Amal Abdel Aleem Morsy and Samah Roshdy Abo El Eneen Abo El Eneen *
Clinical and Chemical Pathology Department, Faculty of Medicine, Al-Azhar University
***Corresponding Author:** Samah Roshdy Abo El Eneen Abo El Eneen, **Phone No.:** (+2) : 01090600157

ABSTRACT

Background: Diabetes mellitus has been described as a metabolic disorder of multiple etiology characterized by chronic hyperglycemia with disturbances of carbohydrate metabolism. Diabetic nephropathy is typically characterized by a gradual increase in urinary albumin excretion. Albuminuria, a marker of glomerular involvement in early renal damage, cannot always detect early diabetic nephropathy (DN).

Aim of the Work: Is to determine the suggested promising diagnostic role of kidney injury molecule-1 (KIM-1) level and β2 microglobulin and how could improve the early diagnosis, predict disease progression and deliver new insights in pathogenic of diabetic nephropathy.

Subjects and Methods: this study included 80 subjects. Group I: sixty (60) patients with T2DM classified into 3 subgroups according to the level of albumin/creatinine ratio (ACR). Thirty (30) diabetic patients with normoalbuminuria (1a), 20 diabetic patients with microalbuminuria (1b) and 10 diabetic patient with macroalbuminuria (1c). Group II: Twenty, age and sex matched, apparently healthy individuals serving as a control group. **Results:** Our results revealed a statistical significant increase in FBS, blood urea, as well as microalbuminuria with GFR decline in patients group when compared to control group. However, serum levels of creatinine were only significantly elevated in diabetic patients with ACR ≥300 mg/g when compared to control group. The results revealed a highly statistically significant increase in urinary KIM and β2 microglobulin levels in micro than normo and in macro than micro albuminuric group.

Conclusion: Our data revealed that tubular biomarkers were increased in T2DM patients with normoalbuminuria when compared with controls.

Keywords: Kidney Injury Molecule-1 - β2-Microglobulin - Diabetic Nephropathy.

INTRODUCTION

Diabetes mellitus (DM) has been described as a metabolic disorder of multiple pathogens characterized by chronic hypoglycemia with metabolic disorders of carbohydrates caused by insulin secretion defects, insulin action or both. Type 2 diabetes includes individuals with insulin resistance (IR) and usually insulin (and not absolute) deficiency [(1)].

Diabetic nephropathy is a chronic condition that develops over many years and is characterized by a gradual increase in urinary albumin secretion. DN is one of the severe complications that occur in diabetics and is associated with an increased risk of death from all causes, cardiovascular disease and progression to end stage renal disease (ESRD), requiring costly renal replacement therapy in the form of dialysis or transplantation[(2)].

There is a preclinical stage of diabetic nephropathy, characterized by urinary albumin excretion rate that are not detectable by standard laboratory methods unless it is in excess of 300 mg/day; that is distinctly abnormal. The range (30-300) mg/day has been referred to as microalbuminuria and is the first laboratory evidence of diabetic renal disease [(3)].

In clinical practice, most commonly used markers of renal disease and progression of DN are serum creatinine, estimated glomerular filtration rate, and proteinuria or albuminuria[(4)]. Albuminuria, a marker of glomerular involvement in early renal damage, cannot always detect early DN. Thus, more sensitive and specific markers in addition to albuminuria are needed to predict the early onset and progression of DN [(5)].

Significant efforts have been made to identify serum or urine biomarkers which can be clinically detected in early stages of DN and progressive kidney function decline in diabetic patients. The research community is focusing on a different strategy to enhance the sensitivity of biomarkers to predict patients who will develop DN or are at risk of progressing to ESRD [(6)].

Kidney injury molecule-1 (KIM-1), a discovered transmembrane tubular protein, is markedly induced in renal injury including acute kidney injury (AKI) and chronic kidney disease (CKD). There are many characteristics of KIM-1 making it an ideal biomarker for kidney injury. For example, KIM-1 is not expressed in normal kidney but specifically expressed in injured proximal tubular cells, and such an expression can persist until the damaged cells have completely recovered [(7)].

Beta 2 microglobulin is a low molecular weight protein that is released at a constant rate and is filtered by the glomerulus, absorbed and hardened by ductile tubes. Therefore, it is theoretically considered an appropriate biomarker for kidney weakness. In fact, tubular involvement may precede glomerular involvement because many of these proteins and tubular enzymes can be detected even before the

Received:27/1/2019
Accepted:27/2/2019

appearance of a small amino acid and rise in serum creatinine [4].

AIM OF THE WORK

Is to determine the suggested promising diagnostic role of KIM-1 level and β_2 microglobulin and how could improve the early diagnosis, predict disease progression and deliver new insights in pathogenesis of diabetic nephropathy.

