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Assessment of urinary nephrin level as an early predictor of diabetic nephropathy in type 2 diabetes mellitus patients

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ABSTRACT

End-stage kidney disease is largely caused by diabetic nephropathy (DN), which affects 40% of people with type 2 diabetes mellitus (T2-DM). A podocyte protein called nephrin may be a useful biomarker for early detection of podocyte damage that occurs in the early stages of DN. This study aimed to assess urinary nephrin as early marker for diagnosis of DN in individuals with T2-DM. The study involved sixty T2-DM patients with duration less than five years from time of diagnosis and twenty healthy subjects. Based on their urinary albumin/creatinine ratio, the T2-DM participants were divided into three groups: Group (Gp1) patients with macroalbuminuria (n=20), Gp2: patients with microalbuminuria (n= 20) and Gp3: patients with normoalbuminuria (n=20). In addition, twenty apparently healthy subjects were enrolled as control group. Fasting blood glucose (FBG) and postprandial blood glucose (PPBG), glycated hemoglobin (HbA1c), homeostasis model assessment-estimated insulin resistance (HOMA-IR), kidney function and lipid profile were measured. Blood glucose levels showed high significant difference among patients and healthy individuals. FBG and PPBG were significantly increased in T2-DM patients with macroalbuminuria than T2-DM patients with microalbuminuria, normoalbuminuria and control groups. All groups of T2-DM patients had significantly higher urinary nephrin levels than healthy controls (p<0.001). There was significant positive correlation between urinary nephrin, FBG, PPBG, HbA1c, urea, creatinine, albumin-to-creatinine ratio (ACR), HOMA-IR, cholesterol, triglycerides (TG) and LDL. There was significant negative correlation between urinary nephrin, estimated glomerular filtration rate and HDL**.** Significantly elevated urinary nephrin levels may serve as a diagnostic and prognostic indicator for DN.

Keywords: Diabetic nephropathy, Nephrin level, Predictor, T2-DM patients

1. Introduction

The most prevalent consequence of type 2 diabetus mellitus (T2-DM) is diabetic nephropathy (DN), which affects 40% of individuals with T2-DM. DN is marked by proteinuria, hypertension, and a gradual decline in kidney function. DN increases the need for dialysis, kidney transplants, and other forms of renal replacement therapy, and it is a major cause of end-stage renal disease (ESRD) (Umanath and Lewis, 2018; Pugliese et al., 2020). The kidneys are among the most susceptible organs to the effects of diabetes due to their narrow and small arteries, which are impacted by the high prevalence of the condition over time. Serious conditions like kidney failure can develop in some people if their kidney function is not monitored. Dialysis or a kidney transplant are the only available treatment options in these situations (Chebib and Torres, 2021). More than half of cases of ESRD are caused by DN, which is the primary cause of acquired podocyte damage. It damages the glomerular filtration

barrier, which is made up of podocytes, the glomerular basement membrane, and fenestrated endothelial cells (Zhong et al., 2024). Many patients with DN see a decline in the glomerular filtration rate (GFR) before albuminuria appears, or even in the absence of albuminuria. DN is categorized by either albuminuria or reduced GFR, as some individuals experience kidney function decline without albuminuria (García-Carro et al., 2021). The glomerular capillaries are surrounded by specialized epithelial cells called podocytes. Podocyte damage and population decline are frequently seen in the initial phases of DN. Nephrin may serve as an early indicator of diabetic nephropathy, highlighting the importance of podocyte preservation or repair (Valverde et al., 2022). To slow the progression of DN in T2-DM, kidney problems must be identified early.

