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Comparative Study on Nephrin, Transforming Growth Factor-β and Some Biochemical Markers for Predicting Type 2 Diabetic Nephropathy

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Comparative Study on Nephrin, Transforming Growth Factor-β and Some Biochemical Markers for Predicting Type 2 Diabetic Nephropathy

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Abstract

Background: Diabetic Nephropathy (DN), is a microvascular complication of the diabetes mellitus type 1 or 2 and the major reason of end-stage kidney disease, which is distinguished by an increase in urine albumin excretion (microalbuminuria) and/or a decreased glomerular filtration rate (GFR) or both. Microalbuminuria has limited diagnostic role in early-stage diabetic nephropathy, because renal damage usually occurs before proteinuria. Therefore, more sensitive and specific biomarkers are needed for early detection of Diabetic Nephropathy.

Materials and Methods: A case-control study involved 180 participants aged 40-≥70 years, 60 individuals were healthy, and 120 had type 2 DM. The participants were divided into three groups according to the urinary albumin/ creatinine ratio (ACR): 40 patients with normoalbuminuria, 40 patients with microalbuminuria, and 40 patients with macroalbuminuria.

Results: The diabetic nephropathy was found in male more than female and the majority of patients were in age group 60-≥70 years. A significant difference in mean ± SD of age, Body mass index (BMI), and duration of diabetes $P \leq 0.001$, macroalbuminuria, microalbuminuria, and normoalbuminuria groups show a statistically higher serum nephrin and TGF-β in comparison to the healthy controls. Nephrin and TGF-β are strongly associated with blood urea and serum creatinine and an inversely associated with the glomerular filtration rate in all diabetic groups. Receiver operating characteristic (ROC) curve analysis for DN detection. Nephrin and TGF-β showed high sensitivity and specificity.

Conclusion: Elevated levels of nephrin and TGF-β in type 2 diabetic patients have been reported in the current study. These findings proposed that nephrin and TGF-β could be an early diagnostic markers for detection of diabetic nephropathy.

Keywords: Diabetes mellitus type 2, Diabetic nephropathy, Predicting, Nephrin, Transforming growth factor-β

1. Introduction

Diabetes mellitus (DM), is a common disease that includes a complex and heterogenous group of chronic metabolic diseases characterized by hyperglycemia resulting due to defects in insulin secretion, insulin action, or both. Chronic hyperglycemia of DM is associated with long-term damage, dysfunction, and failure of multiple organs, especially the kidneys, nerves and blood vessels (Jasim *et al.*, 2022). Type1 diabetes is a chronic disease that occurs when the pancreas does not produce enough insulin to control blood sugar levels. Type 2 diabetes, is a metabolic disease characterized by high blood sugar due to insulin resistance in target tissues and pancreatic cell dysfunction. Type 2 is the most common form of diabetes, accounting for 90–95% of all diabetes cases (Mohammed *et al.*, 2022). Diabetes remains the main cause of the renal disease (Salman & Hamzah, 2022). Diabetic nephropathy, also known as diabetic kidney disease(DKD), is characterized by increased in urinary albumin excretion (microalbuminuria) and/or

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a decreased glomerular filtration rate (GFR) or both, up to 25% of patients newly diagnosed with T2DM already develop one or more complications of DM (Azagew *et al.*, 2024). The molecular pathophysiology of DN included a complex interactions between hyperglycemia-induced metabolic, hemodynamic and the inflammatory factors. These factors change the function and morphology the walls of blood vessel and interact with adjacent cells causing the renal endothelial dysfunction, which plays a major role in the DN development. Early changes in DN include increases in kidney size, glomerular volume, and glomerular filtration rate, followed by the accumulation of glomerular extracellular matrix, increased urinary albumin excretion, glomerular sclerosis and tubular fibrosis (Abbas *et al.*, 2022). DKD is diagnosed by clinical biomarkers like serum creatinine, blood urea, estimated glomerular filtration rate, and albuminuria is important indicator for kidney dysfunction (Devarajan *et al.*, 2022).

Nephrin, a 180-KD trans-membrane protein that has been found as an essential biomarker for predicting diabetic kidney disease and the severity of podocyte injury. Nephrin is necessary for the proper functioning of the renal filtration barrier which forms the main component of slit diaphragm. Nephrin is arranged in a precise pattern that form pores that allows filtration of blood and prevents albumin and macromolecules from filtration. Decrease in nephrin expression has been suggested to podocyte loss and linked to the progression of kidney disease (Devanath *et al.*, 2022).