SUBJECTS AND METHODS

This study was conducted on 80 subjects. They were divided into sixty (60) patients with T2DM diagnosed according to **American Diabetic Association (ADA) Guidelines** [8] and twenty (20) age and sex matched apparently healthy controls. The patients were recruited from outpatient's clinic and inpatients of Endocrinology Department at Al-Zahraa University Hospital, from 8/2016 to 4/2017.**On the 2-basis of albumin/creatinine ratio (ACR),** patients were categorized into three subgroups. **Subgroup (1a)**: (30) patients with normoalbuminuria (ACR<30 mg/g), **subgroup (1b)**: (20) patients with microalbuminuria (ACR 30 – 299 mg/g) and subgroup **(1c)**: (10) patients with macroalbuminuria (ACR ≥300 mg/g).

Exclusion Criteria:

Patients on renal replacement therapy (hemodialysis or peritoneal dialysis) and patients with causes of nephropathy rather than diabetes. In addition patients with active inflammatory disease including pneumonia, urinary tract infection, endocarditis, rheumatoid arthritis and cancer were all excluded from the study.

Ethical approval:

Our study was approved by the Researches Ethics Committee at Faculty of Medicine, Al-Azhar University. Written consent was obtained from all patients before the study, all individuals included in this study were subjected to the following:

1- **Full history taking and thorough clinical examination. 2- Laboratory investigations which included:** The analytical assay for serum fasting glucose, 2hpp blood glucose, urea, creatinine, calculation of urinary albumin creatinine ratio (ACR) were done on Cobas c311 auto–analyzer system, using commercial kits supplied by Roche Diagnostics, glomerular filtration rate by creatinine clearance, urine examination by dipsticks, and urinary KIM-1 and β_2 microglobulin by enzyme linked immunosorbent (ELISA) method.

Sampling:

1- **Urine Samples:**

Morning mid-stream urine samples were obtained from all studied subjects, using disposable clean dry cups without preservatives. Every specimen was divided into 2 portions. The first portion was for immediate urine examination by dipstick test for urine analysis. Test results were used as a primary rough method for detection of normoalbuminuria, microalbuminuria, and macroalbuminuria, and then urinary protein level was accurately determined by albumin /creatinine ratio. The second portion was centrifuged at 600 rpm for 10 minutes, the supernatant was separated and divided into 2 Eppendorfs and refrigerated at -20°C to be used for the assay of urinary (u) KIM-I and u β_2 microglobulin. The 24 hours urine samples were used for estimation of urinary. Patients were instructed to record the start and end time before starting urine collection. Two milliliter (2 ml) of 24 hour voided urine samples was taken. Samples have been centrifuged at 1000 rpm for 10 minutes and used for estimation of creatinine in urine.

Venous Blood Samples:

About five milliliters (5 ml) of venous blood were collected under complete aseptic condition from all included subjects in the study after fasting (6-8) hours. The withdrawn samples were left for 30 minutes in water bath at 37°C then centrifuged at 1500 rpm for 15 minutes. Serum was separated and used for subsequent assay of urea, creatinine, and fasting blood glucose. Another sample was taken later on from each subject for estimation of 2hpp blood glucose.

Statistical analysis

All statistical calculations were done using computer program SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) release 15 for Microsoft Windows (2006).

- Descriptive statistics of the different studied groups were done using the mean and standard deviation for parametric data (age, duration of disease, FBS, 2hpp, creatinine, creatinine clearance, uKIM-1 and uβ2 microglobulin). While median and inter-quartile range (IQR) were used for non-parametric data (urinary albumin and albumin /creatinine ratio). **Student t"** test value was used to assess the statistical significance of the difference between two study group means[9]**,** was used to assess the statistical significance difference between more than 2 study group means. **Kruskall Wallis Test:** was used in case of non-parametric data. **Post-hoc Test:** was used for comparisons of all possible pairs of group means. Chi^2 test was used to compare qualitative data. Spearman correlation coefficient was used for correlations between variables. P value ≤ 0.05 were considered statistically significant. **Receiver Operating Characteristic-curve (ROC-curve):** was used to assess the diagnostic performance of the studied parameter for discriminating patients from controls [10]**.** Chi-square (x^2) test of significance was used in order to compare proportions between two qualitative parameters.

RESULTS

Statistical comparison for age and sex (**Table 1)** revealed no statistically significant difference (p> 0.05).

Table (1): Comparative statistics between studied patients' subgroups as regards age and sex

		Normo albuminuria (1a)	**Micro albuminuria (1b)**	**Macro albuminuria (1c)**	**Control group**	**Test value**	**P-value**	**Sig.**
		No.= 30	**No.= 20**	**No.= 10**	**No.= 20**			
Age (years)	Mean±SD	49.03 ± 7.74	50.40 ±7.04	49.70 ± 5.98	45.90 ± 8.25	1.344•	0.266	NS
	Range	35 – 67	40 – 60	40 – 60	36 – 60			
Sex	Male	14 (46.7%)	6 (30.0%)	5 (50.0%)	11 (55.0%)	2.761*	0.430	NS
	Female	16 (53.3%)	14 (70.0%)	5 (50.0%)	9 (45.0%)			

*: Chi-square test; •: One Way ANOVA Test, P-value > 0.05 Non significant (NS)

Statistical comparison for duration of disease between patients' subgroups (**Table 2)** revealed statistically significant difference (p>0.05). Post-hoc test was used and **revealed a significant difference between normo- and** microalbuminuria**.** There was no statistically significant difference between micro and macroalbuminuria subgroups (p> 0.05).