Although microalbuminuria is prevalent in various clinical diseases such urinary tract infections, cardiovascular illness, non-diabetic people, it was formerly thought to be the gold standard for early detection of DN. Additionally, many individuals with T2-DM may experience kidney issues despite not showing signs of microalbuminuria (Kostovska et al., 2020). In diabetic patients, new urine biomarkers may reveal elevated levels before microalbuminuria manifests, which makes them crucial for identifying nephropathy in individuals with normoalbuminuria. (Gluhovschi et al., 2016)**.** Nephrin is an essential part of podocytes, which together with the glomerular basement membrane and endothelial cells make up the glomerular filtration barrier (Brinkkoetter et al., 2013). The glomerular basement membrane, fenestrated endothelial cells, and a podocyte monolayer on the urinary side make up this barrier (Welsh and Saleem, 2010). Nephrin is vital for the stability of the filtration barrier and for podocyte maturation during the development of the glomerulus (Doné et al., 2008). Podocytes separate from the glomerular basement membrane as a hallmark of early podocyte structural change. Severe glomerular damage crucial for clinical purposes to identify podocyte injury as soon as possible (Kandasamy et al., 2014). In many patients with diabetic nephropathy, GFR is decreased without or before the development of albuminuria. Therefore, diabetic nephropathy is referred to as either albuminuria or low GFR, as some patients with deterioration of renal function without albuminuria (Jwad et al., 2022*)*. Rangaswamaiah et al. (2022) found that the urinary nephrin significantly elevated in normoalbuminuria group only when compared to urinary albumin-to-creatinine ratio (ACR) and it is positive association with kidney damage. Persistent microalbuminuria is the gold standard for early DN diagnosis; to alleviate the burden of chronic kidney disease in T2-DM, it is imperative to identify innovative biomarkers for the early detection of DN and the progression to ESRD. This study aimed to measure the levels of urine nephrin in patients with T2-DM and investigate its potential use as an early diagnostic indicator for DN. This study concluded significantly elevated levels of urinary nephrin might be used for early diagnosis and prognostic marker for nephropathy in T2-DM.

2. Materials and methods

Study design

This cross-sectional study involved sixty T2-DM patients with duration less than 5 years from time of diagnosis and twenty healthy subjects matched in age and sex. All patients who took part in this study gave their written, informed permission. The research ethics committee at Tanta University, Faculty of Medicine granted approval for this study under approval code 34901/9/21.

Exclusion criteria were any patient with T2-DM with duration >5 years, T1-DM, gestational diabetes, patient with ketoacidosis, any evidence of cardiac or hepatic disease, and any patient with malignancy. All subjects (n=80) were classified into four equal groups: Group (Gp) 1: included apparently healthy subjects as control group, T2-DM patients with normoalbuminuria were included in Gp2, Gp3 with microalbuminuria, and Gp4 with macroalbuminuria. ACR in normoalbuminuria <30 mg/g, microalbuminuria 30-300 mg/g and macroalbuminuria >300 mg/g.

Samples and methods

Blood sera were separated for biochemical analysis. Blood glucose level including (fasting (FBG) and post prandial blood glucose (PPBG)

were determined using INDIKO PLUS according to the method of Tietz (1995). Labelled, and identified peripheral venous blood samples in the following ways: using an EDTA tube for glycated hemoglobin (HbA1c). HbA1C was determined using Siemens Dimension, RxL Max automated glycated hemoglobin analyzer (Reversed- phase cation exchange chromatography). Kidney function test (serum creatinine and blood urea) was determined according to the method of of Thomas, (1998). Complete lipid profile using INDIKO PLUS. Triacylglycerol (TG) was determined according to the method of Fossati and prencipe (1982), Serum total cholesterol (TC) was determined according to the method of Allain et al. (1974).

Homeostasis model assessment–Insulin resistance (HOMA-IR) was estimated according to the equation: (Fasting insulin (μU/ml) ×fasting glucose (mg/dl) ÷405

Insulin was measured in serum or plasma samples by gold-standard enzyme-linked immunosorbent assay (ELISA) methods (Shen and Prinyawiwatkul, 2019).

Urine samples was collected from the patients to perform chemical analysis by using dipsticks and to determine Albumin/ Creatinine Ratio. Urinary dipsticks were used to first analyse the chemical composition of a 10-milliliter sample of midstream morning urine that had been collected in sterile containers. Albumin levels were determined using the turbidimetric method (da Trindade et al., 2021), while creatinine levels were determined using the Jaffe reaction (Delanghe and Marijn, 2011). The supernatant from the remaining urine was separated by centrifuging it at 1000g for 20 minutes. It was then stored at -80 °C for subsequent nephrin quantification. Urinary albumin/creatinine ratio was measured by the following equation: ACR in (mg/g) = Albumin in (mg/dl) /creatinine (g/dl).

eGFR was measured by the coakroft equation: creatinine clearance (CrCl) (male) = (140-age X weight in kg) / (serum creatinine X 72), if female multiply by 0.85, according to (Brinkkoetter et al., 2013). The human nephrin ELISA kit from Wuhan Fine Biotech, China, was used to measure urinary nephrin (NPHS1) using an enzyme-linked immune-sorbent assay (ELISA) kit (Şambel et al., 2022).

