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Urinary Nephrin as a Marker of Nephropathy in Obese Prediabetic Patients

Hanan Mohammed Amer
Professor of Internal Medicine - Diabetes & Endocrinology, Faculty of Medicine, Ain Shams University, Cairo, Egypt

Maram M. Aboromia
Professor of Internal Medicine - Diabetes & Endocrinology, Faculty of Medicine, Ain Shams University, Cairo, Egypt

A A ElSherbini
Professor of Internal Medicine - Diabetes & Endocrinology, Faculty of Medicine, Ain Shams University, Cairo, Egypt

Hanan Mahmoud Ali
Assistant Professor of Internal Medicine - Diabetes & Endocrinology, Faculty of Medicine, Ain Shams University, Cairo, Egypt

Zainb Yousry Helal
Masters degree in internal medicine Ain Shams University, Cairo, Egypt

Mina Michaeil Nesim
Lecturer of Internal Medicine - Diabetes & Endocrinology, Faculty of Medicine, Ain Shams University, Cairo, Egypt

***Abstract*---**Background: One of the most important complications of obesity and prediabetes is nephropathy. In the Eastern Mediterranean area, the incidence of obesity is always growing. In Saudi Arabia, Egypt, Kuwait, the United Arab Emirates, and Bahrain, the highest level of obesity is found in 15-year-old adults and also in older adults. Obesity and overweight are prevalent in the preceding countries at rates ranging from 69% to 77% in men and 74% to 80% in women, making it critical to distinguish nephropathy in these patients for a better prognosis. Objective: To assess the level of urine nephrin as a nephropathy marker in obese and prediabetic patients. Patients and Methods: This was cross-sectional observational research that included 90 people. Their ages varied from 30 to 70 years old, and they came from Dar El-Shefa hospital's outpatient clinic between

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***Corresponding author**: Helal, Z.Y.; Email: zainbhelal@yahoo.com*
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December 2019 and May 2020. The study was explained to all participants, and their informed consent was acquired before the start of the trial. The subjects were split into three groups: group I (thirty normoalbuminuric obese non-diabetic patients), group II (thirty normoalbuminuric obese pre-diabetic patients), and group III (thirty healthy control). Results: Our findings revealed a highly statistically significant difference in nephrin levels between the three studied groups, with a P-value (0.001) higher in group II (obese prediabetic patients), followed by the group I (obese non-prediabetic patients), than in group III (control group). Conclusion: This study shows that nephrin levels in urine are increased in obese prediabetic patients even before albuminuria, which indicates early nephropathy, drawing attention to the possibility of using urinary nephrin as a marker of nephropathy. And this gives us an opportunity for early intervention and prevention of complications.

***Keywords*---**chronic kidney disease, nephrin, obesity, and prediabetes.

Introduction

When there is kidney damage or the estimated glomerular filtration rate (eGFR) becomes less than 60 ml/min/1.73m2, then we can term the disease as chronic kidney disease (CKD). CKD is associated with high risks like progression to end-stage renal disease (ESRD), which demands transplantation or dialysis, and cardiovascular risk. Diabetics account for approximately thirty to fifty percent of all ESRD patients worldwide *(1)*.

The intermediate stage of normal glucose tolerance (NGT) is prediabetes, which could shortly progress to a diabetic stage with more prevalence of great risks in the form of chronic complications that affect the heart, eyes, and kidneys. Diabetics account for 30 to 50% of people with ESRD *(2)*. There is an increase in obesity incidence globally that affects public health. CKD could be promoted by effects of obesity like features of metabolic syndrome, including hypertension, insulin resistance, and inducing hyperfiltration of glomeruli *(3)*.

Diabetic Nephropathy could be detected early by using the gold standard test of microalbuminuria. The endothelium, podocytes, and glomerular basement membrane are considered the protective filtration barriers which are damaged, leading to the presence of microalbumin, Furthermore, the fact that structural damage may occur before microalbumin excretion reduces the diagnostic accuracy of the test. Podocyte injury is the sole component of the glomerular filtration barrier that is indicated by the presence of nephrin in urine *(4)*. Nephrin is a critical component of the slit diaphragm, which is situated between the foot processes of the podocytes and is responsible for the regulation of blood flow. It is likely to be expelled first in the event of a disruption to the filtration barrier since it is a component of the renal filtration diaphragm *(5)*.