Table (2): Comparative statistics between studied patients' subgroups for duration of disease using ANOVA test

		Normoalbuminuria (1a)	**Microalbuminuria (1b)**	**Macroalbuminuria (1c)**	**Test value**	**P-value**	**Sig.**
		No.= 30	**No.= 20**	**No.= 10**			
Duration of disease (year)	Mean±SD	10.23 ± 4.50	12.40 ± 4.41	13.70 ± 4.08	3.409	0.04	S
	Range	5 – 25	6 – 20	8 – 20			
	Range	5 – 26	27 – 272	310 – 500			
Post hoc analysis by LSD							
	Normo vs micro		**Normo vs macro**	**Micro vs macro**			
Duration of disease (year)	0.016		0.131	0.683			

P-value <0.05: Significant (S)

Comparative statistics of biochemical parameters (FBS, 2hpp, urea and creatinine) between patients' subgroups (T**able 3)** revealed a highly statistically significant increase in FBS and urea. But creatinine and 2hpp revealed no statistical significance difference between the studied groups. Post-hoc test was used and reveled significant difference between normo- and macroalbuminuria and between micro- and macroalbuminuria as regard FBS. Also the test revealed a significant difference between normo and micro-, normo- and macro- and between micro- and macroalbuminuria respectively.

Table (3): Comparative statistics of biochemical parameters (FBS, 2hpp, urea and creatinine) between patients subgroups using ANOVA test

		Normo albuminuria	**Micro albuminuria**	**Macro albuminuria**	**Test value**	**P-value**
		No.= 30	**No.= 20**	**No.= 10**		
FBS (mg/dl)	Mean ± SD	147.83 ± 7.61	150.60 ± 5.05	198.50 ± 7.14	3.371•	0.041
2hpp (mg/dl)	Mean ± SD	176.37 ± 4.88	188.75 ± 10.44	244.00 ± 7.79	2.149•	0.126
Urea (mg/dl)	Mean ± SD	20.63 ± 5.99	26.95 ± 1.18	35.40 ± 7.51	7.978•	0.001
Creatinine (mg/dl)	Mean ± SD	0.75 ± 0.1	0.75 ± 0.1	0.94 ± 0.08	1.151•	0.324
Post hoc analysis by LSD						
	Normal vs micro		**Normal vs macro**	**Micro vs macro**		
FBS (mg/dl)	0.863		0.001	0.029		
Urea (mg/dl)	0.040		0.001	0.040		

P-value >0.05: Non significant (NS); P-value <0.05: Significant (S); P-value< 0.01: highly significant (HS)
•: One Way ANOVA test

Table (4) and figure (1): shows comparative statistics of creatinine clearance, albumin in urine and albumin creatinine ratio between patients' subgroups. It revealed a highly statistically significant decrease in creatinine clearance. Albumin in urine and albumin/creatinine ratio revealed a highly statistically significant increase. Post-hoc test was used and revealed a significant difference between normo- and microalbuminuria and between normo- and macroalbuminuria as regard creatinine clearance. However, no significant difference was observed among micro- and macroalbuminuria as regard creatinine clearance. Also the test revealed a significant difference between normo- and micro-, normo- and macro and between micro- and macroalbuminuria as regard albumin in urine and albumin/creatinine ratio**.**

Table (4): Comparative statistics of creatinine clearance, albumin in urine and albumin creatinine ratio between patients' subgroups

		Normo albuminuria	**Micro albuminuria**	**Macro albuminuria**	**Test value**	**P-value**
		No.= 30	**No.= 20**	**No.= 10**		
Creatinine clearance (ml/min)	Mean ± SD	90.13 ± 6.96	67.70 ± 7.87	62.20 ± 8.74	13.498•	0.001
Albumin in urine (mg/24h)	Median (IQR)	8.5(6-13)	72(39.5-100)	375(320- 415)	49.250≠	0.001
Albumin/creatinine ratio	Median (IQR)	12(7-16)	67.5(48-100)	804.5(410 -945)	49.208≠	0.001
Post hoc analysis by LSD						
	Normo vs micro		**Normo vs macro**		**Micro vs macro**	
Creatinine clearance (ml/min)	0.001		0.003		0.473	
Albumin in urine (mg/24h)	0.001		0.001		0.001	
Albumin/ creatinine ratio u KIM (ng/L)	0.026		0.001		0.001	

≠: Kruskal–Wallis; •: One Way ANOVA Test, P-value > 0.05 Non significant, P-value < 0.05 Significant, P-value < 0.01 Highly significant