Statistical analysis

SPSS v28 (IBM©, Armonk, NY, USA) was employed to conduct the statistical analysis. The data's normality was evaluated using histograms and the Shapiro-Wilks test. For parametric data, ANOVA and Tukey's test were employed, whereas for non-parametric data, the Kruskal-Wallis and Mann-Whitney tests were employed. Categorical data was analyzed using chi-square tests. The relationship between two quantitative variables was investigated using Spearman's correlation. Diagnostic accuracy was assessed using receiver operating characteristic curve (ROC) analysis; acceptable performance was indicated by Area under the curve (AUC) values greater than 50%. P-values below 0.005 were statistically significant.

Results

Glucose level and glycaemic control

FBG and PPBG, were significantly higher in diabetic patients with macroalbuminuria, diabetic patients with microalbuminuria and diabetic patients with normoalbuminuria compared to control group (p<0.001). There was no significant difference between groups normoalbuminuria and microalbuminuria and between microalbuminuria and macroalbuminuria but there was significant difference between groups normoalbuminuria and macroalbuminuria. There was significantly higher in HbA1c in diabetic patients with macro-albuminuria compared to diabetic patients with microalbuminuria, diabetic patients with normoalbuminuria and control group. HOMA-IR was significantly higher in diabetic patients with macro-albuminuria compared to diabetic patients with microalbuminuria, diabetic patients with normoalbuminuria and control group p value (<0.001) (Fig. 1 and table. 1).

Lipid profile, cholesterol, TG, LDL and HDL

Cholesterol, TG and LDL were significantly higher in diabetic patients with macroalbuminuria compared to diabetic patients with microalbuminuria, diabetic patients with normoalbuminuria and control group (p<0.001). HDL was significantly decreased in diabetic patients with macroalbuminuria compared to other groups (table.2 and Fig. 2).

Table 1. FBG, PPBG, HbA1C %, and HOMA-IR in studied groups.

Parameters	Control	Macroalbuminuria	Microalbuminuria	Normoalbuminuria	*P* value
FBG (mg/dL)	90.15±11.72	146.2 ± 26.62	161.8 ± 26.17	172.15±32.42	<0.001*
PPBG (mg/dL)	123.3±14.59	250.45±42.43	285.5±21.03	272.7±86.94	<0.001*
HbA1c (%)	5.84±0.39	6.97±0.18	7.66±0.37	8.67±0.34	<0.001*
HOMA-IR	1.72±0.33	5.05±0.72	4.9±0.6	4.9±0.71	<0.001*

Data is presented as mean ± SD; *: significant as *p* value ≤ 0.05. FBG: fasting blood glucose, PPBG: Postprandial blood glucose, HbA1c: glycated hemoglobin, HOMA-IR: Homeostatic Model Assessment of Insulin Resistance.