Aim of the work

The purpose of this study is to determine the amount of urine nephrin in normoalbuminuric, obese, prediabetic individuals as a marker of nephropathy.

Patients and Methods

This was cross-sectional observational research that included 90 people. Their ages varied from 30 to 70 years old, and they came from Dar El-Shefa hospital's outpatient clinic between December 2019 and May 2020. The study was explained to all participants, and their informed consent was acquired before the start of the trial.

Sample size

This research enrolled 90 individuals who were split into three groups: Group I: 30 obese individuals who are neither prediabetic nor diabetic. 30 obese prediabetic individuals were included in Group II. Group III: 30 healthy, non-obese individuals served as the control group.

Inclusion criteria: (30-75 years old).BMI (>30 for groups I and II). Group II suffers from pre-diabetes.
Exclusion criteria: Any nephrological disorder (obstructive, glomerular, tubular). females who are pregnant smokers. Urinary tract infections late stage of heart failure. Late-stage of liver disease Are you under the age of thirty or over the age of 75.
Sample collection: People were chosen by simple randomization.
Ethical consideration: Respect for people, respect for the autonomy and dignity of participants. Justice-participants should be selected from groups of people whom the research may benefit. Procedure for conducting research. After informed consent, The following procedures have been performed on all patients:

- Complete medical history, paying special attention to age, nephropathy symptoms, current illness, medications, and any previous medical history.
- 2-Full clinical examination with special emphasis on blood pressure measurement, BMI calculation, waist/hip ratio calculation, edema examination, and signs of insulin resistance as acanthosis negricans.
- 3- Laboratory assessment of the nephrine level in urine by ELISA. Fasting insulin by ELISA [to calculate HOMA-IR]. Hb A1C measurement by quantitative colorimetric analysis of glycohemoglobin in the blood, 2HPP. glucose oxidase test for fasting blood glucose, lipid profile [total cholesterol, HDL, LDL, and TG] by colorimetric and fluorometric measurement kit, GFR was calculated using the MDRD-4 equation, and in urine, the albumin/creatinine ratio was measured. An analysis of urine was done.

Methodology
Our study was done in three steps

The first step was to choose 100 obese people according to inclusion and exclusion criteria, then we did labs. Albumin/creatinine ratio to exclude

albuminuria. estimated GFR to rule out renal disease. Urine analysis to rule out UTI. Second step: after exclusion of patients with albuminuria, renal impairment, and UTI, we did labs for FBS, 2HPP, and HbA1C for 80 people to differentiate between prediabetics and non-prediabetics.

Then we randomly collected 30 normoalbuminuric obese non-prediabetic patients and 30 normoalbuminuric obese prediabetic patients. Third step: we fulfilled the labs' recommendations for our study (urine nephrin, lipid profile, and fasting insulin).

Urinary nephrin level estimation

Midstream urine and blood sera from the previous morning were used. Untreated plastic containers were used to collect urine samples (10 mL). After centrifuging the blood for 10 minutes at 3.000 rpm, the serum was collected in 1.5 mL Eppendorf tubes. In the first instance, urine dipsticks were used to conduct a chemical analysis of the freshly collected urine. Then, using a turbidimetric technique on a ChemWell biochemical analyzer, the microalbumin content in urine was determined, and the creatinine concentration was determined using the Jaffe reaction (2910 Awareness Technology, Inc.). Once the centrifuged urine samples were collected, they were stored at-80 degrees Celsius until the nephrin concentration could be determined.

