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Assessment the level of Urinary nephrin as an early biomarker in Diabetic Nephropathy

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Abstract

Most people with diabetes have hyperglycemia, or uncontrolled blood sugar, which can lead to a number of dangerous side effects, such as diabetic nephropathy. Finding new indicators that can give early diagnosis and prevent consequences has become more crucial as a result of the lack of early detection. Many kidney disorders originate and advance as a result of damage or dysfunction to podocyte cells. This increases the possibility of employing podocyte cell product targets, particularly in urine, as practical clinical markers for kidney disease diagnosis and surveillance. A cross-sectional study will be carried out to a group of 70 diabetic patients with diabetic nephropathy 20 healthy control. A total of 25 mL urine was collected. Alb/Cr ratio in a random urine sample . Serum creatinine, serum albumin and HbA1C. Urinary Albumin / creatinine ratio. Urinary podocalyxin, nephrin and podocin were determined by using commercially available ELISA test. According to the study, 50% of patients with normoalbuminuria have greater urine Nephrin levels than the healthy control group. Nephrin levels are also higher in the former group. These results imply that Nephrin initially manifests in the urine before microalbumin in the early stages of kidney injury. According to the data, both the UM/CR and CKD staging categories had significantly higher urine nephrin levels than the healthy control group. Urinary nephrin levels gradually increased with CKD stage, particularly in stages two and three, and it was shown that urine nephrin was more sensitive than the UM/CR ratio for early kidney damage identification. Diabetes patients had much greater amounts of nephrin, and podocalxyin in their urine compared to the healthy control group.

1. Introduction

Although there is no precise time frame for diabetic kidney disease (DKD), patients with diabetes who were monitored for ten years in the UK were found to develop microalbuminuria at a rate of 2% year following their diabetes diagnosis (1) .

For a long time, measuring urinary albumin and glomerular filtration rate (GFR) was considered the key to diagnosing kidney disease. Due to the lack of early diagnosis, the search for new markers that can provide early diagnosis and avoid complications has become increasingly important. podocyte cells contribute to maintaining the blood-urine barrier, preventing proteins and large molecules from entering the urinary ultrafiltrate. Therefore, podocyte cell injury or dysfunction plays an important role in the development and progression of many kidney diseases(2) . This increases the possibility of employing podocyte cell product targets, particularly in urine, as practical clinical markers for kidney disease monitoring and diagnosis. Numerous earlier investigations have shown that the quantity of podocyte-associated chemicals in the urine may be able to predict the prognosis of DKD. The Nephrin is among the most significant. Nephrin is a 180 kDa transmembrane protein that is present on the exterior of podocyte foot processes. Nephrin is a component of the glomerular filtration barrier (GFB), which is formed by podocytes in the vascular space and GBM as a monolayer of fenestrated endothelium (3). Through its interaction with CD2AP, nephrin interacts with the actin cytoskeleton. (4), and it also colocalizes and binds to the actin cytoskeleton and podocin. Urinary nephrin protein and mRNA have been investigated in individuals with different

glomerulopathies and may be useful clinical indications of podocyte destruction. In those with type 2 diabetes who were discovered to have normoalbuminuria, urine nephrin was proven to be a more sensitive indication of diabetic nephropathy than urine albumin testing. (5,6).

In order to determine which of these measures is more sensitive and crucial for the early identification of the disease, we examined the roles of urinary Nephrin and other podocyte markers with GFR and urinary albumin/creatinine ratio. Furthermore, the majority of earlier research concentrated on determining the amount of these markers after a kidney biopsy, which is a difficult and complicated procedure (7,8). Therefore, to show their effectiveness in early and accurate disease identification, we will evaluate the level of these markers in urine and compare them with traditional diagnostic techniques

2. Material and Methods

A cross-sectional study will be carried out to a group of 70 diabetic patients with diabetic nephropathy 50 male and 20 female and 20 healthy control group 15 male and 5 female . All the subjects enrolled in this study were diagnosed under specialist supervision. Serum and urine samples will obtain from all participants.

A total of 25 mL urine was collected and centrifuged at 2000 rpm for 10 min. The supernatant was aliquoted to volume to 0.5 ml. . A mixture of glacial acetic acid, ethanol, and double-distilled water was added up to a total of 1 mL. Samples were stored at -20°C and aliquots were thawed for ELISA assays.