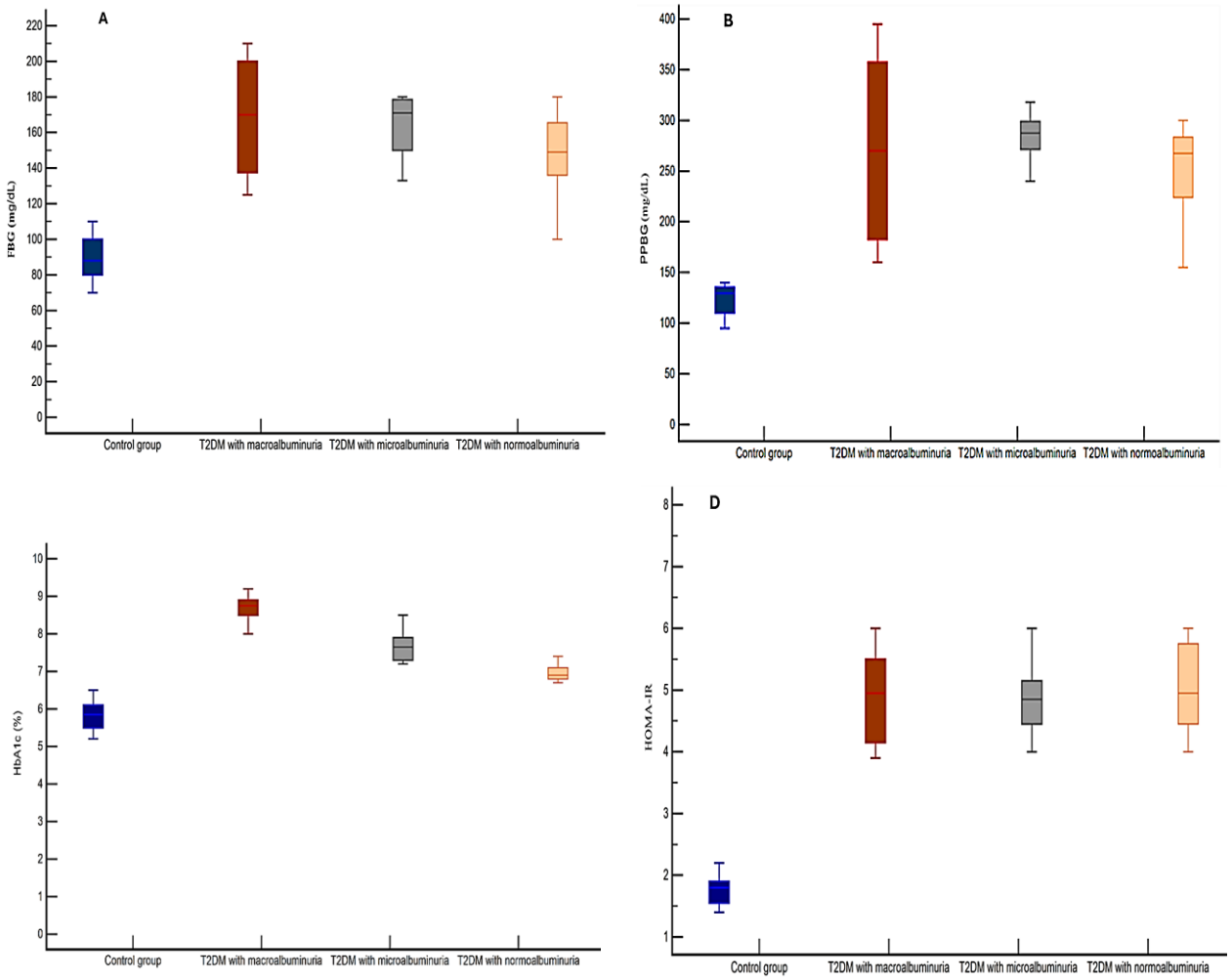

Fig. 1. Boxplots show FBG (A), PPBG (B), HbA1c (C) and HOMA-IR (D) among the studied groups

Table.2. Cholesterol, TG, LDL and HDL in studied groups.

Parameters	Control	Macroalbuminuria	Microalbuminuria	Normoalbuminuria	P value
Cholesterol (mg/dL)	164.45±21.39	178.95±42.7	184.7±41.53	238.25±41.78	<0.001*
TG (mg/dL)	149.85±29.07	162.8±38.81	169.9±38.5	192.2±30.48	<0.001*
LDL (mg/dL)	87.83±13.69	145.35±37.13	155.67±31.72	166.86±32.17	<0.001*
HDL (mg/dL)	42.65±4.96	41.9±5.34	41.5 ± 7.13	38.4 ± 4.92	<0.001*

Data is presented as mean ± SD. *: significant as P value ≤ 0.05.TG: Triglyceride, LDL: low-density lipoprotein, HDL: high-density lipoprotein.

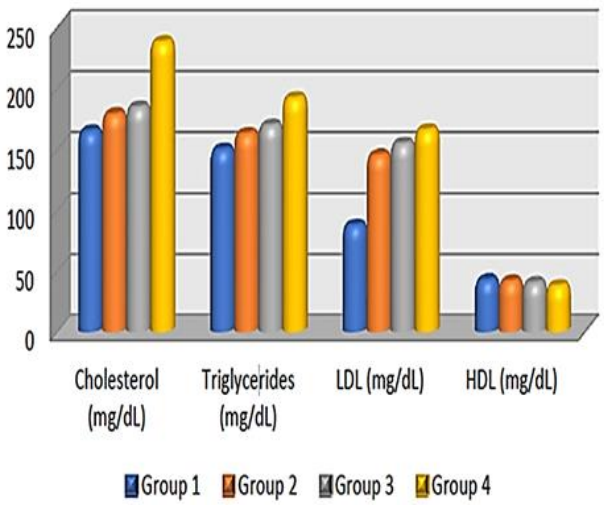

Fig.2. Lipid profile of the studied groups

Renal function, urea, creatinine, ACR, and eGFR

Urea, creatinine, ACR show higher levels in DM with macroalbuminuria compared to diabetic patients with microalbuminuria, diabetic patients with normoalbuminuria and control group. Diabetic patients with macroalbuminuria show higher levels of urea and creatinine compared to diabetic patients with microalbuminuria, diabetic patients with normoalbuminuria and control group with (p<0.001). The level of urea and creatinine in both diabetic patients with microalbuminuria and diabetic patients with normoalbuminuria showed no significant difference. eGFR was significantly lower in diabetic patients with macroalbuminuria compared to other groups and statistically higher in diabetic patients with microalbuminuria compared to other groups (table. 3).