To determine the amount of nephrin present in urine, researchers used kits that were readily available from commercial sources (Exocell Inc., Philadelphia, PA). In this technique, polyclonal antibodies against nephrin are utilized in conjunction with an indirect competitive ELISA. Anti-nephrin rabbit antibodies compete with nephrin antigens extracted from urine and immobilized nephrin antigens (at the bottom of polystyrene plates). The presence of bound antibodies is detected using an antirabbit HRP (HRP-horseradish peroxidase) reaction. Following washing, the 450 nm wavelength is used to measure the amount of residual antibody conjugate bound to immobilized nephrin antigens. An increase in urine color intensity is inversely proportional to an increase in nephrin concentration. Nephrin concentrations were determined using a standard curve that was created using industry-standard reference materials. The results are given in nanograms per milliliter of liquid.

Statistical analysis

The information was gathered, edited, coded, and placed into the IBM SPSS version 23 statistical package for social science research. When the quantitative data was determined to be parametric, the mean, standard deviations, and ranges were provided as the results of the analysis. Aside from quantitative factors, qualitative variables were provided as numbers and percentages as well. To compare groups using qualitative data, the Chi-square test and Fisher exact test were used. The Fisher exact test was used instead of the Chi-square test only when the anticipated count in any cell was determined to be less than 5. The One Way Analysis of Variance (ANOVA) was used to compare more than two distinct sets of data that included quantitative data and parametric distribution, followed by post hoc analysis using the LSD test when the difference was statistically

significant. The correlation coefficients between two quantitative parameters within the same group were calculated using the Spearman correlation coefficients. It was decided on the cut-off point between the two groups using the receiver operating characteristic curve (ROC), which measured the sensitivity, specificity, positive predictive value, negative predictive value, and area under the curve of each group (AUC). The confidence interval was set at 95 percent, with a 5 percent margin of error considered acceptable. As a result, the p-value was determined to be significant in the following ways: P > 0.05 indicates that the difference is not significant (NS). P values less than 0.05 are considered significant (S). P <0.01 indicates a statistically significant difference (HS).

Results

Three groups participated in our study: Group I consists of 30 obese individuals who are not prediabetic or diabetic. Group II consists of 30 obese pre-diabetic individuals. Group III consists of 30 healthy, non-obese individuals who serve as a control group. The demographic data of the three studied groups in the current shown in table (1). The laboratory data of the three studied groups in the current shown in table (2).

The current study revealed that there is a highly statistically significant difference between the three investigated groups in terms of BMI and WHR, with group II having the highest BMI and WHR followed by the group I and finally group III, with a p-value of 0.000. While there is no statistically significant difference between the three examined groups in terms of age, gender, and HTN (p-values = 0.209, 0.248, and 0.075, respectively) as shown in table (3)

The results of the current study revealed that there is a highly statistically significant difference between the three investigated groups in terms of Homa-IR, HbA1C, 2HPP, FBS, Total CH, LDL, HDL, and TG with a P-value of <0.001 between the three studied groups. The post hoc analysis using the LSD test was done as shown in table (4). As demonstrated in this study, there is a highly statistically significant difference between the three investigated groups in terms of fasting insulin and eGFR, with a P-value of less than 0.001 being greater in group II, followed by group I, and finally group III, Post hoc analysis using LSD test was done as shown in table (5).

Concerning nephrin, the current study revealed that there is a highly statistically significant difference discovered between them, with a P-value less than0.001 being greater in group II, followed by group I, and finally group III, Post hoc analysis using LSD test shows that there is a highly statistically significant difference between obese non-prediabetic (group I) and obese prediabetic (group II) regarding nephrin with P-value <0.001 being higher in group II than group I and there is a highly statistically significant difference between Obese non-Prediabetic (group I) and the control (group III) regarding nephrin with P-value <0.001 being higher in group I than group III, Also there is a highly statistically significant difference between Obese Prediabetic (group II) and the control (group III) regarding nephrin with P-value <0.001 being higher in group II than groupIII as shown in table (6).