Diabetic patients were divided into four groups according to their stage of kidney disease . There are 5 stages of kidney disease. Determination the stage of kidney disease based on the presence of kidney damage and glomerular filtration rate (GFR) normal , mild, moderately , sever , and kidney failure.

Another division based on the albuminuria in which all patients included in the study were divided into Normoalbuminuria , Microalbuminuria and. Macroalbuminuria . In the present study we prefer to measure the Alb/Cr ratio in a random urine sample . Serum creatinine, serum albumin and HbA1C. Urinary Albumin / creatinine ratio. Urinary nephrin and podocaxlyin were determined by using commercially available ELISA test according to the manufacturer's instructions.

3. Results

According to age and sex Our study revealed that there was non-significant difference between the studied groups as regards demographic data including mean age and sex between patients and control groups . The study reveals that the majority of diabetic patients were in older age with long duration of diabetes (64.27 ± 10.9) as shown in table (1) .

The current study demonstrated that cases were significantly higher regarding the levels of UA/C ratio and HBA1c parameters in diabetic patients than that of healthy group table (1). Also the present study confirmed that there was a decline in glomerular filtration rate in patients than that of control group.

Table (1) showed the characteristics of diabetic patients and control groups

parameters	Groups	No.	Mean	Std. D.	P Value
AGE	diabetic patients	70	64.27	10.9	
	heathy control	20	61.70	13.1	Non. sig.
eGFR	diabetic patients	70	63.1	25.2	
	heathy control	20	98.0	4.5	Sig.
UA/C ratio	diabetic patients	70	232.6	167.3	.
	heathy control	20	20.7	5.7	Sig.
FBS	diabetic patients	70	97.5	19.3	
	heathy control	20	91.9	8.2	Non sig.
HBA1C	diabetic patients	70	7.4	.74	
	heathy control	20	5.5	.53	Sig.

An important finding of the present study was that the patients group had higher Urinary Nephrin and Podocalyxin levels than the control group table (2) . In addition, a significant higher Urinary Nephrin and PCX levels were found in patients with macroalbuminuria than in those with normoalbuminuria and microalbuminuria .fig (1). However, our results showed a strong positive correlation between urinary Nephtin level and UACR, fig (4)

StructTable (2) showed the levels of podocyte markers in diabetic patients and control group.

Parameters	group	No.	Mean	Std. D.	P value
U. PCX/UCre. ng/umol	diabetic patients	70	36.0	13.2	
	heathy control	20	15.1	5.1	Sig.
U. Nephrin/UCre. ng/umol/l	diabetic patients	70	16.3	5.0	
	heathy control	20	2.4	.9	Sig.

Our study revealed that patients with normoalbuminuria have higher urinary Nephrin. Level than healthy control group as well as revealed that 50% of patients with normoalbuminuria have higher urine Nephrin levels. These findings suggest that in the early stages of kidney damage urinary nephrin first appears in the urine preceding microalbumin.

The results obtained showed that urinary nephrin and pdocaxlyin level was significantly increased in both UM/CR and CKD staging subgroups compared with the healthy control group. A gradual increase in urinary nephrin level with CKD stage, especially in 2 and 3

stages, and the higher sensitivity of urinary nephrin as compared to UM/CR ratio in early detection of kidney damage was demonstrated.

Table (3) laboratory level of parameters in different groups

parameter	Healthy control No. 20	Normo Albuminuria No.20	Micro Albuminuria N0.30	Macro Albuminuria N0.40	P Value
Age	61.9 ± 13.1	65.7± 11.1	64.5 ± 11.3	63.8 ± 10.5	Non Sig.
eGFRml/min.	98.0 ± 4.5	92.6 ± 6.5	82.2 ± 13.1	37.7 ± 11.0	Sig .
UA/Cre.ratio	20.7 ± 5.7	27.7± 6.3	96.0 ± 45.7	414.8 ± 61.1	Sig.
HBA1c	5.5 ± 0.5	6.5± 0.5	7.5 ± 0.7	7.4 ± 0.8	Sig.
FBG	91.9 ± 19.3	91.4 ± 8.2	98.6 ± 18.3	± 20.8 96.2	Non sig
Pcx/U Cre. ng/umol/l	15.1 ± 5.1	18.4± 6.4	26.1 ± 6.1	49.1± 7.0	sig
Nephrin/Ucre. ng/umol/l	2.4 ± 0.9	5.6± 1.9	13.1 ± 2.8	20.5± 4.0	sig