Urinary nephrin level among studied groups

Urinary nephrin level was significantly higher in diabetic patients with macroalbuminuria than diabetic patients with microalbuminuria, diabetic patients with normoalbuminuria and control group (P<0.001). Urinary nephrin level was significantly higher in diabetic patients with microalbuminuria than diabetic patients with normoalbuminuria and control group (P<0.001).

Table 3. Urea, creatinine, ACR, eGFR, and urinary nephrin levels in studied groups

Parameters		Control	Macroalbuminuria	Microalbuminuria	Normoalbuminuria	*p* value
Urea (mg/dl)		22.5±5.4	30.15 ± 6.23	27.4 ± 2.23	76.25±15.01	<0.001*
Creatinine(mg/dl)		0.78±0.2	0.96 ± 0.21	0.98±0.1	2.03±0.39	<0.001*
ACR (mg/g)	**M ± SD**	14.45±2.93	20.8 ± 6.42	75.62±70.73	419.55±17.57	<0.001*
	Median (IQR)	14.5(12-16.5)	21.5(15-25.75)	44.5(35-70.275)	410(405.2-435.2)	
eGFR (ml/min)		138.92±15	121.99±18.51	92.23 ± 10.61	53.03 ± 5.45	<0.001*
Urinary Nephrin (ng/ml)		1.04(0.99-1.16)	2.78(1.74 - 3.44)	4.72 (2.8 - 5.59)	9.55 (8.92 - 10.26)	<0.001*

Data are presented as mean ± SD. IQR: interquartile range; *: significant as P value ≤ 0.05. ACR: albumin creatinine ratio, eGFR: estimated glomerular filtration rate

Correlation between urinary nephrin and different parameters of the studied patients

Urinary nephrin levels were strongly positively correlated with HbA1c, urea, creatinine, ACR, HOMA-IR, cholesterol, triglycerides, LDL, FBG, PPBG, and urea ($P \leq 0.001$). There was significant negative correlation between urinary nephrin level and eGFR ($r= -0.747$, $P <0.001$) and HDL ($r=-0.302$, $P = 0.006$) (table.4).

Diagnostic accuracy of urinary nephrin level in diagnosis of diabetic nephropathy in T2-DM patients (A).

Urinary nephrin can significantly diagnose DN in T2-DM patients at cut off >3.52, with 85% sensitivity, 90% specificity, 89.5% positive predictive value (PPV) and 85.7% negative predictive value (NPV) (Fig.3).

Diagnostic accuracy of urinary nephrin level in diagnosis of normoalbuminuria to microalbuminuria in T2-DM patients (B)

Urinary nephrin can significantly differentiate DM with normoalbuminuria from DM with microalbuminuria in T2-DM patients at cut off >1.28, with 85% sensitivity, 35% specificity, 56.7% PPV and 70% NPV (Fig.3).

Diagnostic accuracy of urinary nephrin level in diagnosis of microalbuminuria to macroalbuminuria in T2-DM patients (c)

Urinary nephrin can significantly differentiate DM with microalbuminuria from DM with macroalbuminuria in T2-DM patients at cut off ≤1.4, with 100% sensitivity, 85% specificity, 87% PPV and 100% NPV (Fig.3).

Diagnostic accuracy of ACR, eGFR and urinary nephrin in prediction of diabetic nephropathy in T2-DM patients (D, E and F)

Urinary nephrin was the only variable that significantly predict the incidence of DN in T2-DM patients at cut off >1.2, with 90% sensitivity, 85% specificity, 85.7% PPV and 89.5% NPV. ACR and eGFR were insignificant predictors for the incidence of DN in T2-DM. (Fig.3).