The current study showed that the best cut-off point between control group VS group I & II was > 170 with a sensitivity of 98.3%, specificity of 96.6%, and area under the curve (AUC) of 97% as shown in table (7). This study demonstrated a highly statistically significant positive association between nephrin level and the following variables: BMI, WHR, HOMA-IR, HbA1c, 2HPP, FBS, total cholesterol, LDL cholesterol, triglycerides, and fasting insulin in the individuals with type 2 diabetes with P-value <0.001 and there is no statistically significant relation between nephrin level and age and eGFR level of the studied subjects with P-value = 0.893 and 0.495 respectively, Also there is a negative correlation between nephrin level and HDL cholesterol between all the studied subjects as shown in table (8).

In the current study, logistic regression analysis for predictors of independent factors that affect nephrin level showed that 2HPP is the only independent factor that affects the level of nephrin level. An increase in the 2HPP level independently increases nephrin level as shown in table (9).

Table 1
Descriptive data of the studied groups regarding demographic data, anthropometric measures, and clinical data

		Group I (Obese non Prediabetic) No.= 30	Group II (Obese Prediabetic) No.= 30	Group III (Control group) No.= 30
Age (years)	Mean±SD	44.46 ± 4.23	42.03 ± 6.01	46.23 ± 14.04
	Range	30 – 50	30 – 49	30 – 69
Sex	Female	12 (40.0%)	17 (56.7%)	11 (36.7%)
	Male	18 (60.0%)	13 (43.3%)	19 (63.3%)
BMI (kg/m^2)	Mean±SD	34.23 ± 3.51	41.00 ± 4.73	24.03 ± 0.89
	Range	30 – 42	30 – 48	23 – 26
Waist hip ratio	Mean±SD	1.00 ± 0.14	1.15 ± 0.12	0.88 ± 0.05
	Range	0.83 – 1.4	0.96 – 1.3	0.8 – 0.99
Hypertension (mmHg)	Negative	26 (86.7%)	25 (83.3%)	30 (100.0%)
	Positive	4 (20.0%)	5 (16.7%)	0 (0.0%)

Table 2
Descriptive data of the studied groups regarding laboratory data

		Group I (Obese non Prediabetic) No.= 30	Group II (Obese Prediabetic) No.= 30	Group III (Control group) No.= 30
Homa-IR	Mean±SD	2.81 ± 1.11	7.04 ± 0.86	1.50 ± 0.27
	Range	0.91 – 4.87	4.93 – 8.57	0.98 – 1.88
Hb A1C (%)	Mean±SD	5.07 ± 0.32	6.03 ± 0.21	4.94 ± 0.45
	Range	4.5 – 5.5	5.7 – 6.4	4.2 – 5.5
2HPP (mg/dl)	Mean±SD	129.47 ± 7.69	159.73 ± 9.71	125.80 ± 6.63
	Range	111 – 139	145 – 179	115 – 138
FBS (mg/dl)	Mean±SD	85.47 ± 7.27	118.93 ± 4.20	88.07 ± 5.93
	Range	74 – 96	110 – 125	75 – 96

Total cholesterol (mg/dl)	Mean±SD	204.67 ± 29.12	230.53 ± 26.65	165.30 ± 11.01
	Range	150 – 285	180 – 276	150 – 195
LDL cholesterol (mg/dl)	Mean±SD	143.10 ± 20.77	163.37 ± 21.84	94.70 ± 3.90
	Range	100 – 200	125 – 200	85 – 100
HDL cholesterol (mg/dl)	Mean±SD	42.17 ± 10.18	41.00 ± 6.21	53.77 ± 4.56
	Range	5 – 56	35 – 55	45 – 59
Triglycerids (mg/dl)	Mean±SD	155.63 ± 26.38	193.03 ± 30.84	146.03 ± 7.01
	Range	99 – 200	150 – 266	133 – 160

Table (1)
Comparison between the three studied groups regarding demographic data and characteristics of the studied patients