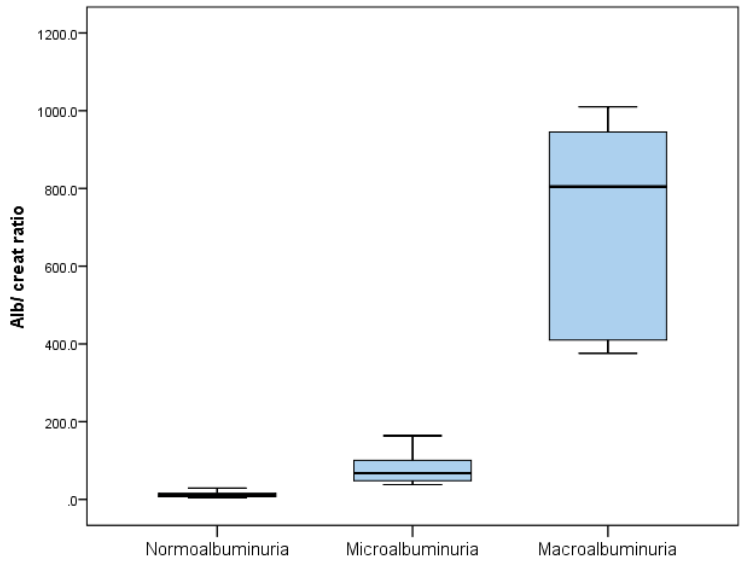

Figure (1): Box–plot chart showing the median value of albumin creatinine ratio in patient subgroups.

Table (5) revealed a highly statistically significant increase in u KIM and u β_2 Micro-globulin ($p<0.001$). Post-hoc test was used and revealed significant difference between normo and microalbuminuria, normo and macro and between micro and macroalbuminuria as regard u β_2 microglobulin and u KIM ($p<0.001$).

Table (5): Comparative statistics of u KIM and u β_2 microglobulin between patients' subgroups

		Normo albuminuria	**Micro albuminuria**	**Macro albuminuria**	**Test value**	**P-value**
		No.= 30	**No.= 20**	**No.= 10**		
	Range	4 – 29	38 – 244	376 – 1010		
u KIM-1 (ng/L)	Mean ± SD	27.60 ± 5.33	40.05 ± 6.83	78.30 ± 3.34	165.888•	0.001
u β_2 Microglobulin (mg/ml)	Mean ± SD	2.97 ± 0.96	3.95 ± 0.85	5.50 ± 0.22	10.397•	0.001
Post hoc analysis by LSD						
		Normal vs micro	**Normal vs macro**	**Micro vs macro**		
u KIM-1 (ng/L)		0.001	0.001	0.001		
u β_2 Microglobulin (mg/ml)		0.032	0.001	0.012		

One Way ANOVA Test , P-value > 0.05 Non significant , P-value < 0.05 Significant

Table (6) and figure (2) shows correlation study between u KIM-1 and all studied parameters in group 1a using Spearman correlation coefficient. The data revealed significant positive correlation between u KIM1 and albumin in urine ($p<0.012$).

Table (6): Correlation between u KIM-1 and all studied parameters among subgroup 1a

	u. KIM (ng/L)	
	rs *	**P-value**
u KIM1 (ng/L)	–	–
u. β2 microglobulin (mg/ml)	-0.433	0.056
Age (years)	0.065	0.785
Duration of disease (year)	0.262	0.265
FBS (mg/dl)	0.305	0.191
2hpp (mg/dl)	0.098	0.680
Urea (mg/dl)	0.088	0.714
Creatinine (mg/dl)	0.107	0.654
Creatinine clearance (ml/min)	-0.063	0.793
Albumin in urine (mg/24h)	0.452*	0.012
Albumin/creatinine Ratio	-0.212	0.369

rs*: Spearman's correlation coefficient.

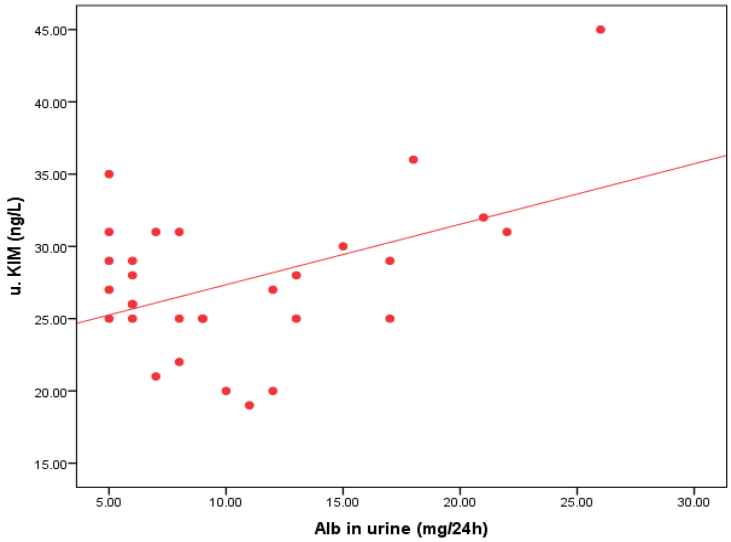

Figure (2): Positive correlation between u KIM1 and urinary albumin in subgroup Ia.

