Original Article

Study of Urinary Transferrin as a Marker of Diabetic Kidney Disease

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Abstract:

Objectives: The aim of the present study is to assess urinary transferrin as a marker of DKD and its correlation with different clinical predictive factors of DKD progression. **Patients and Methods:** 60 T2DM patients recruited from the internal medicine wards and outpatient clinic of Alexandria Main University Hospital divided in to 3 groups. Group A, consisted of 20 T2DM patients with normal albumin excretion (>30mg/24h), group B, 20 T2DM patients with moderately increased albumin excretion (30-299mg/24h), group C, 20 T2DM patients with severely increased albumin excretion (<300mg/24h). And 20 sex & age matched healthy subjects were included as control.Urinary transferrin was measured using quantitative sandwich Enzyme-Linked Immunosorbent Assay (ELISA) technique. Urinary creatinine was measured by buffered jaffé reaction. Urinary transferrin to creatinine ratio (TFCR) was calculated and expressed in terms of µg/gm. Results The mean urinary TFCR was significantly higher in the 3 studied patient groups (3.40 ± 2.08, 8.63 ± 4.44, 13.91 ± 9.35 µg/gm respectively) in comparison to the control group (0.64 ± 0.58) with p value (0.003, < 0.001, >0.001 respectively). Urinary TFCR was significantly higher in group B than in group A (p1=0.011) and in group C than in group A (p2>0.001), while there was no statistically significant difference in urinary TFCR between group B and C (p3=0.234).14 patients (70%) in group A had the level of urinary TFCR exceeding the upper limit in the control group (2.01 µg/gm).

There was statistically significant positive correlation between TFCR and urinary albumin to creatinine ratio (ACR) in group C and total sample of the 4 studied groups with a p value >0.001, also there was statistically significant negative correlation between TFCR and estimated GFR (eGFR) in group C and total sample of the 4 studied groups with a p value >0.001.m Conclusion Urinary transferrin could be considered as early marker of DKD progression.

Key words: Diabetic Kidney Disease, End Stage Renal Disease, Urinary TFCR.

Introduction :

Diabetes mellitus (DM) is a complex, chronic disorder that occurs when there are raised blood glucose levels as the body cannot produce any or enough insulin or use insulin effectively.[1] The prevalence of DM is increasing globally. It affects about 8.8 %of total world population by 2017 with higher prevalence in urban areas versus rural areas.[2]

Generally, chronic complications of diabetes are divided into microvascular complications and macrovascular complications.[3]

Diabetic kidney disease (DKD) is one of the most serious microvascular diabetic complications with significant impact on morbidity and mortality.[4]

Diabetic kidney disease was previously known as diabetic nephropathy (DN) and is defined as DM with albuminuria (ratio of urine albumin to creatinine ≥ 30 mg/ gm), impaired glomerular filtration rate (GFR) (< 60 mL / min/ 1.73 m2), or both. Today, DKD includes not only DN but also other renal insults that occurs as a direct result of DM such as, atheroembolic disease, ischemic nephropathy and interstitial fibrosis.[5]

DKD is diagnosed by the persistent presence of elevated urinary albumin excretion (albuminuria), low eGFR, or other manifestations of kidney damage.[6]

Elevated urine albumin excretion (UAE) is considered as a well-established glomerular marker of diabetic nephropathy. It is considered normal to mildly increased when it is less than 30 mg/day (formerly known as normoalbuminuria), between 30and 300 mg/day, moderately increased albuminuria (formerly known as microalbuminuria), and above 300 mg/day, severely increased albuminuria (formerly known as macroalbuminuria).[7] The rate of progression from micro to macroalbuminuria in type 2 diabetic patients is 2-3% annually. Screening for DKD should include measurements of serum creatinine, estimation of GFR and screening for microalbuminuria.[8]

Although microalbuminuria is considered to be the best predictor of renal and cardiovascular complications of DM, many earlier, more sensitive and specific markers of kidney damage have been studied for better diagnosis and treatment of DKD at an earlier stage to prevent the progression to ESRD.[9]