Table 4: Correlation between urinary nephrin and different parameters of the studied patients

	Urinary Nephrin	
	R	***P* value**
Age (years)	-0.134	0.236
FBG (mg/dL)	0.534	<0.001*
PPBG (mg/dL)	0.484	<0.001*
HbA1c (%)	0.816	<0.001*
Urea (mg/dl)	0.602	<0.001*
Creatinine (mg/dl)	0.656	<0.001*
ACR (mg/g)	0.791	<0.001*
eGFR (mil/min)	-0.747	<0.001*
HOMA-IR	0.351	0.001*
Cholesterol (mg/dL)	0.601	<0.001*
Triglycerides (mg/dL)	0.485	<0.001*
LDL (mg/dL)	0.616	<0.001*
HDL (mg/dL)	-0.302	0.006*

r: correlation coefficient, * Significant p value <0.05, FBG: Fasting blood glucose, PPBG: Postprandial blood glucose, HbA1c: Glycated hemoglobin, ACR: Albumin creatinine ratio, GFR: Glomerular filtration rate, HOMA-IR: Homeostatic Model Assessment of Insulin Resistance.

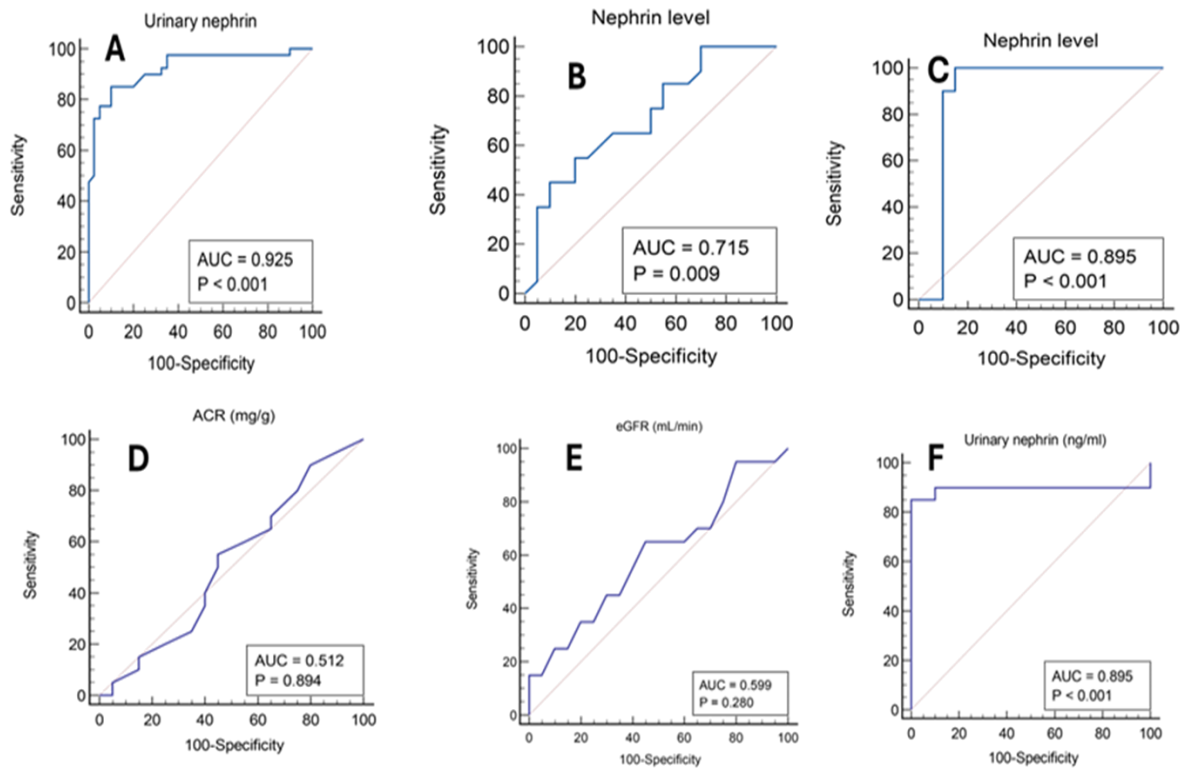

Fig. 3. ROC curve of (A) urinary Nephrin in diagnosis of diabetic nephropathy (B) urinary Nephrin in diagnosis of normoalbuminuria to microalbuminuria, (C) urinary Nephrin in diagnosis of microalbuminuria to macroalbuminuria (D) Albumin Creatinine ratio, (E) : estimated glomerular filtration rate, and (F) urinary Nephrin in prediction of diabetic nephropathy in T2-DM patients

4. Discussion

In the current study, blood glucose levels showed high significant difference among patients and healthy subjects. FBG and PPBG were significantly increased in diabetic patient with macroalbuminuria than diabetic patient with microalbuminuria, normoalbuminuria and control groups. This was in agreement with Richard et al. **(**2018) who reported that, higher FBG and PPBG were linked to higher urinary albumin level. Kondapi et al. (2021) confirmed the role of hyperglycemia in diabetic complications and explained that long term hyperglycemia caused by insulin metabolism disorder was a reason for diabetic nephropathy and pathological changes in kidney. However, there was no significant difference between FBG and PPBG in both of diabetic with normoalbuminuria and diabetic with microalbuminuria these was in agreement with Kondapi et al. (2021) who noticed that the diabetic status remains unaltered from normoalbuminuria to microalbuminuria but increased in macroalbuminuric group, thus uncontrolled diabetes status in macroalbuminuric group has resulted in progressive renal damage with macroalbuminuria.