Transforming growth factor–beta (TGF-β), is a protein that perform many cellular functions involving cell growth control, cell proliferation, and apoptosis. This protein modulates or regulates extracellular matrix production and stimulates glomerular mesangial and epithelial cells to produce extracellular matrix proteins. TGF-β, is a growth factor involved in diabetic nephropathy pathogenesis, that causes mesangial extension by promoting glomerular mesangial hypertrophy and inducing extracellular matrix expansion (Wang *et al.*, 2022).

Aims: This study aimed to assess the levels of nephrin, Transforming growth factor-β and other biochemical markers for the prediction of DN in patients with type 2 diabetes mellitus.

2. Materials and methods

2.1. Study design

Case-control study was carried out on patients previously diagnosed with T2DM who visited the Diabetes and Endocrinology Center and Kidney Diseases and Transplantation Center in the Medical City in Baghdad from April 2024 to the end of July 2024.

The study included 180 participants: 60 individuals were healthy, and 120 individuals with type 2 diabetes mellitus were divided into three groups according to the albumin-to-creatinine ratio (ACR), as following: normoalbuminuria, 40 individuals with ACR < 30 mg/g, microalbuminuria, 40 individuals with ACR 30–300 mg/g, macroalbuminuria, 40 individuals with ACR > 300 mg/g.

Diabetic nephropathy is defined as a derangement in renal function with an estimated GFR < 60 ml/min/1.73 m3, and kidney damage according to estimation of ACR ≥ 30 mg/g (Tuttle *et al.*, 2014). This test was performed directly on each patient by taking a random spot urine and measuring urine creatinine using Urinalysis Hybrid FUS-3000 Plus.

2.2. Sample collection

After an overnight fast, venous blood samples were taken from each patient in a sitting position via disposable syringes. After a tourniquet was used, ten milliliters of blood were drawn from each person, and 8 milliliters were slowly pushed into a gel and clot activator disposable tube without anticoagulant and allowed to coagulate for 10–15 minutes before being centrifuged to obtain serum for testing fasting blood sugar, urea and creatinine and 2 milliliters in EDTA tubes for measuring HbA1c via a Tosoh automated G8 HPLC analyzer and the remaining serum was stored at 80°C. ELISA technique used for measuring nephrin and TGF-β levels in serum. The glomerular filtration rate (GFR) was calculated via the CKD-EPI Creatinine-Cystatin Equation (2021) online calculator.

2.3. Inclusion criteria

- All diabetic patients age 40->70 years for both gender were diagnosed by physicians and measurement the fasting blood glucose level and HbA1c, according to the WHO criteria.
- All diabetic patients were newly diagnosis with kidney disease due to diabetes mellitus.

2.4. Exclusion criteria

- Patients with gestational diabetes; lactating women; and patients on hypolipidemic drugs, such as statins were excluded from this study.
- In addition, chronic obstructive pulmonary disease, cancer, HIV, treatment with immunosuppressive drugs, chemotherapy or radiotherapy, patients take antioxidant will effect on level of cytokines.

Table 1. The clinical characteristic of all studied groups.

Clinical Variables	Control (N = 60)	Diabetic Patients			P-Value
		Normo-albuminuria (N = 40)	Macro-albuminuria (N = 40)	Macro-albuminuria (N = 40)	
Age(Years)					
Mean ± SD	49.42 ± 8.56	58.88 ± 8.36	60.28 ± 8.38	60.68 ± 9.81	<0.001** (HS)
Gender					
Male	35	20	24	24	0.76 (NS)
Female	25	20	16	16	
DM Duration (Years)					
Mean ± SD	___	10.40 ± 4.79	15.88 ± 6.31	18.28 ± 6.11	<0.001** (HS)
BMI (kg/cm^2)					
Mean ± SD	25.69 ± 2.65	30.23 ± 2.64	31.42 ± 3.5	30.18 ± 3.29	<0.001** (HS)

- Patients definitely diagnosed with other types of chronic renal disease such as IgA nephropathy or membranous nephropathy, in addition patients with obstruction, infection, or injury in the urinary tract or other condition affecting urinary albumin.

2.5. Ethical approval

Before commencing this study, all participants supplied written consent. The Diabetes and Endocrinology Center at the Medical City in Baghdad ethics committee approved the study on April, 2024.

2.6. Statistical analysis

Patient data were analyzed via SPSS version 26.0 software (Statistical Packages for Social Science, version 26, Chicago, USA). Descriptive statistics presented as the means and SDs. ANOVA was used to estimate the difference in the level of numeric data, and the chi-square test was used to estimate the associations between qualitative variables. Pearson's correlation analysis was performed to test the correlation of serum nephrin with other biochemical parameters. Receiver operating characteristic (ROC) curves were constructed to study the diagnostic accuracy of the markers for identifying DN in diabetic patients compared with healthy controls. The cutoff value with the best combination of sensitivity and specificity was determined from the ROC curve. A p-value of less than 0.05 was considered statistically significant.

