The Egyptian Journal of Hospital Medicine (April 2022) Vol. 87, Page 2113-2118

Role of Nephrinuria in Early Diagnosis of Type 2 Diabetic Nephropathy at Zagazig University Hospitals

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ABSTRACT

Background: Diabetic nephropathy can be detected early with the help of the nephrin biomarker.

Objective: The aim of the current work was to assess the level of nephrin in the urine of type 2 diabetic patients as a biomarker of early detection of diabetic nephropathy.

Patients and Methods: This study included a total of sixty-six type 2 diabetic patients and 22 apparently healthy control subjects, attending at Departments of Clinical Pathology and Diabetic Clinic, Internal Medicine Department, Zagazig University Hospitals. The included participants were divided into four groups; **Group A** (control) consisted of 22 apparently healthy control subjects, **Group B** consisted of 22 type 2 diabetic patients with normo-albuminuria, **Group C** consisted of 22 type 2 diabetic patients with micro-albuminuria, and **Group D** consisted of 22 type 2 diabetic patients with macro-albuminuria. Urinary nephrin level was assessed among all participants.

Results: The urine nephrin and the urine nephrin/creatinine ratio showed highly statistically significant differences between the study groups. Post hoc test showed that urine nephrin and urine nephrin/creatinine ratio were highly elevated in group B when compared to group A, also were elevated in group D when compared to group C and group D when compared to group B. Correlation matrix showed that there was significant positive correlation between duration of diabetes (years) with urine nephrin.

Conclusion: It could be concluded that even in diabetic patients with normal albuminuria, urinary nephrin is elevated because it precedes albuminuria. Diabetic nephropathy can be diagnosed earlier with the help of this marker.

Keywords: Nephrin, Diabetic Nephropathy, Urinary nephrin, renal disease.

INTRODUCTION

Diabetes's prevalence has reached epidemic proportions, and by 2035, it is predicted to impact more than 350 million individuals around the world [(1)].

For End-Stage Renal Disease and Chronic Kidney Disease, Diabetic Nephropathy (DN) is the most common cause. In part, this is due to an increase in type 2 diabetes, which is linked to obesity [(2)].

At least 20-40 percent of type 2 diabetic individuals develop evident nephropathy in the first two decades after the onset of diabetes without any intervention; around 20 percent develop end-stage renal disease [(3)].

If albumin levels in the urine and the Glomerular Filtration Rate (GFR) decline, you have diabetes-related nephropathy, much like any other chronic kidney illness [(4)]. One-third of diabetic nephropathy patients find improvement in their kidney damage due to medication, and this is frequently linked to better diabetes and hypertension control. Some individuals with microalbuminuria and overt nephropathy improve with medication [(5)].

In addition to exercise-induced albuminuria, urinary tract infections, severe illnesses, and heart failure all contribute to the confusion. Albuminuria is unable to reliably predict diabetic kidney damage because it can be seen in the urine of non-diabetics as well [(6)]. As a result, a brand-new biomarker is needed, one that is incorporated into the kidney's structure. Many renal indicators have been investigated for the early prediction of renal impairment through the years [(7)].

The renal podocytes express nephrin, a 180-kD transmembrane protein. Congenital nephrotic syndrome of the Finnish variety was the first to be found to have it [(8)]. Due to its role in the renal filtering diaphragm and its location between the foot processes of the podocytes, nephrin is likely to be expelled first if the filtration barrier is damaged [(9)]. Diabetic nephropathy can be detected early with the help of the nephrin biomarker [(7)].

The aim of the current work was to assess the level of nephrin in the urine of type 2 diabetic patients as a biomarker of early detection of diabetic nephropathy.

PATIENTS AND METHODS

This study included a total of sixty-six type 2 diabetic patients and 22 apparently healthy control subjects, attending at Departments of Clinical Pathology and Diabetic Clinic, Internal Medicine Department, Zagazig University Hospitals. This study was conducted between Oct 2019 to April 2020.

The Eighty-eight included participants were divided into four groups; **Group A** (control) consisted of 22 apparently healthy control subjects with their median age 51 years, **Group B** consisted of 22 type 2 diabetic patients with normo-albuminuria with their median age 49.5years, **Group C** consisted of 22 type 2 diabetic patients with micro-albuminuria with their median age 43 years, and **Group D** consisted of 22 type 2 diabetic patients with macro-albuminuria with their median age 56.