As regard HbA1c, there was significant difference among patient groups and control. HbA1c was significantly higher in diabetic patient with macroalbuminuria than diabetic patient with microalbuminuria, normoalbuminuria and control group.

These results were in agreement with Zhang et al. (2020) who reported that, HbA1c was increased in diabetic patients than control and HbA1c was also related to grades of albuminuria, highlighted that early intensive glucose control is important in delaying the development of subsequent diabetes related complications. In the present study, as regard HOMA-IR, there was significant difference among patient groups and control. HOMA-IR was significantly higher in diabetic patient with macroalbuminuria than diabetic patient with

microalbuminuria, normoalbuminuria and control group. This result was in agreement with Saleem et al. (2019)who reported that, HOMA-IR was significantly different among diabetic patients and control group, and it is inversely related to renal function. In this study, as regard lipid profile there was statistically significant difference between control and patient groups. In contrast, Saleem et al. (2019) found that, there were no significant differences in lipid profile among diabetic patients and control group. There was statistically significant difference among patient groups and control groups regarding cholesterol. Cholesterol was significantly increased in diabetic patient with macroalbuminuria than diabetic patient with microalbuminuria, normoalbuminuria and control group. This result was in agreement with Sapkota et al. (2021) they reported that, cholesterol was significantly higher in patients with macroalbuminuria compared to those with microalbuminuria and normoalbuminuria. They suggested that DM accelerates abnormal lipoprotein metabolism which causes the progression of DN. There was statistically significant difference between patient groups regarding TG and LDL.

TG and LDL were significantly higher in diabetic patients with macroalbuminuria compared to microalbuminuric, normoalbuminuric patients and control group. In agreement with the result of Mansoor et al. (2022) reported that, TG and LDL were significantly increased in the patients with diabetic nephropathy in comparison with control group. Also, Kawanami et al. (2021) noticed that dyslipidemia was highly prevalent among diabetic patients with nephropathy and explained that lipids may damage vascular, mesangial and tubular cells of kidneys, underlying dysglycemia among diabetic patients further accelerates the renal damage induced by dyslipidemia, thus dyslipidemia and nephropathy act synergistically in worsing the clinical condition and increase the risk of renal consequence among diabetic patients.

In the current study, there was statistically significant difference among patient groups and control group regarding HDL. It was significantly lower in diabetic patients with macroalbuminuric compared to microalbuminuric, normoalbuminuric and control group. This result was in agreement with Mansoor et al., 2022 who reported that, HDL was decreased in the patients with diabetic nephropathy compared to healthy control. Many factors are known to affect lipid levels in diabetes as carbohydrate metabolism directly affects lipid metabolism. Rai et al. (2018) suggested that increased lipolysis promote quick breakdown of HDL. Elevated lipids may cause glomerular and tubulointerstial injury thus contributing to progression of DN.

As regard renal functions there was statistically significant difference between control and patient groups. There was statistically significant difference between patient groups regarding urea and creatinine. They were significantly higher in macroalbuminuric group compared to microalbuminuric, normoalbuminuric and control groups.

In agreement with this result, Al-Hazmi et al. (2020) reported that, serum urea and creatinine were significantly higher in patients' groups than control group. Saleem et al. (2019) found that, urea and creatinine were markedly higher in diabetic patients with macroalbuminuria compared to the other microalbuminuric, normoalbuminuric and control groups. Additionally, in this study there was no significant difference regarding creatinine between normoalbuminuric, microalbuminuric groups. Vanholder et al. (2018) explained this finding stated that serum creatinine remains unaltered until 50-60% of the kidney damage has taken place.

In this study, there was statistically significant difference between diabetic patients and control group regarding ACR and eGFR. eGFR was significantly lower in macroalbuminuric patients compared to microalbuminuric, normoalbuminuric and control groups. This result came in line with Jwad et al. (2022) who reported that diabetes mellitus causes microvascular complications including diabetic nephropathy through hyperglycemia which leads to hyperfiltration and hence increased glomerular filtration rate. Later as the disease

progresses the patient might progress into end stage renal disease.