Urinary transferrin (TF) has been considered to be a sensitive marker of DKD progression. Transferrin is iron-binding plasma glycoprotein that control the level of free iron in biological fluids. The liver is the main site of transferrin synthesis but other tissues and organs, including the brain, also produce transferrin.[10] Transferrin is very similar in weight to albumin but it is less anionic and has an isoelectric point (pI) one unit higher than albumin, therefore, it is expected to be filtered through the glomerular barrier more readily.[11] Among type 2 diabetic patients, urinary transferrin significantly increases with the progression of glomerular damage proven by biopsy and it has been shown that some type 2 diabetic patients with diffuse glomerular lesions despite normal UAE, had microtransferrinuria.[12] Furthermore, increased urinary transferrin excretion could predict the development of microalbuminuria in normoalbuminuric type 2 diabetic patients.[13]

Moreover, recent advances in the same issue have been introduced to assess newly studied risk markers for DKD depending on certain clinical and biochemical characteristics associated with progressive kidney damage in both T1DM and T2DM.[14] Elevated baseline haemoglobin A1c (HbA1c), elevated systolic blood pressure, albuminuria grade, early GFR decline, serum uric acid, presence of concomitant microvascular complications (most especially retinopathy), sex category, age and duration of diabetes have been considered as established risk markers in prediction of DKD.[15] While dyslipidemia, elevated body mass index (BMI), smoking status, hyperfiltration, decrease in vitamin D and haemoglobin level, have been proposed as potential risk markers for DKD with further research required to assess the nature of any relationship. Though clinical use of these clinical predictive factors remains restricted.[16]

Patients and methods:

After obtaining the approval of the ethics committee of the Faculty of Medicine of Alexandria University, 60 T2DM patients with different levels of UAE from the internal medicine wards and outpatient clinics of Alexandria Main University hospital divided into 3 group each includes 20 patients and 20 healthy controls were recruited. An informed consent from the participant subjects was taken before conducting the study. Patients with advanced kidney diseases (defined as eGFR < 30 ml/min), kidney diseases other than DKD, liver insufficiency and patients on corticosteroid therapy were excluded.

Data including name, age, sex, duration of DM, past medical history, and data obtained from clinical examination including fundus examination were recorded at enrollment. Laboratory investigations included complete blood picture, lipid profile, renal function test, liver function test, fasting blood glucose, HbA1c, serum uric acid, urinary ACR were done and eGFR were calculated using CKD-EPI formula.[17]

Urinary transferrin level was estimated using quantitative sandwich ELISA technique and urinary transferrin to creatinine ratio (TFCR) was calculated and expressed in terms of µg/gm.

Results:

Table (I): Comparison between the studied groups regarding gender, age and BMI

	Group A (n=20)	Group B (n=20)	Group C (n=20)	Control (n=20)	p
Sex					
Male	6 (30%)	8 (40%)	11 (55%)	9 (45%)	**0.447**
Female	14 (70%)	12 (60%)	9 (45%)	11 (55%)	
Age (years)	50.45[a] ± 8.13	53.15[a] ± 11.10	57.75[a] ± 7.48	39.80 ± 11.63	**<0.001***
BMI (kg/m²)	29.5(21 - 43)	28.5 (22 – 52)	29 (22 – 42)	26 (24 – 30)	**0.053**

Qualitative data were described using number and percentage. Normally quantitative data was expressed using Mean ± SD while abnormally distributed data was expressed using Median (Min. – Max.).

a: Significant with Control group

b: Significant with Group A

c: Significant with Group B

Table (II): Comparison between the studied groups according to history and clinical data.