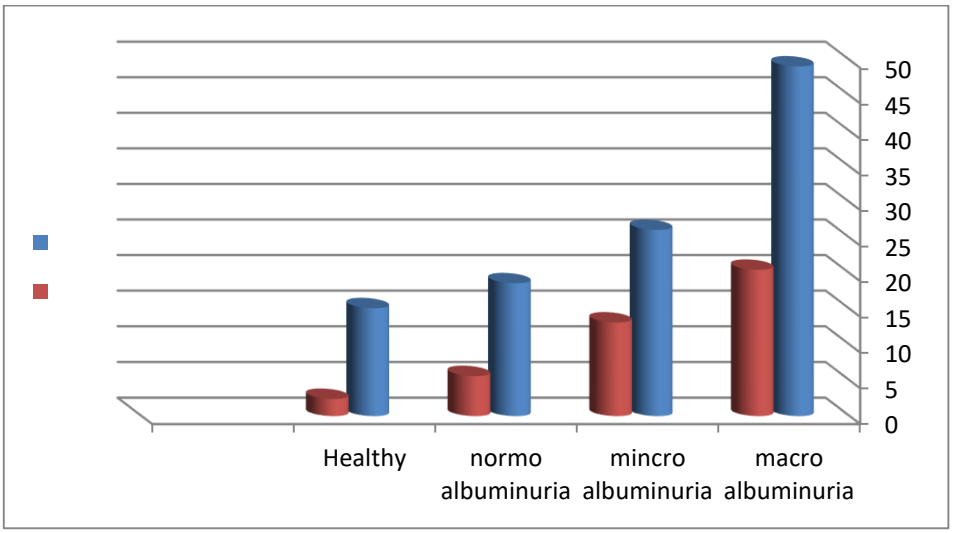

Fig. 1. urinary nephrin and podocaxlyin levels in subgroups of patients divided according to UM/CR

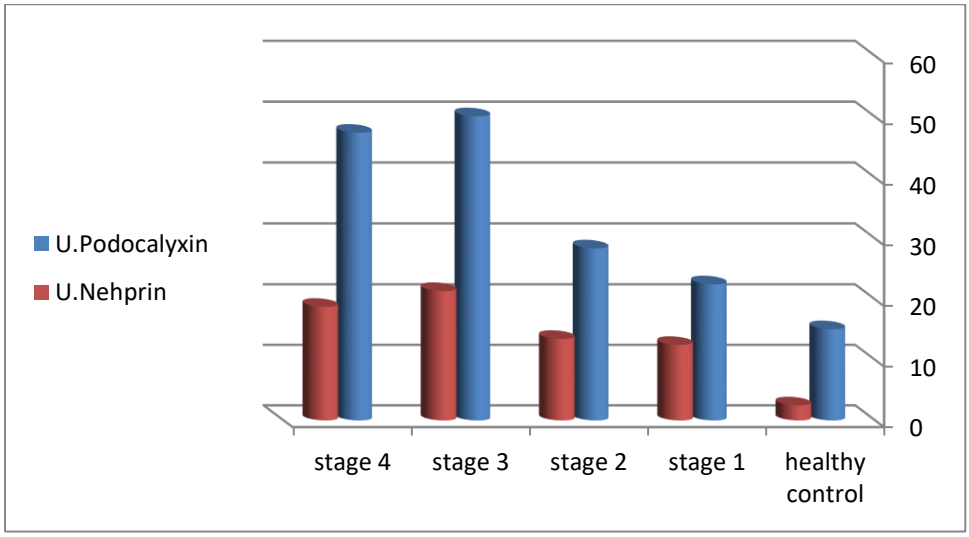

Fig. 2. urinary Nephrin and podocaxlyin levels in subgroups of patients divided according to CKD stage.

The current study showed a negative correlation between U. Nephrin and eGFR in the case and control group while a strog positive correlation was found between U.Nephrin and albumin/creatnine ratio . As shown in figure (5,6).