Received: 25/11/2021
Accepted: 23/1/2022

Inclusion criteria: Type 2 diabetic patient, and age > 18 years.

Exclusion criteria: Hypertension, chronic renal failure, recent use of any antibiotics for two weeks ago, patient with urinary tract infection, and age <18 years.

All patients were subjected to:

Full history taking: Name, age, sex, residence, medical history of chronic and metabolic diseases, date of examination and/or admission, contact information and other habits of medical interest.

Clinical examination: General examination, local examination and neurovascular examination.

Laboratory investigations: Glycated hemoglobin, kidney function tests, creatinine in urine, and microalbumin in urine

Special laboratory investigations: Urinary nephrin levels by (ELISA).

***Ethical Consideration*:**

This study was ethically approved by Zagazig University's Research Ethics Committee. Written informed consent of all the participants was obtained and submitted them to Zagazig University (ZU-IRB#6459). The study protocol conformed to the Helsinki Declaration, the ethical norm of the World Medical Association for human testing.

Statistical analysis

The collected data were coded, processed and analyzed using the SPSS (Statistical Package for Social Sciences) version 22 for Windows® (IBM SPSS Inc, Chicago, IL, USA). Data were tested for normal distribution using the Shapiro Walk test. Qualitative data were represented as frequencies and relative percentages. Chi square test ($\chi 2$) to calculate difference between two or more groups of qualitative variables. Quantitative data were expressed as mean ± SD (Standard deviation). Independent samples t-test was used to compare between two independent groups of normally distributed variables (parametric data). P value < 0.05 was considered significant.

RESULTS

Statistically, there are no significant variations regarding age and gender amongst the groups studied **(Table 1).**

Table (1): Demographic data of studied groups:

items	Studied groups				KW Test	p
	Group A (n=22)	Group B (n=22)	Group C (n=22)	Group D (n=22)		
Age (years) Mean ±SD Median (range)	47.9±15.7 51 (19-69)	47.09±17.8 49.5 (20-76)	45.27±18 43 (18-72)	48.45±16.9 56 (18-69)	0.24	0.97
Sex No (%) Female Male	9(40.9) 13(59.1)	15(68.18) 7 (31.82)	12(54.55) 10(45.45)	10(45.45) 12(54.55)	χ^2=3.8	0.28

KW =Kruskall Wallis test χ^2 chi square test of significant s=significant p<0.05

There was a statistically significant differences between the patient groups and the control group in terms of serum creatinine and uric acid, but no difference was found between any of the patient groups. Unlike plasma urea nitrogen, there were no statistically significant variations in blood urea **(Table 2).**

Table (2): Comparison between studied groups as regard serum creatinine, urea and uric acid:

Variables	Studied groups				F	p	Post hoc(P)	
	Group A (n=22)	Group B (n=22)	Group C (n=22)	Group D (n=22)			B&C	B&D
Creatinine (mg/dl) Mean ±SD	0.78±0.1	0.90±0.16	0.99±0.19	1.5±0.18	3.62	0.016	0.85	0.99
	*P=0.005		**P=0.99					
Urea (mg/dl) Mean ±SD	15±3.8	26.4±5.9	28.6±3.48	30±4. 8	0.55	0.65	-	-
Uric acid (mg/dl) Mean ±SD	4.3±0.6	5.1±0.99	5.2±0.94	5.5±0.95	5.2	0.002	0.99	0.66
	*P=.0.018		**P=.0.49					

#F (Anova test). P<0.05=significant. P>0.05=nonsignificant ##(Date expressed Mean ±SD with f test)

###Post hoc test used to detect significant. (*P= Group A & Group B groups). (**P= Group C & Group D)

This study found a statistically significant difference in urine creatinine levels across the groups (P=0.043), as well as significant differences in urine nephrin and the urine nephrin to creatine ratio (P0.001). A post hoc test found that urine nephrin and the urine nephrin/creatinine ratio were significantly higher in group B when compared to group A, and also in group D when compared to group C. **(Table 3).**

Table (3): Comparison between studied groups as regard urine creatinine, Micro albumin/ creatinine ratio, urine nephrin and urine nephrin /creatinine ratio:

Variables	Studied groups				Test ofsig	P	Post hoc(P)	
	Group A (n=22)	Group B (n=22)	Group C (n=22)	Group D (n=22)			B&C	B&D
Urine creatinine (mg/dl) Mean ±SD	89.5 ± 16.25	108.98 ± 19.36	78.8± 11.36	56.1±8.12	KW=8.2	0.043	0.38	0.01
	*P=.0.29		**P=.0.3					
Micro-albumin/creatinine ratio (ug/mg) Mean ±SD	10.8±2.21	8.4±1.81	87.2±13.21	1743±216.11	KW=73.7	0.0001	0.0001	0.0001
	*P=.0.23		**P=.0.0001					
Urine nephrin (ng/ml) Mean ±SD	11.5±1.3	20.9±2.9	21.4±2.7	24.3±3.8	F=85.6	0.0001	0.53	0.0001
	*P=0.0001		**P=0.001					
Urine Nephrin /creatinine ratio (ng nephrin /mg creatinine) Mean ±SD	12.6±2.31	21.9±4.33	25.3±4.27	45.1±8.31	KW=34.2	0.0001	0.49	0.005
	*P=.0.0001		**P=.0.046					

Urine nephrin at cut off ≥ 14.4 can discriminate between healthy subjects and diabetic patient with normoalbuminuria with sensitivity 100% and specifity 100%. Also shows that urine nephrin / creatinine ratio at cut off ≥ 13.96 can discriminate between healthy subjects and diabetic patient with normoalbuminuria with sensitivity 81.8% and specifity 77% **(Table 4).**

Table (4): Validity data of urine nephrin and urine nephrin/creatinine ratio as marker to detect diabetic nephropathy:

	Cut off	Group B	Group A	Sensitivity	Specificity	PPV	NPV	Accuracy
Urine nephrin (ng/ml)	≥ 14.47	22	0					
	<14.47	0	22	100%	100%	100%	100%	100%
Urine nephrin/ creatinine Ratio (ng nephrin /mgcreatinine)	≥ 13.96	18	5	81.8%	77%	78%	81%	79.5%
	<13.96	4	17					

ROC curve of both urinary nephrin and urinary nephrin/creatinine ratio to discriminate healthy subjects from diabetic patients with normoalbuminuria with an AUC, 1 and 0.82 for urine nephrin and urine nephrin/creatinine ratio) respectively. So, urine nephrin was very good parameter to discriminate healthy subjects from normoalbuminuric diabetic patients and urine nephrin/creatinine ratio was good parameter to discriminate healthy subjects from normoalbuminuric patient **(Figure 1)**

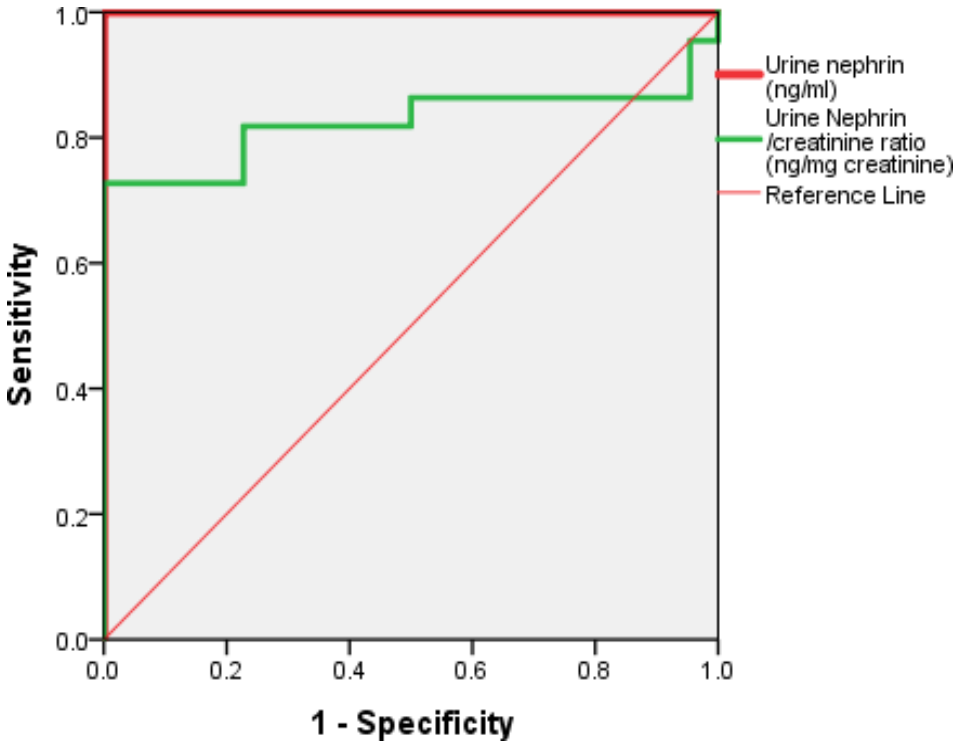

Figure (1): Roc curve for urine nephrin and urine nephrin/creatinine ratio as diagnostic markers of diabetic nephropathy.