	Group A (n=20)	Group B (n=20)	Group C (n=20)	Control (n=20)	p
Duration of DM (years)	7 (0.5 - 25)	10 (0.5 - 23)	11.5 (2 - 20)		**0.467**
HTN	12[a] (60%)	13[a] (65%)	18[ab] (90%)	3 (15%)	**<0.001***
Systolic BP	127.5 ± 15.52	135.5[a] ± 21.64	148.5[a] ± 20.59	117.0 ± 14.55	**<0.001***
Diastolic BP	80.0 ± 6.49	86.0[a] ± 13.14	93.0[ab] ± 13.80	75.50 ± 7.59	**<0.001***
Smoking status					
No	17 (85%)	13 (65%)	11 (55%)	17 (85%)	
Smoker	2 (10%)	6 (30%)	6 (30%)	3 (15%)	**0.187**
X-Smoker	1 (5%)	1 (5%)	3 (15%)	0 (0%)	
Retinopathy	a	a	ab		
No	14 (70%)	9 (45%)	5 (25%)	20 (100%)	
Non-proliferative	4 (20%)	4 (20%)	4 (20%)	0 (0%)	**<0.001***
Proliferative	2 (10%)	7 (35%)	11 (55%)	0 (0%)	

Qualitative data were described using number and percentage. Normally quantitative data was expressed using Mean ± SD while abnormally distributed data was expressed using Median (Min. – Max.).

a: Significant with Control group
b: Significant with Group A
c: Significant with Group B

Table (III): Comparison between the studied groups according to different laboratory investigations

	Group A (n=20)	Group B (n=20)	Group C (n=20)	Control (n=20)	p
Urea (mg/dl)	23.8 (15.8 – 38.8)	29[ab](13 - 49)	31[ab](13 - 83)	22.5 (15 - 42)	**0.008***
Cr (mg/dl)	0.72 (0.5 – 1.1)	0.8[a](0.4 – 1.5)	0.85[ab](0.44 – 1.96)	0.62 (0.42 - 1)	**0.014***
S .uric acid (mg/dl)	3.65 (2.7 – 5.8)	4.2 (2 - 8)	5.45 (2.8 - 13)	4.05 (2.4 – 7.8)	**0.080**
FBG (mg/dl)	176.5[a](98 - 360)	188.5[a](98 - 305)	190[a](93 - 302)	89.5(68-121)	**<0.001***
HbA1c %	8.71[a] ± 1.59	9.01[a] ± 2.34	9.29[a] ± 1.47	4.98 ± 0.69	**<0.001***
Cholesterol (mg/dl)	215[a](110 - 413)	201 (103 - 269)	195(111 - 426)	166.5(115 - 265)	**0.024***
TG (mg/dl)	118 (64 - 320)	138(38 - 431)	160.5[a](66 -723)	94.5 (58 -203)	**0.040***
LDL (mg/dl)	130[a](81 - 331)	114[a](56 - 211)	118.5[a](76 -194)	85[a](56 - 131)	**<0.001***
HDL (mg/dl)	48.90 ± 9.63	46.80 ± 11.57	42.30 ± 10.80	50.05 ± 10.12	**0.108**
eGFR (ml/min/1.73m^2)	99.7(76.9 – 143.6)	93.4[a](51.60 -234)	80.2[a](37.4-156.1)	139.4(59.5-233.7)	**0.002***
ACR (mg/gm)	5.9(2.9 – 18.1)	38.85[ab](32.5 - 105)	531.5[abc](332-3399)	12.65(2.7-35)	**<0.001***
Urinary.TF(ng/ml)	2.45[a](0.76 – 5.3)	6.15[ab](0.7 – 10.6)	12.2[ab](0.8 – 23.4)	0.45(0 - 1)	**<0.001***
TFCR (µg/gm)	2.97[a](0.69 – 8.43)	9.08[ab](0.57 – 16.61)	13.29[ab](0.88 – 39.79)	0.57(0 – 2.01)	**<0.001***

Normally quantitative data was expressed using Mean ± SD while abnormally distributed data was expressed using Median (Min. – Max.).

a: Significant with Control group; b: Significant with Group A; c: Significant with Group B

Table (IV): Correlation between TFCR with different parameters in each group and total sample