Table (7) and figure (3) shows ROC-AUC, which denoted sensitivity for diagnosis of DN among T2DM patients at cutoff value > 18 ng/L with sensitivity, specificity, PPV and NPP values= 100%. The curve showed urinary KIM-1 is an excellent biomarkers (AUC=1.000).

Table (7): The diagnostic performance of u.KIM-1 (ng/L) and β_2 microglobulin (mg/ml) in discriminating patients group from control group

Parameter	AUC	Cutoff Point	Sensitivity	Specificity	PPV	NPV
KIM (ng/L)	1.000	>18	100.00	100.00	100.00	100.00
β_2 Microglobulin (mg/ml)	0.983	>1.5	93.33	100.00	100.00	83.3

AUC: Area under the curve. PPV: Positive predictive value. NPV: Negative predictive value.

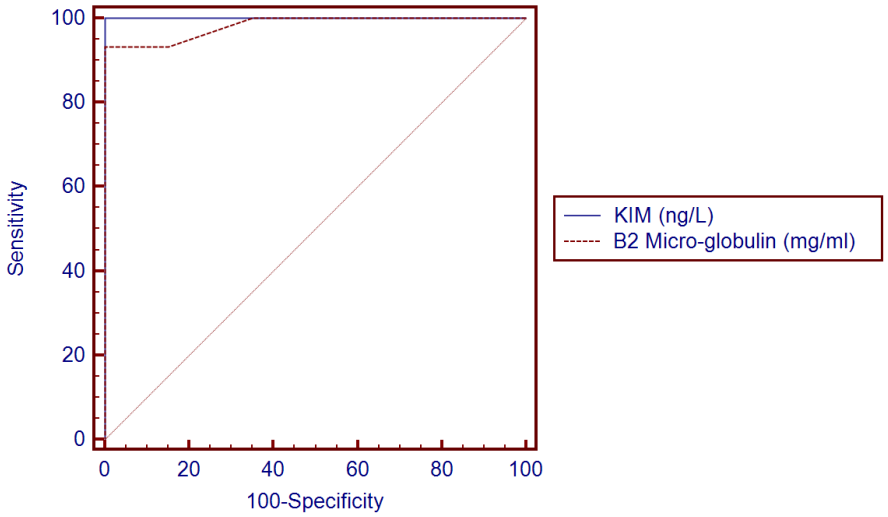

Figure (3): Receiver operating characteristic curve (ROC) analysis showing the diagnostic performance of u KIM-1 and u β2 microglobulin for discriminating patients groups from control group.

DISCUSSION

Diabetes mellitus (DM) has been described as a metabolic disorder of multiple pathogens with chronic hyperglycemia with disorders of carbohydrate metabolism caused by defects in insulin secretion, insulin action or both. Type 2 diabetes includes individuals with insulin resistance (IR) and insulin deficiency (not absolute). DM complications cause increased morbidity, disability and mortality and pose a threat to the economies of all countries, especially developing countries[(11)].

Diabetic nephropathy (DN) is a chronic condition that develops over many years, and is typically characterized by a gradual increase in urinary albumin excretion. DN is a severe complication occurring in diabetic patients and it is associated with an increased risk of all-cause mortality, cardiovascular disease and progression to ESRD, requiring costly renal replacement therapy in the form of dialysis or transplantation [(2)].

Microalbuminuria predicts the onset and progression of diabetic nephropathy. Despite its use as the conventional glomerular biomarker for early detection of diabetic kidney disease, its predictive accuracy is not optimal. Since tubular injury occurs early in the course of diabetic nephropathy, tubular biomarkers should be more sensitive than microalbuminuria as early predictors of the disease [(12)]. Tubular biomarkers can serve as much earlier predictors of diabetic nephropathy than glomerular biomarkers because tubulointerstitial lesions are associated with and may actually precede glomerular injury in the disease [(13)].

The pathological basis of elevated urinary albumin excretion is mainly caused by protein glycation with advanced glycation end products (AGEs) and their deposition, which results in hypertrophy of glomerular and renal system. This in turn leads to the leakage of albumin, the continuous persistent leakage of this protein into urine result in overt DN [(14)].

Studies have shown that in the pathophysiology and progression of diabetic nephropathy not only glomerular but also tubulointerstitial damage is important factors. Some of the first tubular markers suggested were α1 and β2 microglobulin and since then, other markers of tubular damage have been investigated [(15)]. From both experimental and clinical studies KIM-1 is closely related to specific tubular damage and furthermore reflected by the urinary

excretion of KIM-1, as has been confirmed by biopsy studies [16].

The β_2-M readily filtered through the glomerulus and almost completely reabsorbed by the proximal tubular cells where it is metabolized. Plasma concentration not affected by muscle mass or by sex of individuals. Increase in urinary β_2-M indicates tubular dysfunction, and measurement of β_2-M in urine is a sensitive and reliable assay for detecting tubular injury [15].

In our study there were no statistically significant differences between patients subgroups and control group as regard age and sex ($p>0.05$).

Serum creatinine is a measure of kidney function not injury, and it is a late marker for an injury as more than 50% of nephrons must be compromised before changes in the serum creatinine level become evident[17] .