For example, nephrin/creatinine ratio showed a significant negative correlation with the duration of diabetes (years) in group C (r=-0.935 with p-value 0.0001), whereas the correlation matrix in group C showed significant positive correlation between the duration of diabetes (years) and urine nephrine (r=0.47 with p=0.027) **(Table 5).**

Table (5): Correlation matrix between studied parameters among (type 2 diabetic patients with micro-albuminuria):

Type 2 diabeticpatients with micro-albuminuria	Micro albuminuria/creatinine		Urine nephrin		Urine nephrin/creatinineratio	
	(r)	p	(r)	p	(r)	P
Urine nephrin (ng/ml)	0.124	0.582				
Urine nephrin /creatinine ratio (ng nephrin /mg creatinine)	0.16	0.47	**0.45***	**0.036**		
Age (years)	0.16	0.47	0.271	0.222	0.085	0.707
HbA1c	0.244	0.274	0.09	0.69	0.147	0.513
Duration diabetes/yrs	0.13	0.55	**0.47***	**0.027**	**0.49***	**0.02**
Creatinine urine (mg/dL)	-0.09	0.68	-0.305	0.17	**- 0.935**	**0.0001**
Serum creatinine (mg/dL)	0.344	0.126	**.495***	**0.023**	0.008	0.973
Serum Urea (mg/dL)	-.34	0.131	-.096	0.677	-.326	0.149
Serum Uric acid (mmol/day)	0.001	0.996	-0.398	0.074	-.362	0.106

A significant positive correlation was found in the correlation matrix for group D between the number of years with diabetes (r = 0.501, p = 0.018), as well as the ratio of urine nephrin to creatinine (r = 0.67, p = 0.001), while urine creatinine was found to be significantly correlated with both the urine nephrin/creatinine ratio (r = - 0.92, p = 0.0001) and the microalbumin **(Table 6).**

Table (6): Correlation matrix between studied parameters (type 2 diabetic patients with macro-albuminuria):

Type 2 diabeticpatients with macro-albuminuria	Micro albuminuria/creatinine		Urine nephrin		Urine nephrin/creatinineratio	
	(r)	p	(r)	P	(r)	P
Urine nephrin	-.22	0.32		.		
Urine nephrin /creatinine ratio	**.64****	**0.001**	0.199	0.374		
Age	0.185	0.41	-.095-	0.675	0.206	0.358
HbA1c	0.099	0.663	0.168	0.455	0.157	0.484
Duration diabetes	0.28	0.19	**.501***	**0.018**	**.67****	**0.001**
Urine creatinine	**-0.76****	**0.0001**	0.11	0.63	**-0.92****	**0.0001**
Serum creatinine	0.116	0.606	0.085	0.706	0.205	0.361
Serum Urea	**- 0.438***	**0.041**	0.275	0.216	-0.08	0.725
Serum Uric acid	-.082	0.718	-.114	0.613	-.157	0.486

Patients with a duration of diabetes of less than 5 years had significantly higher urine nephrin or urine nephrin/creatinine ratios in all patient groups when they were separated into two groups based on their duration of diabetes **(Table 7).**

Table (7): Relation between duration of diabetes with urine nephrin and urine nephrin/creatinine ratio in patient groups:

Diabetes duration/years	Urine nephrin			Urine nephrin/ creatinine			n.
	Mean ±SD	t	p	Median(range)	M W	p	
Group B							12
≤5	19.4±2.5	2.9	0.008	15.8(5.2-49.62)	2.8	0.005	10
>5	22.6±2.5			30.9(17.7-57.54)			
Group C							11
≤5	20.1±2.1	2.5	0.02	15.3(11.4-42.2)	2.9	0.003	11
>5	22.7±2.6			43(10.56-82)			
Group D							6
≤5	22±3.5	1.8	0.082	23(15.2-44.4)	2.8	0.005	16
>5	25.2±3.7			56.2(14.4-77.2)			

DISCUSSION

International Diabetes Federation (IDF) says that Egypt is one of the top 10 nations having a high prevalence of diabetes in the globe. There will be an increase from 34.6 million to 67.9 million diabetics in the Middle East and North Africa (MENA) region between 2013 and 2035 [(1)]**.** According to the Egyptian renal data system, diabetic nephropathy rose from 8.9% to 14.5% between 1996 and 2001 as a cause of end-stage renal disease [(10)]**.**

Developing a new biomarker that is part of the kidney's structural components will be necessary. It has been found that nephrin, a transmembrane protein, is the best biomarker for predicting diabetic kidney disease as well as the severity of the damage to podocytes [(11)].