		TFCR (µg/gm)				
		Group A (n=20)	Group B (n=20)	Group C (n=20)	Control (n=20)	Total sample (n = 80)
Age (years)	r_s	0.372	0.325	0.244	-0.196	0.559*
	p	0.106	0.163	0.299	0.407	<0.001*
BMI (kg/m²)	r_s	-0.286	-0.391	-0.115	0.069	0.115
	p	0.222	0.089	0.630	0.773	0.309
Duration of DM(years)	r_s	0.442	0.004	0.030		0.183
	p	0.051	0.987	0.899		0.162
Systolic	r_s	-0.010	-0.197	0.195	0.209	0.460*
	p	0.967	0.405	0.411	0.376	<0.001*
Diastolic	r_s	-0.315	-0.263	0.249	0.198	0.463*
	p	0.176	0.263	0.289	0.403	<0.001*
HB (g/dl)	r_s	0.066	0.145	-0.387	0.198	-0.244*
	p	0.782	0.541	0.092	0.402	0.029*
S .uric acid(mg/dl)	r_s	0.004	0.086	0.239	-0.153	0.216
	p	0.987	0.719	0.311	0.520	0.054
HbA1c %	r_s	0.158	0.062	0.133	-0.013	0.606*
	p	0.505	0.796	0.578	0.956	<0.001*
Cholesterol (mg/dl)	r_s	-0.146	-0.283	-0.409	-0.209	-0.014
	p	0.539	0.227	0.073	0.377	0.903
TG (mg/dl)	r_s	-0.170	-0.258	-0.349	-0.256	0.122
	p	0.474	0.272	0.132	0.276	0.282
LDL (mg/dl)	r_s	-0.132	-0.020	-0.141	-0.107	0.184
	p	0.578	0.932	0.552	0.655	0.103
HDL (mg/dl)	r_s	0.082	-0.076	-0.118	0.016	-0.236*
	p	0.731	0.750	0.620	0.947	0.035*
Retinopathy	r_s	0.073	0.045	0.143		0.507*
	p	0.760	0.849	0.548		<0.001*
ACR	r_s	-0.272	-0.026	0.859*	0.030	0.670*
	p	0.246	0.912	<0.001*	0.899	<0.001*
eGFR	r_s	0.047	0.174	-0.732*	0.248	-0.465*
	p	0.845	0.462	<0.001*	0.293	<0.001*

r_s: Spearman coefficient
*: Statistically significant at $p \leq 0.05$

Discussion:

Diabetic Kidney Disease represents a major component of the health burden associated with DM. In many regions, diabetes is considered the main leading cause of ESRD with significant impact on morbidity and mortality specially in DM patients with proteinuria.[18]

A large number of studies support that the risk for rapid progression of DKD and consequently the development of ESRD is strongly associated with the progression of albuminuria in diabetic patients. So that, albuminuria has often been considered as surrogate for hard renal endpoints.[19] However, earlier, more sensitive and specific markers of kidney damage are needed for better diagnosis and treatment of DKD at an earlier stage to prevent the progression to ESRD.[20]

Transferrin is an iron-binding plasma glycoprotein that is very similar in weight to albumin, therefore, it is expected to be filtered more readily through the glomerular barrier. Urinary transferrin is considered to be a more sensitive marker of glomerular damage in diabetic patients.[21]

In this study, the mean urinary TFCR was significantly higher in the 3 studied patient groups in comparison to the control group, the mean urinary TFCR was significantly higher in group B than in group A and in group C than in group A, while there was no statistically significant difference in urinary TFCR between group B and C. Moreover, 70% of patients in group A had the level of urinary TFCR exceeding the upper limit in the control group.

There was statistically significant positive correlation between TFCR and ACR in group C and total sample of the 4 studied groups, while, there was no statistically significant correlation between TFCR and ACR in group A, group B or in control group.

We can conclude from the previous results that high levels of urinary TF is a marker of progression of DKD either with or without albuminuria and this relationship is more established in patients with severely increased albuminuria. Moreover, transferrinuria frequently occurs before the development of microalbuminuria, so it could be considered as a sensitive marker for early detection of DKD in T2DM patients with normal albumin excretion. These results are in agreement with 3 studies that investigated the role of urinary TFCR in assessment of progression of DKD in T2DM.