Our results revealed a statistical significant increase in FBS, blood urea, as well as microalbuminuria with GFR decline in all patients groups when compared to control group. However, serum levels of creatinine were only significantly elevated in diabetic patients with ACR ≥300 mg/g when compared to control group. Our result was in contrast to the studies of **Sheik *et al.*** [18], **Mussap *et al.*** [19] **and El-Attar *et al.*** [20] on T2 diabetic patients. They reported that the decline in renal function in type 2 diabetic patients leads to a reduction in GFR and in a proportional increase in microalbuminuria. There was broad acceptance of microalbuminuria as a marker of increased DN risk. However, assessment of microalbuminuria cannot replace the GFR estimation, because they may represent different aspects of renal damage. Besides, albumin excretion rates are altered by variations in blood pressure and exercise as well as blood glucose levels and there is an intra-individual variability during the evolution of albuminuria, and day-to-day variation[20].

Microalbuminuria is a late manifestation in the course of DN. Its presence is indicative of stage III DN. Furthermore, microalbuminuria is not specific for diabetes or early nephropathy alone but is considered to reflect generalized vascular damage [3].

Diabetic nephropathy is characterized by the presence of large amount of urinary proteins, mostly albumin. In the present study, the progression to micro or macroalbuminuria was more frequent in type 2 diabetic patients. The significant increase in microalbuminuria in T2DM patients with nephropathy was consistent with **Maahs *et al.*** [21], who reported that microalbuminuria can predict the progression to DN. DN starts to develop when urinary albumin excretion values are still within the normoalbuminuric range

In our study there was significant difference in duration of disease between patients' subgroups, macroalbuminuric group showed significant increase in the duration of diabetes when compared to both normoalbuminuric and micro groups. This findings was in line with **Kondaveeti *et al.*** [22], who found that there was a direct relation between the duration of diabetes and the development of microalbuminuria, because long-standing hyperglycemia results in mesangial expansion.

Routinely used measures of renal function, such as levels of blood urea and serum creatinine, increase significantly only after substantial kidney injury occurs and then with a time delay so, sensitive and specific biomarkers are needed to detect early kidney injury. Urine has been examined as a source for biomarkers given its easy availability and reduced complexity when compared with serum [23].

The KIM-1 is a type 1 transmembrane glycoprotein (339 aa). KIM-1 ectodomain is cleaved and shed in a metalloproteinase-dependent fashion. The soluble KIM-1 protein that appears in the urine of humans is about 90 Kd[7].

In our study we found significant increase in urinary KIM-1 level in patients' groups compared to the control group, this results was in agreement with **Van Timmeren *et al.*** [24], who found an increased u-KIM-1 level in diabetic patients compared to control group. Also our result was in line with **Fu *et al.*** [25], who reported higher urinary uKIM-1 in 101 patients with T2DM observed for 5 years as compared with the control group.

In the present study, there was a significant increase in urinary KIM-1 levels in diabetic patients with microalbuminuria and macroalbuminuria than normoalbuminuria patients and control. Our result was in accordance with the studies of **Garg *et al.***[26] and **Petrica *et al***[27] who found that urine KIM-1 levels were elevated more in diabetic patients with microalbuminuria and macroalbuminuria than diabetic with normoalbuminuric. The significant increase of KIM-1 in normo-, micro- and macroalbuminuric groups than controls group is due to injury of proximal tubules with shedding of KIM-1 in urine during tubular injury making it readily detectable in the urine of diabetics

Also our results were matching with **Nielsen *et al.*** [14], in his study he worked on 177 diabetic T2 patients and subdivided into 3 group normoalbuminuric, microalbuminuric and

macroalbuminuric groups. Patients with macroalbuminuric had higher levels of u KIM-1 than normoalbuminuric and microalbuminuric patients.

Our results showed a significant correlation between urinary KIM-1 and albumin in urine of patients with T2DM. This result was in line with **Nielsen *et al.* [14]** who reported that urinary KIM-1 concentration was increased in T2DM patients with normoalbuminuria. So, urinary KIM-1 levels are strongly correlated with nephropathy. Moreover **Conway *et al.* [28]** reported that uKIM-1 was correlated with stringency of glycemic control.

In agreement with our work, **Waanders *et al.* [29],** found a correlation of the KIM-1 and albuminuria in T2DM. However **Zhang *et al.* [30],** found that KIM-1 correlated positively with interstitial damage, inflammation, and serum creatinine, but did not correlate with albuminuria. To explain this, it was suggested that not all albuminuria is accompanied by tubulointerstitial damage and progressive decline in renal function.