		Group I (Obese non Prediabetic) No.= 30	Group II (Obese Prediabetic) No.= 30	Group III (Control group) No.= 30	Test value	P-value	Sig.
Age (years)	Mean±SD	44.46 ± 4.23	42.03 ± 6.01	46.23 ± 14.04	1.594•	0.209	NS
	Range	30 – 50	30 – 49	30 – 69			
Sex	Female	12 (40.0%)	17 (56.7%)	11 (36.7%)	2.790*	0.248	NS
	Male	18 (60.0%)	13 (43.3%)	19 (63.3%)			
BMI (kg/m^2)	Mean±SD	34.23 ± 3.51	41.00 ± 4.73	24.03 ± 0.89	184.792•	0.000	HS
	Range	30 – 42	30 – 48	23 – 26			
Waist hip ratio	Mean±SD	1.00 ± 0.14	1.15 ± 0.12	0.88 ± 0.05	45.719•	0.000	HS
	Range	0.83 – 1.4	0.96 – 1.3	0.8 – 0.99			
Hypertension (mmHg)	Negative	26 (86.7%)	25 (83.3%)	30 (100.0%)	5.185*	0.075	NS
	Positive	4 (20.0%)	5 (16.7%)	0 (0.0%)			

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

Table 4
Comparison between the three studied groups regarding laboratory data

		Group I (Obese non Prediabetic)	Group II (Obese Prediabetic)	Group III (Control group)	Test value•	P-value	Sig.
		No.= 30	No.= 30	No.= 30			
Homa-IR	Mean±SD	2.81 ± 1.11	7.04 ± 0.86	1.50 ± 0.27	366.170	<0.001	HS
	Range	0.91 – 4.87	4.93 – 8.57	0.98 – 1.88			
Hb A1C (%)	Mean±SD	5.07 ± 0.32	6.03 ± 0.21	4.94 ± 0.45	91.422	<0.001	HS
	Range	4.5 – 5.5	5.7 – 6.4	4.2 – 5.5			
2HPP (mg/dl)	Mean±SD	129.47 ± 7.69	159.73 ± 9.71	125.80 ± 6.63	158.253	<0.001	HS
	Range	111 – 139	145 – 179	115 – 138			
FBS (mg/dl)	Mean±SD	85.47 ± 7.27	118.93 ± 4.20	88.07 ± 5.93	295.118	<0.001	HS
	Range	74 – 96	110 – 125	75 – 96			
Total cholesterol (mg/dl)	Mean±SD	204.67 ± 29.12	230.53 ± 26.65	165.30 ± 11.01	57.814	<0.001	HS
	Range	150 – 285	180 – 276	150 – 195			
LDL cholesterol (mg/dl)	Mean±SD	143.10 ± 20.77	163.37 ± 21.84	94.70 ± 3.90	121.313	<0.001	HS
	Range	100 – 200	125 – 200	85 – 100			
HDL cholesterol (mg/dl)	Mean±SD	42.17 ± 10.18	41.00 ± 6.21	53.77 ± 4.56	27.504	<0.001	HS
	Range	5 – 56	35 – 55	45 – 59			
Triglycerides (mg/dl)	Mean±SD	155.63 ± 26.38	193.03 ± 30.84	146.03 ± 7.01	32.717	<0.001	HS
	Range	99 – 200	150 – 266	133 – 160			

Post hoc analysis using LSD test

Parameters	P1	P2	P3
Homa-IR	<0.001	<0.001	<0.001
Hb A1C	<0.001	0.152	<0.001
2HPP	<0.001	0.083	<0.001
FBS	<0.001	0.093	<0.001
TOTAL cholesterol	<0.001	<0.001	<0.001
LDL cholesterol	<0.001	<0.001	<0.001
HDL cholesterol	0.541	<0.001	<0.001
Triglycerides	<0.001	0.122	<0.001

P-value >0.05: Non significant (NS); P-value <0.05: Significant (S); P-value< 0.01: highly significant (HS)
•: One Way ANOVA test
P1: Comparison between Obese non Prediabetic and Obese Prediabetic
P2: Comparison between Obese non Prediabetic and Control group
P3: Comparison between Obese Prediabetic and Control group

Table 5
Comparison between the three studied groups regarding F.insulin and e.GFR of the studied patients