The first one is by Randy et al[22] who observed higher level of TFCR in patients with T2DM than in control group and also found a highly significant correlation between urinary albumin and transferrin excretion in patient groups. They concluded that urinary transferrin excretion is increasing with the degree of albuminuria with highly significant correlation to urinary albumin excretion in T2DM with overt proteinuria.

The second study is by Cheung et al[23] who found that, 61% of normoalbuminuric diabetic patients had high TFCR (higher than the 95th percentile value seen in healthy subjects). TFCR was significantly higher in the patient groups than in control group. Moreover, they observed that TFCR was significantly higher in patients with clinical retinopathy and it correlated with arterial BP. They concluded that transferrinuria is very common in T2DM patients and frequently occurs before microalbuminuria and suggested that it could be a sensitive marker of incipient nephropathy.

The third study is more recent by Narita et al[24] who studied the parallel increase in urinary excretion rates of four markers including transferrin in normoalbuminuric T2DM patients and found that TF excretion rate was significantly increased in the normoalbuminuric patient group than in control group, so they suggested that increased urinary TF excretion could predict a future development of microalbuminuria in normoalbuminuric T2DM patients.

The results of the previous studies and our study were nearly similar. The possible interpretations of these findings are that proteinuria in DN has been shown to result from a disturbance in glomerular size selectivity to different proteins. Therefore, increased urinary TF excretion, which is somewhat larger, that often precede the onset of microalbuminuria in diabetic subjects could be explained by altered glomerular size selectivity to these two proteins , while in patients who already developed albuminuria, the urinary transferrin excretion has a linear relationship with UAE.[25]

Moreover, in T2DM, transferrinuria precedes biopsy proven tubulointerstitial changes found in diabetic kidney as tubular reabsorption of TF results in release of reactive iron which produce oxidative stress on the tubular epithelium. Transferrin excretion was also associated with some markers of proximal tubule damage such as alpha-1-microglobulin and urinary N-Acetyl-β-D glucosaminidase (NAG) in diabetic patients but it is not yet clear if TF is the cause of tubular damage or transferrinuria is secondary to decreased tubular reabsorption.[26]

In our study, there was also statistically significant negative correlation between TFCR and eGFR in group C and total sample of the 4 studied groups,

while there was no statistically significant correlation between TFCR and eGFR in group A, group B or in control group.

Jiang et al[27] found that, urinary levels of glomerular, including transferrin, and tubular damage markers of DKD were higher in T2DM patients with slightly decreased eGFR , interestingly, they also found that some of these markers including transferrin, were already elevated in diabetic patients with relatively normal eGFR (≥90 mL/min/1.73 m2) suggesting that these markers are potential sensitive markers for early diabetic kidney damage.

They also demonstrated that all damage markers were significantly negatively associated with eGFR and positively associated with albuminuria but only glomerular not tubular markers lose their significant negative correlation with eGFR after adjustment of albuminuria and other confounding factors like HbA1c.[27]

In the current study, the prevalence of hypertension was significantly higher in the 3 studied patient groups than in control group. the prevalence of hypertension was also significantly higher in group C than in group A. This is coinciding with the results of Bakris et al[28], who found that the prevalence of hypertension in DN increases at each stage of CKD, approaching 90% for ESRD patients. This could be explained by interaction of multiple mediators that result in renal sodium reabsorption and peripheral vasoconstriction such as activation of the renin-angiotensin-aldosteron system (RAAS), upregulation of endothelin (ET-1), upregulation of reactive oxygen species (ROS), and downregulation of nitric oxide (NO) which are important factors that result in development of hypertension and progression of diabetic renal and cardiovascular events.[28]