The present study included 88 subjects, 42 males and 46 females. There were no statistically significant differences between patients and control as regard age and sex. Our results went with **Shahid *et al.*** [(7)] who found that out of 78 patients, 37 were males and 41 were females.

In terms of serum creatinine and uric acid, a statistically significant differences were found between the patient groups and the control group. However, no statistically significant difference was found as regard serum urea.

These results agreed with **Ezz and Abd El Azeem** [(12)] as diabetic patients with nephropathy had higher serum creatinine levels than those in the control group (2.8 versus 0.78 mg/dl) were found to be considerably higher than those of diabetic patients without the condition (0.94) (2.8 versus 0.94 mg/dl).

As regard urine creatinine, our study showed that it was significantly decreased in diabetic nephropathy patients especially patients with macroalbuminuria when compared to control.

These results coincide with **Ezz and Abd El Azeem** [(12)] since DN patients had lower urinary creatinine levels than diabetics and control groups, the researchers concluded that DN patients had lower urinary creatinine levels. They concluded that DN patients had lower urinary creatinine levels than diabetics and controls because their levels of urine creatinine were significantly lower than those of diabetics and controls.

Our study showed that there were high statistically significant differences between patients and

control as regard urine nephrin and urine nephrin /creatinine ratio. Urine nephrin and urine nephrin/creatinine ratio were elevated in group B (normoalbuminuric) when compared to group A (control), also were highly elevated in group D (macroalbuminuric) when compared to group C (microalbuminuric) and group B.

Patari *et al.* [13] stated that 30 percent of normoalbuminuric patients, 17 percent of microalbuminuric patients, and 28 percent of macroalbuminuric patients were found to have nephrinurea; none of the control subjects had nephrinurea.

Jim *et al.* [14] 100 percent of diabetics with micro- and macro-albuminuria, as well as 54 percent of those with normo-albuminuria, had nephropathy, and this was seen in all of them.

In our study, ROC curve of both urinary nephrin and urinary nephrin/creatinine ratio can discriminate healthy subjects from diabetic patients with normoalbuminuria with an (AUC) 1 and 0.82 for urine nephrin and urine nephrin/creatinine ratio respectively. So, urine nephrin was very good parameter to discriminate healthy subjects from normoalbuminuric diabetic patients with sensitivity and specificity of 100% and urine nephrin/creatinine ratio was good parameter to discriminate healthy subjects from normoalbuminuric patients with sensitivity of 81.8% and specificity of 71%.

The present study showed that there was significant positive correlation between duration of diabetes and urine nephrin or urine nephrin / creatinine ratio, also patients when subgrouped according to duration of diabetes to > 5 or ≤ 5. There was statistically significant increase in urine nephrin or urinary nephrin / creatinine ratio in patients suffering from diabetes more than 5 years with p value of 0.008 for urine nephrin and 0.005 for urine nephrin / creatinine ratio. Our results went with **Shahid *et al.*** [7] who reported that nephrinuria aslo correlated with duration of diabetes

Our results showed that there was significant positive correlation between urine nephrin / creatinine ratio and microalbuminuria / creatinine ratio in the macroalbuminuric group (D). **Jim *et al.*** [14] agreed with our results as they found that nephrinuria correlated with albuminuria (p = 0.001). So, they concluded that nephrinuria is considered as biomarker of preclinical diabetic nephropathy. Also, **Ng *et al.*** [15] stated that nephrinuria was strongly associated with albuminuria. **Moon *et al.*** [16] supporting nephrinuria as an early indicator for diabetic nephropathy in patients

CONCLUSION

It could be concluded that even in diabetic patients with normal albuminuria, urinary nephrin is elevated because it precedes albuminuria. Diabetic nephropathy can be diagnosed earlier with the help of this marker.

Financial support and sponsorship: Nil.
Conflict of interest: Nil.

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