Urinary beta-2-microglobulin (β2-M) was investigated in this study as a potential biomarker in the detection of early nephropathy in type 2 diabetes. Researchers found that evaluation of GFR and urinary albumin excretion are not ideal for determining renal damage in diabetic subjects and about 20% of patients with diabetic nephropathy remain normoalbuminuric despite a reduction in GFR **[32].**

In our study the level of u β2-microglobulin was significantly higher in the diabetic patients than in controls. Our finding agree with **Apakkan, [31],** who found that u β2-microglobulin was significantly higher in the diabetic patients than in controls. Also increased u β2-microglobulin levels in diabetic patients were reported by **Ekrikpo *et al* [32].**

Our study revealed that u β2-microglobulin excretion was significantly higher in the patients, while albumin excretion was still in normal range in the urine of diabetic patients, which indicated that the increase in urinary β_2-M precedes the stage of albuminuria**.** Also urinary excretion of β_2-M was significantly higher in the patient with normoalbuminuria than the controls, indicating the presence of tubular injury in early diabetic patients. In addition, urinary excretion of β2-microglobulin increased progressively from normoalbuminuria to macroalbuminuria, indicating its value in predicting progression of DN.

Our result was in agreement with the studies of ***Petrica et al*[27]. *Nikolov et al.* [33],** **Fiseha and Tamir [34]** and **Piwowar *et al* [35]** on T2DN patients. They reported that a high amount of u β2-microglobulin in the diabetic patients with micro and macroalbuminuria compared with normoalbuminuria and control.

Receiver operating characteristic curve (ROC) analysis shows the diagnostic performance of u KIM-1 and u β2 microglobulin for discriminating patients groups from control group. At a cutoff value **>18 for u KIM-1**, the diagnostic sensitivity was 100, diagnostic specificity was 100, positive predictive value was100 and negative predictive value was100. At cutoff value **>1.5 for u β2 microglobulin**, the diagnostic sensitivity was 93.33, diagnostic specificity was 100, positive predictive value was 100 and negative predictive value was 83.3. Also ROC-AUC**, which denoted sensitivity for diagnosis of DN among T2DM patients; the curve showed urinary KIM-1 is an excellent biomarkers (AUC=1.000).**

CONCLUSION

Our data revealed that tubular biomarkers (KIM-1 and β2 microglobulin), were increased in T2DM patients with normoalbuminuria when compared with controls, indicating that renal tubular damage precede glomerular injury in diabetic kidney disease. Urinary excretion of KIM-1 and β2 microglobulin increased progressively from normoalbuminuria to macroalbuminuria, indicating its value in predicting progression of DN. Both of them are more sensitive and specific than u albumin in early diabetic stage, with higher diagnostic sensitivity and specificity to KIM-1 than β2 microglobulin.

REFERENCES

1. **American Diabetes Association (2014):** Nephropathy in diabetes. Diabetes Care, 27(1): s79-83.
2. **Gariani K, Seigneux S and Pechereb A (2012):** Diabetic nephropathy : an update. Rev Med Suisse, 8: 473-479.
3. **Mongensen CE and Schimitz O (2016)**: The diabetic kidney : from hyperfiltration and microalbuminuria to end –stage renal failure, Medicine Clinicals of North Amireca, 7(2): 1465-1492.
4. **Radovan H,, Robert E, Sebastjan B and Nina H (2015):** Biomarkers of Renal Disease and Progression in Patients with DiabetesJ Clin Med., 4(5): 1010–1024.
5. **Campion CG, Sanchz- Ferras O and Batchu SN (2017):** Biomarkers of nephropathy, Canadian Journal of Kidney Health and Disease, 4: 1-18.
6. **Temesgen F and Zemenu T(2016):** Urinary Markers of Tubular Injury in Early Diabetic Nephropathy. International Journal of Nephrology, https://www.hindawi.com/journals/ijn/2016/4647685/abs/