		Group I (Obese non Prediabetic)	Group II (Obese Prediabetic)	Group III (Control group)	Test value	P-value	Sig.
		No.= 30	No.= 30	No.= 30			
Fasting insulin (mIU/L)	Mean±SD	13.47 ± 5.05	23.97 ± 2.71	6.90 ± 1.32	192.815•	0.000	HS
	Range	5 – 22	16 – 29	5 – 9			
e. GFR (ml/min /1.73m 2)	Mean±SD	114.40 ± 6.94	120.33 ± 8.76	107.30 ± 6.18	23.495•	0.000	HS
	Range	90 – 125	90 – 130	90 – 115			

Post hoc analysis using LSD test			
Parameters	P1	P2	P3
Fasting insulin	<0.001	<0.001	<0.001
e. GFR	0.002	0.000	0.000

P-value >0.05: Non significant (NS); P-value <0.05: Significant (S); P-value< 0.01: highly significant (HS)
•: One Way ANOVA test
P1: Comparison between Obese non Prediabetic and Obese Prediabetic
P2: Comparison between Obese non Prediabetic and Control group
P3: Comparison between Obese Prediabetic and Control group

Table 6
Comparison between the three studied groups regarding nephrin

		Group I (Obese non Prediabetic)	Group II (Obese Prediabetic)	Group III (Control group)	Test value•	P-value	Sig.
		No.= 30	No.= 30	No.= 30			
Nephrin	Mean±SD	519.17 ± 184.73	1066.33 ± 280.03	147.00 ± 146.60	176.331	<0.001	HS
	Range	170 – 930	675 – 1725	75 – 900			

Post hoc analysis using LSD test		
P1	P2	P3
<0.001	<0.001	<0.001

P-value >0.05: Non significant (NS); P-value <0.05: Significant (S); P-value< 0.01: highly significant (HS)
•: One Way ANOVA test
P1: Comparison between Obese non Prediabetic and Obese Prediabetic
P2: Comparison between Obese non Prediabetic and Control group
P3: Comparison between Obese Prediabetic and Control group

Table 7
Receiver operating characteristic curve (ROC) for nephrin level in differentiation between the three studied groups

	Cut off point	AUC	Sensitivity	Specificity	+PV	-PV
Control Vs Patients	>170	0.979	98.33	96.67	98.3	96.7

Table 8
Correlation of nephrin level with demographic, anthropometric, and laboratory data of the studied subjects

All Cases	Nephrin	
	r	P-value
Age (years)	0.014	0.893
BMI (kg/m2)	**0.857****	0.000
WHR	**0.680****	0.000
Homa-IR	**0.781****	0.000
Hb A1C (%)	**0.650****	0.000
2HPP (mg/dl)	**0.704****	0.000
FBS (mg/dl)	**0.541****	0.000
Total cholesterol (mg/dl)	**0.736****	0.000
LDL cholesterol (mg/dl)	**0.783****	0.000
HDL cholesterol (mg/dl)	**-0.493****	0.000
Triglycerides (mg/dl)	**0.663****	0.000
Fasting insulin (Miu/L)	**0.781****	0.000
e. GFR (ml/kg/1.73m2)	0.207	0.113

Table 9
Multivariate Linear regression analysis

	Unstandardized Coefficients		Beta	t	Sig.
	B	SE			
BMI	17.249	8.980	0.262	1.921	0.061
WHR	-192.042	326.168	-0.082	-0.589	0.559
Homa-IR	35.762	149.955	0.237	0.238	0.813
Hb A1C	42.451	127.012	0.066	0.334	0.740
2HPP	**7.928**	**3.283**	**0.394**	**2.415**	**0.020**
FBS	-0.343	8.009	-0.017	-0.043	0.966
TOTAL CH	-2.227	2.543	-0.193	-0.875	0.386
LDL	4.879	3.289	0.324	1.483	0.145
HDL	1.653	4.261	0.039	0.388	0.700
TG	0.914	1.310	0.088	0.698	0.489
F. insulin	-8.348	36.918	-0.157	-0.226	0.822

Discussion

In the current study, which aimed to evaluate the level of urinary nephrin as a marker of nephropathy in obese prediabetic patients, We selected 90 normoalbuminuric subjects who were divided into 3 groups: Group I (30 obese non-prediabetic or diabetic people), Group II (30 obese prediabetic patients), and Group III (30 healthy non-obese people as a control group). We observed that there was a highly statistically significant difference between the three studied groups regarding nephrin, which was higher in group II (obese prediabetic patients), followed by the group I (obese non-phrin-groups non-control group).