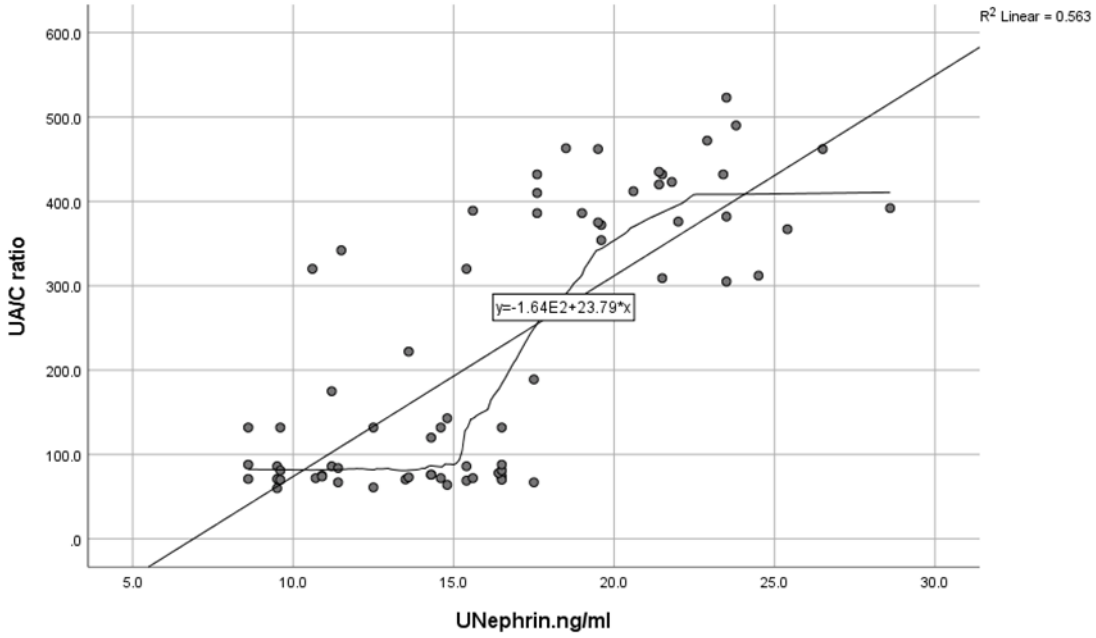

Fig. 3. show the correlation between U. Nephrin and UA/C ratio serum levels in diabetic patients group.

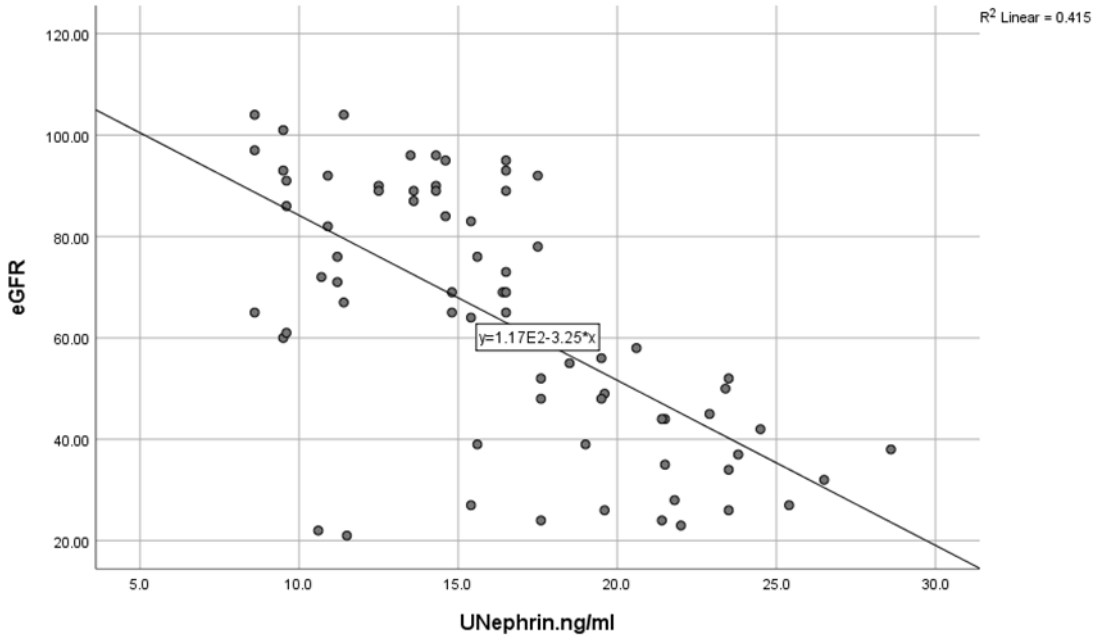

Fig. 4. show the correlation between U. Nephrin and eGFR serum levels in diabetic patients group