In the present study, on comparing urinary nephrin level in the studied groups, it was revealed that significant higher level was found in diabetic patients with macroalbuminuria than diabetic patients with microalbuminuria, diabetic patients with normoalbuminuria and control group. Also, urinary nephrin level showed positive correlation with FBG, PPBG, HbA1c, HOMA-IR, cholesterol, TG, LDL, urea, creatinine and ACR. However urinary nephrin level showed negative correlation with HDL and GFR. These results were in agreement with Kondapi et al. (2021) who reported that, urinary nephrin level showed positive correlation with albuminuria, albumin creatinine ratio, serum creatinine and fasting plasma glucose and negative correlation with eGFR.

In the present study, ROC analysis of urinary nephrin level in diagnosis of diabetic nephropathy in T2-DM was found to be a differentiating marker in different grades of albuminuria in diabetic patients, as it could differentiate between normoalbuminuria and microalbuminuria, it could also differentiate between microalbuminuria and macroalbuminuria. ROC curve analysis of ACR, eGFR and urinary nephrin level in diagnosis of diabetic nephropathy in T2-DM showed urinary nephrin was the only variable that can significantly predict the incidence of diabetic nephropathy in T2-DM patients. ACR and eGFR were insignificant predictors for the incidence of diabetic nephropathy in T2-DM patients. Rangaswamaiah et al. (2022) found that, there was a significantly elevated level of urinary nephrin in T2-DM mellitus patients when compared to controls. The urinary nephrin significantly elevated in normoalbuminuria group only when compared to urinary ACR and it is positive association with kidney damage. This study concluded significantly elevated levels of urinary nephrin might be used for early diagnosis and prognostic marker for nephropathy in T2-DM mellitus. Veluri et al. (2022), found that, the diagnostic sensitivity and specificity of urinary nephrin level for diabetic nephropathy were 100 and 88%, respectively, and urinary albumin creatinine ratio was 43 and 76% respectively. His findings suggest that nephrin levels are strongly and positively associated with nephropathy in T2-DM mellitus patients and it has a greater potential to be an early predictable marker of nephropathy than urinary albumin creatinine ratio. Mesfine et al. (2023), found that, the urinary nephrin level as a predictor of diabetic nephropathy the sensitivity was 90% and specificity was 62%. He concluded his study with that, urinary nephrin level may be a promising marker for the detection of early glomerular injury. Urinary nephrin level could provide an important addition to a panel of novel markers to help in the detection of acute and chronic renal injury.

In agreement with Kostovska et al. (2020)**,** these results rendering urinary nephrin as good diagnostic and prognostic marker for diabetic nephropathy. In order to explain urinary nephrin role in occurrence and prognosis of diabetic nephropathy. Gorriz and Martinez-Castelao et al. (2012) found that albumin, which is normally filtered by the glomeruli, causes damage to the tubular cells, leading to tubule interstitial fibrosis with a decrease in the rate of kidney function. Patients with microalbuminuria have more chances of spontaneously reverting back to normal. Complete remission is highly unlikely in patient with macroalbuminuria, but the extent of the progression of DN can be delayed (Kondapi et al., 2021).

Thus, early detection of renal involvement in patient with T2-DM is important for timely treatment and to slow the disease progression to ESRD. As nephrin is one of the necessary Proteins for a proper function of the slit diaphragm. Nephrin is also important in regulating podocyte insulin sensitivity; its cytoplasmic domain enables the docking of glucose transporters GLUT1 and GLUT4 with vesicle-associated membrane protein-2, thus facilitating insulin signalling (Kravets et al., 2020). Hyperglycaemia disrupts podocytes both structurally and functionally, leading to excretion of nephrin which is present in the glomerular filtration barrier (Kondapi et al., 2021). Therefore, preventing podocyte injury or

promoting podocyte repair is crucial for improving diabetic kidney (Ma et al., 2023).

Conclusion

Urinary nephrin level is increased in diabetic patients compared to normal control group. Moreover, urinary nephrin level is related to degree of urinary albumin excretion, as its level is higher in diabetic patients with macroalbuminuria than those with microalbuminuria and in diabetic patients with microalbuminuria than those with normoalbuminuria. These results suggest that urinary nephrin level could be used as a diagnostic and prognostic marker for diabetic nephropathy.

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