This result coincides with many results which aim to determine whether urinary nephrin is a biomarker for diabetic nephropathy as *dos Santos et al.* [6] study in which 83 overweight or obese patients and 18 healthy controls were included in the study. Nephrin and other podocyte markers are expressed in urine. It was found that urinary nephrin and other podocyte markers were higher in adults with obesity class III than in other groups (P 0.05). Podocyte mRNA levels were also higher in patients with obesity class I or II than in controls, nephrin (P-value 0.021). After it was discovered that hyperinsulinemia was correlated with podocyturia, it was discovered that higher insulin levels were associated with obesity, regardless of the degree of obesity. So, even with normal urinary albumin excretion rates, severe obesity and hyperinsulinemia were linked to higher urinary expression of podocyte-associated mRNAs.

Another study conducted by Wang et al. [7] found similar results when they examined urine nephrin levels (measured by mRNA expression) in diabetic patients with and without kidney damage, as well as in nine healthy individuals. The researchers observed that nephrinuria was more common in diabetics with nephropathy, and they concluded that measuring nephrin could help with the clinical classification of diabetic nephropathy patients.

Nascimento et al. [8] studied urine nephrin in 15 healthy controls and 67 diabetics and found similar results. Also, this result coincides with *Jim et al.'s* [9] study. It looked at urine nephrin levels in 66 diabetics at various stages of chronic kidney disease (CKD) and albuminuria levels and compared them to 10 healthy controls. This result disagrees with Nakamura et al. [10], A study was done on 60 patients, including 10 normoalbuminuric diabetic patients, who did not find podocytes in the urine of normoalbuminuric type I diabetic patients. This opposition to our study may be due to the very low number of subjects included in the study, or nephrin may be released as a free molecule before podocytes detach.

In the current study, we found that there was a highly statistically significant positive correlation between nephrin level and HbA1c, 2HPP, and FBS of the studied subjects. This result coincides with Alaa et al. [11] study which found that in diabetic nephropathy, Nephrin showed a significant positive correlation with glucose levels. Also, this result coincides with the results of dos Santos et al. [6] who showed significant positive correlations between urinary nephrin and other podocyte markers and FPG, HbA1c.

In the current study, we found that there was a highly statistically significant positive correlation between nephrin level and HOMA-IR and fasting insulin of the

studied subjects. This result coincides with dos Santos et al. [6] in which the results showed significant positive correlations between urinary nephrin and other podocyte markers and serum insulin.

Using a receiver operating characteristic curve (ROC) for nephrin level in differentiation between the three studied groups, we found that the best cut off point between the control group and VS group I & II was > 170 pg/ml with a sensitivity of 98.3%, a specificity of 96.6% and an area under the curve (AUC) of 97%. This result coincides with the Kostovska et al. [12] study in which, according to the results of the ROC analysis, nephrin has a total predicted probability of 96 percent in people with DN. This means high discriminatory power between healthy subjects and patients with DN. Additionally, this finding indicates that nephrin, when used as a urine biomarker in the early identification of DN, has great sensitivity and specificity. Using Logistic regression analysis for predictors of independent factors that affect nephrin level, we showed that 2HPP is the only independent factor that affects the level of nephrin level. An increase in the 2HPP level independently increases the nephrin level.

Conclusion

This study shows that nephrin levels in urine are increased in obese prediabetic patients even before albuminuria, which indicates early nephropathy, drawing attention to the possibility of using urinary nephrin as a marker of nephropathy that gives us an opportunity for early intervention and prevention of complications.

Conflict of interest

There are no conflicts of interest declared by the authors, and there was no financial support from any institution. We declare that we have received no money or grants for this research.

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