As regards diabetic retinopathy (DR), the prevalence of DR in our study was significantly higher in the 3 studied patient groups than in control group and also it was significantly higher in group C than in group A. This implies that DR is strongly associated with the progression of albuminuria. These results coincide with the results of Zhang et al[29] who found that patients with DKD and DR were more likely to have higher levels of serum creatinine and proteinuria, and decreased eGFR and he concluded that there was a strong associations between the presence of DR and the renal outcomes in T2DM patients. These findings suggested a potential common pathway between the DR and DKD, as the presence of retinopathy may represent diabetic related systemic microvascular damage that lead to both breakdown of the blood vessel–retinal tissue barrier and progressive renal dysfunction. Furthermore, the presence of DR may identify individuals who may be at increased risk for DN or more serious renal microvascular damage.[30]

As regards the association of HbA1c with DKD, we found that the level of HbA1c was statistically significant higher in the 3 studied patient groups than in control group. It was also higher in group C than in group A and B but without a statistically significant difference.

In a study by Kaminska et al[31], urinary albumin excretion was higher in T2DM patients with poor glycemic control (HbA1c ranged between 6.6 and 10%) than in T2DM patients with good glycemic control (HbA1c ranged between 6.1 and 6.5%) suggesting that the urinary albumin excretion rises with increased glycemia and percentage of glycosylated haemoglobin.

Hyperglycemia is essential for development of DKD, and glycemic control is the primary determinant of the onset of nephropathy. The UKPDS (United Kingdom Prospective Diabetes Study) in type 2 diabetes have revealed a strong relationship between glycemic control and risk of the development of diabetic microvascular complications, although there is no easily defined HbA1c threshold. However, there has been only limited evidence suggesting that glycemic control slows the rate of GFR decline and retards progression to ESRD.[32]

As regards lipid profile, we found no statistically significant difference between the 3 studied patient groups as regards total serum cholesterol, serum triglycerides (TG) and serum low density lipoproteins (LDL). However, total serum cholesterol was statistically significant higher in group A than in control group, serum TG was

statistically significant higher in group C than in control group, serum LDL was statistically significant higher in the 3 studied patient groups than in control group while there was no statistically significant difference between the 4 studied groups as regards serum high density lipoproteins (HDL).

These results coincides partially with Shoji et al[33] who reported no differences between diabetic patients with normoalbuminuria and those with microalbuminuria as regards total serum cholesterol, serum TG and serum LDL, but that not for those with macroalbuminuria. However, in another study by Al-Jamei et al[34], they found significant increase in the serum levels of total serum cholesterol and serum LDL in diabetic patients with macroalbuminuria than those with microalbuminuria and normoalbuminuria. Moreover, they found significant increase in serum TG and decrease in serum HDL in patients with microalbuminuria and macroalbuminuria than those with normoalbuminuria, but they found no significant difference between microalbuminuric and macroalbuminuric patient groups as regards serum TG and HDL.

Dyslipidemia in DM results from insulin resistance and defective insulin action on lipoprotein metabolism. Thus, there is increased lipolysis with increased production of very low density lipoproteins (VLDL), TG, LDL, and rapid breakdown of HDL. dyslipidemia also have been found to facilitate glomerulosclerosis under hyperglycemic conditions.[35]

The current study also demonstrated a statistically significant positive correlation between TFCR and both systolic and diastolic BP, retinopathy and HbA1c. These results are in agreement as regards BP and retinopathy with the study mentioned above that is by Cheung et al(23) who reported a significant positive correlation between TFCR and both arterial BP and clinical retinopathy supporting that TF may be a sensitive marker for detecting diabetic complications. However, the same study was against our study as regards HbA1c, as they found no significant correlation between TFCR and HbA1c, suggesting that excess transferrinuria is not explained by short or medium term fluctuations in glycemic control but more probably by an intrinsic abnormality of glomerular function. However, more studies with larger sample sizes are needed to prove relationship between urinary TFCR and different clinical predictive markers of DKD.

Conclusion:

Urinary transferrin is considered as early and sensitive marker of DKD progression and can predict the occurrence of albuminuria and other diabetic complications.

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