3. Results

3.1. The clinical characteristic of all the study groups

In Table 1 Mean and SD of age for diabetic patients (normo-, micro, and macroalbuminuria) and controls were (58.88 ± 8.36, 60.28 ± 8.38, 60.68 ± 9.81) and (49.42 ± 8.56) respectively, with a high significant difference ($p < 0.001$). No significant difference between the diabetic patients and the healthy controls regarding the gender, the number male were (68) and the number of females were (52) were diabetic patients and 35 male with 25 female as control. The table also show the duration of T2DM was high significant difference (P-value < 0.001) between the diabetic patients (normo, micro, and macroalbuminuria), the mean duration of DM was significantly higher in those with macro- and microalbuminuria (18.28 ± 6.11, and 15.88 ± 6.31) than those with normoalbuminuria (10.40 ± 4.79).The majority of patients with macroalbuminuria had duration of diabetes >10 years. P-value of body mass index was (<0.001), there were high significant differences in BMI value between the diabetic patients (macro-, micro-, and normoalbuminuria) and control groups, the mean were (30.18 ± 3.29, 31.42 ± 3.5, 30.23 ± 2.64) and (25.69 ± 2.65) respectively.

3.2. Evaluation the biochemical parameters in all the study groups

Through the Table 2, show the mean and SD of FBS, HbA1c, blood urea, serum creatinine and eGFR. Results of the estimated glomerular filtration rate (eGFR) (ml./min./1.73 m^2) in patients' and healthy groups revealed high significant difference among patients (macro, micro and normoalbuminuria) and control group, mean was (45.22 ± 14.7, 64.59 ± 22.36, 89.4 ± 8.07) and (110.08 ± 10.32) respectively. Mean and SD of blood urea (mg/dl) results in the patients' and control group, mean were (92.7 ± 31.43.37, 59.08 ± 18.79, 34.3 ± 6.23) and (26.68 ± 5.76) respectively, with highly significant differences ($p < 0.01$). Serum creatinine (mg/dl) results in the patients' and control group, mean were (2.84 ± 1.21, 1.5 ± 0.73, 0.78 ± 0.18) and (0.66 ± 0.17) respectively, with highly significant differences ($p < 0.01$). Mean and SD

Table 2. Mean ± SD of biochemical markers in all the studied groups.

Clinical Variables	Control (N = 60)	Diabetic Patients			P-Value
		Normo-albuminuria (N = 40)	Macro-albuminuria (N = 40)	Macro-albuminuria (N = 40)	
eGFR (ml./min./1.73m^2)	110.08 ± 10.32	89.4 ± 8.07	64.59 ± 22.36	45.22 ± 14.70	<0.001** (HS)
Blood Urea (mg/dl)	26.68 ± 5.76	34.3 ± 6.23	59.08 ± 18.79	92.7 ± 31.43.37	<0.001** (HS)
S. Creatinine (mg/dl)	0.66 ± 0.17	0.78 ± 0.18	1.5 ± 0.73	2.84 ± 1.21	<0.001** (HS)
HbA1c %	5.16 ± 0.40	8.03 ± 1.57	8.86 ± 1.64	8.77 ± 1.99	<0.001** (HS)
FBS (mg/dl)	93.28 ± 6.30	196.27 ± 49.23	218 ± 56.77	255.75 ± 97.8	<0.001** (HS)

Table 3. Mean ± SD of nephrin and TGF-β in all the studied groups.

Clinical Variables	Control (N = 60)	Diabetic Patients			P-Value
		Normo-albuminuria (N = 40)	Macro-albuminuria (N = 40)	Macro-albuminuria (N = 40)	
Nephrin (pg/mL)	46.86 ± 11.8	127.16 ± 59.39	165.83 ± 73.71	244.61 ± 129.2	<0.001** (HS)
TGF-β (pg/mL)	25.91 ± 8.09	68.71 ± 21.77	86.31 ± 30.8	121.71 ± 41.44	<0.001** (HS)

of HbA1c% in the patients' and control group were (8.77 ± 1.99, 8.86 ± 1.64, 8.03 ± 1.57) and (5.16 ± 0.40) respectively, with highly significant differences ($p < 0.01$). Mean and SD of FBS in the patients' and control group were (255.75 ± 97.8, 218 ± 56.77, 196.27 ± 49.23) and (93.28 ± 6.30) respectively, with highly significant differences ($p < 0.01$).