4. Discussion

In line with previous research suggesting that urinary nephrin may be a useful indicator for the early detection of secondary proteinuric kidney disease (9,10) , the current study found that diabetic patients' nephrin levels were significantly higher than those of the control group. Nephrin plays a crucial role in regulating the selective permeability of urine proteins and is a component of the slit-diaphragm in glomeruli. The glomerular filtration barrier consists of podocytes, basement membrane, and endothelial cells; nephrin is an essential component of both. The presence of nephrin in urine may be caused by injury to podocytes (11).(

Micro and macroalbuminuria were associated with elevated urine nephrin levels in this research compared to normoalbuminuria. This study's results are in line with those of Verma et al. and Jam et al., who have conducted comparable research (12,13) . Patients with macroalbuminuria had higher urine nephrin levels than those with microalbuminuria(14). Patients with various glomerulopathies have also had urinary nephrin protein and nephrin mRNA tested, which have shown promise as clinically relevant indicators of podocyte damage (15,16). urine nephrin was shown to be a more sensitive indicator of diabetic nephropathy than urine albumin measurement in individuals with type 2 diabetes who were found to have normoalbuminuria (17) . The reorganization of the podocytes' skeleton may be impacted by a number of factors, including changes in nephrin's amino acid composition, molecular damage, charge irregularity, and reduced expression (18). The slit-diaphragm might be destroyed and proteinuria produced if this impacts the function of the associated protein molecule (19).

Urine podocyte-specific proteins such nephrin show that podocytes alone have been damaged, rather than the other two parts of the glomerular filtration barrier (20). So, podocyte damage is believed to be present before microalbuminuria and proteinuria appear. Podocyte proteins, including nephrin, are seen as better indicators of diabetic nephropathy than microalbuminuria, and they appear earlier in the diagnostic process (17).Our study revealed that patients with normoalbuminuria have higher urinary Nephrin. Level than healthy control this finding suggest that urinary nephrin appears earlier than albuminuria in early renal diseaseas shown in table (3) and fig (1).

Figures 3 illustrate the results of the study's correlation analysis, which revealed a very favorable relationship between urine nephrin and urinary albumin/creatinine ratio. This indicates that while urine albumin excretion rises in diabetes individuals, urinary nephrin excretion also increases. Our investigation found that diabetic individuals with microalbuminuria had significantly higher urine nephrin levels than those with macroalbuminuria. This result agreed with what had been found in an earlier investigation by Patari et al. (19) . who documented elevated amounts of urine nephrin in individuals with advanced-stage diabetes .

The present study revealed that the nephrin urinary level was strongly associated with eGFR. As shown in fig (4) in consistent with a study done by Daniel PK Ng et al.(21) , nephrinuria was independently associated with the logarithmic form of an albumin-creatinine ratio (ACR) and estimated glomerular filtration rate (eGFR). Nephrinuria was associated with these traits in type 2 diabetic patients with normoalbuminuria (22) . Another study also showed an association between biomarkers of podocyte damage like urinary nephrin and vascular endothelial growth factor with normoalbuminuria in patients with type 2 diabetes mellitus (23) . Urinary nephrin was found to be a sensitive and specific marker for diagnosing early diabetic nephropathy than microalbumin in another study(24) .

5. Conclusions:

Diabetes patients had much greater amounts of Nephrin in their urine compared to the healthy control group, according to the data. Urinary nephrin was shown to be more sensitive than UM/CR ratio in detecting diabetic nephropathy in its early stages, and our work shows that its level gradually increases with chronic kidney disease stage, particularly in late stages of renal disease.

6. Authors contribution

Kanaan Faisal Yassin: data collection , work performance , methodology, and manuscript writing.
Sami A Zbaar : Supervision, project administration, critical review, and final approval of the manuscript.

Conflect of interrest

No conflict of interest

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