7. **Zhang Z, Humphreys BD and Bonventre JV (2013):** Shedding of the urinary biomarker kidney injury molecule-1 (KIM-1) is regulated by MAP kinases and juxta-membrane region. J Am Soc Nephrol., 18: 2704-2714.
8. **American Diabetes Association (2018):** Classification and diagnosis of diabetes: Standards of Medical Care in Diabetes, Diabetes Care, 41: S13–S27.
9. **Bowalekar SK (1994):** Statistics in medical research--IV. Sampling distribution, statistical testing of hypothesis and student's t-test. Journal of Postgraduate Medicine, 40(1): 46-51.
10. **Zweig MH and Campbell G (1993):** Receiver-operating characteristic (ROC) plots: a fundamental evaluation tool in clinical medicine. Clinical Chemistry, 39(4): 561-577.
11. **Papatheodorou K, Papanas N, Banach M *et al.* (2016):** Complications of diabetes. J Diabetes Res., 2016: 3.
12. **Vergouwe Y, Soedamanh SS and Zgibor J (2010):** Progression to microalbuminuria in diabetes: development and validation of a prediction rule. Diadetologia, 53(2): 254-62.
13. **Uwaezuoke N, Muoneke V and Mbanefo N (2018):** Tubular Biomarkers as Diagnostic Tools in Diabetic Kidney Disease: A Review of Published Evidence, International Journal of Nephrology and Kidney Failure, 4(2): 1-5.
14. **Nielsen SE, Anderson S and Zdunek D (2011):** Tubular markers do not predict the decline in glomerular filtration rate in diabetic patients with overt nephropathy. Kidney Int., 79(10): 1113-1118.
15. **Zeng X, Hossain D, Bostwick DG *et al.* (2014):** Urinary β2-Microglobulin is a Sensitive Indicator for Renal Tubular Injury. SAJ Case Rep., 1: 103-106.
16. **Aslan O, Demir M and Koseoglu M (2016):** Kidney Injury Molecule Levels in Type 2 Diabetes Mellitus. J Clin Lab Anal., 30(6): 1031-1036.
17. **Euan A S, Neeraj D, James W D and David J W(2013):** Measurement of renal function in patients with chronic kidney diseaseBr J Clin Pharmacol., 76(4): 504–515.
18. **Sheik Mise K, Hoshino J, Ueno T *et al.* (2016):** Prognostic value of tubulointerstitial lesions, urinary N-acetyl- β-d-glucosaminidase, and urinary β2-microglobulin in patients with type 2 diabetes and biopsy-proven diabetic nephropathy. Clin J Am Soc Nephrol., 11: 593-601.
19. **Mussap M, Vestra MD and Fioretto VP (2012):** Cystatin C is a more sensitive marker than creatinine for the estimation of GFR in type 2 diabetic patients, Kidney Int., 61: 1453-1461.
20. **El-Attar HA, Khalil GI and Gaber EW (2017):** Human Kidney Injury Molecule-1 (Kim-1) Level as an Early Marker for Diabetic Nephropathy in Egyptian Type 2 Diabetic Patients**,** Journal of Renal Medicine, 1(1): 31-43.
21. **Maahs DM, Snively BM, Bell RA *et al.* (2007):** Higher prevalence of elevated albumin excretion in youth with type 2 than type 1 diabetes: the SEARCH for diabetes in youth study. Diabetes Care, 30: 2593–8**.**
22. **Kondaveeti SK, Mishra S, Kumar R *et al.* (2013):** Evaluation of glycated albumin and microalbuminuria as early risk markers of nephropathy in type 2 diabetes mellitus. J Clin Diagn Res., 7(7): 1280–3**.**
23. **Hong CY, Chia KS and Ling S (2000):** Urinary protein excretion in Type 2 diabetes with complications. Journal of Diabetes and its Complications, 14(5): 259–265**.**
24. **Van Timmeren MM, Van den Heuvel MC, Bailly V *et al.* (2007):** Tubular kidney injury molecule-1 (KIM-1) in human renal disease. J Path., 212(2): 209–17**.**
25. **Fu WJ, Xiong SL and Fang YG (2012):** Urinary tubular biomarkers in short-term type 2 diabetes mellitus patients: a cross-sectional study. Endocrine, 41: 82–88.
26. **Garg VM, Kumar HS, Mahapatra A *et al.* (2015):** Novel urinary biomarkers in pre-diabetic nephropathy. Clinical and Experimental Nephrology, 19(5): 895–900.
27. **Petrica L, Vlad A, Gluhovschi G *et al.* (2014):** Proximal tubule dysfunction is associated with podocyte damage biomarkers nephrin and vascular endothelial growth factor in type 2 diabetes mellitus patients: a cross-sectional study. PLoS One, 9: e112538**.**
28. **Conway BR, Manoharan D, Manoharan D *et al.* (2012):** Measuring urinary tubular biomarkers in type2 diabetes does not add prognostic value beyond established riskfactors. Kidney Int., 82: 812–818.
29. **Waanders F, Vaidya VS and Van Goor H (2009):** Effect of rennin-angiotensin-aldosterone system inhibition, dietary sodiumrestriction, and/or diuretics on urinary kidney injury molecule-1 expression in non-diabetic kidney disease: Aostoc Analysis of arandomized controlled trial. Am J Kidney Dis., 53: 16-25.
30. **Zhang Z, Humphreys BD and Bonventre JV (2013):** Shedding of the urinary biomarker kidney injury molecule-1 (KIM-1) is regulated by MAP kinases and juxta-membrane region. J Am Soc Nephrol., 18: 2704-2714.
31. **Apakkan M, Aksun D, Ozmen B *et al.* (2004):** β2-microglobulin and cystatin C in type 2 diabetes: assessment of diabetic nephropathy. Experimental and Clinical Endocrinology and Diabetes, 112(4): 195–200.
32. **Ekrikpo UE, Effa E, Akpan E *et al.* (2017):** Clinical Utility of Urinary β2-Microglobulin in Detection of Early Nephropathy in African Diabetes Mellitus Patients. International Journal of Nephrology, 4093171: 8.
33. **Nikolov G, Boncheva M, Gruev T *et al.* (2013):** Urinary biomarkers in the early diagnosis of renal damage in diabetes mellitus patients. Scripta Scientifica Medica, 45(3): 58-64.
34. **Fiseha T and Tamir Z (2016):** Urinary Markers of Tubular Injury in Early Diabetic Nephropathy Int Nephrol., 2016: 46-85.
35. **Piwowar A, Knapik-Kordecka M, Buczynska H *et al.* (2017):** Plasma cystatin C concentration in noninsulin-dependent diabetes mellitus: relation with nephropathy, Arch Immunol Ther Exp., 47: 327-31.