3.3. Evaluation the levels of nephrin and TGF-β in all the study groups

Table 3 presented a statistically significant difference in levels of serum nephrin between the groups, macroalbuminuria group show a statistically higher serum nephrin (244.61 ± 129.2 pg/ml) compared with microalbuminuria (165.83 ± 73.71 pg/ml) and normoalbuminuria (127.16 ± 59.39 pg/ml), also normoalbuminuria group had higher level of serum nephrin in compares with control (46.86 ± 11.8). A statistically significant difference in levels of serum TGF-β, macroalbuminuria revealed increased TGF-β (121.71 ± 41.44 pg/ml) compared with microalbuminuria (86.31 ± 30.8 pg/ml) and normoalbuminuria (68.71 ± 21.77 pg/ml), also normoalbuminuria group had higher level compared with control (25.91 ± 8.09 pg/ml), P-value < 0.001.

Table 4. Correlation between Nephrin, TGF-β and RFTs in macroalbuminuria.

	Nephrin	TGF-β
Macroalbuminuria		
Urea		
Pearson Correlation	0.547	0.715
P-value	0.000	.000
Creatinine		
Pearson Correlation	0.373	0.570
P-value	0.001	.000
eGFR		
Pearson Correlation	−0.346	−0.476
P-value	0.029	.002

Table 5. Correlation between Nephrin and TGF-β with RFTs in microalbuminuria.

	Nephrin	TGF-β
Microalbuminuria		
Urea		
Pearson Correlation	0.400	0.423
P-value	0.010	.000
Creatinine		
Pearson Correlation	0.354	.402
P-value	0.025	0.010
eGFR		
Pearson Correlation	−0.366	−0.423
P-value	0.020	.007

3.4. Correlation between nephrin, TGF-β levels and RFTs in the diabetic patients

The correlation in three diabetic groups was assessed by using Pearson correlation coefficient. In macroalbuminuria, microalbuminuria and normoalbuminuria groups, serum nephrin and TGF-β have a significant positive correlation with urea and creatinine and negative correlation with the eGFR, as presented in Tables 4 to 6 and Fig. 1.

3.5. Evaluation of the ROC curve for serum nephrin and TGF-β on the diabetic groups

ROC curve analysis. In comparison to healthy people, nephrin had a strong capacity to predict

Table 6. Correlation between Nephrin and TGF-β with RFTs in normoalbuminuria.

	Nephrin	TGF-β
Normoalbuminuria		
Urea		
Pearson Correlation	0.504	0.408
P-value	0.001	.009
Creatinine		
Pearson Correlation	0.503	.320
P-value	0.001	.044
eGFR		
Pearson Correlation	−0.545	−0.326
P-value	0.000	.040

nephropathy in T2DM patients. The value of (AUC = 0.90 and $p < 0.001$) in normoalbuminuria group indicates that nephrin has the capacity to predict DN in individuals who are free of illness, with higher sensitivity of 70% and specificity of 94%. Nephrin has a high ability to distinguish between microalbuminuria and healthy group with higher sensitivity of 97% and specificity of 95% through the value of (AUC = 0.97 and $p < 0.001$). As a result, nephrin has a good ability to discriminate between macroalbuminuria and healthy group, with better sensitivity of 96% and specificity of 100%. We also exhibited the values of (AUC = 1.000 and $p < 0.001$) Fig. 2. TGF-β revealed the best cut-off points were >38, >52.7 and >73.5 pg/ml in normo-, micro-, and macroalbuminuria groups respectively. Sensitivity and specificity were 92.5%, 91.7% in normo-, 100%, 95% in micro-, and 97.5%, 100% in macroalbuminuria Fig. 3.

4. Discussion

DN is the major cause of end-stage kidney disease. Albumin in urine is the gold standard for diagnosing nephropathy (Surya *et al.*, 2021). Highly significant differences were found when the ages of the patients and controls were compared, indicating that older people are developed T2DM. These results are similar to those of other studies showing differences between diabetic patients and controls. As people age, many body functions change, and the body can no longer control metabolism and organ secretion (Bohan, 2024). Additionally, previous findings revealed that the prevalence of glucose intolerance [prediabetes and T2DM] increased in patients aged 45 years and older (Chachan *et al.*, 2022). Another study reported that most of DM patients aged 46–55 years (Amelia *et al.*, 2021). Older age, hypertension, lower eGFR, higher levels of BMI, HbA1c are use as significant risk factors for incident CKD in those with diabetes (Sabanayagam *et al.*, 2023). With regards to the sex, the present study found no significant differences in all the studied groups and most of patients are males, one of the studies reported that male sex is an independent risk factor for DKD and males have 50% higher chance of progression of disease to ESRD as compared to females, this can be explained by the absence of hormones estrogen and progesterone, which have a protective effect on kidneys (Al-Zahrani *et al.*, 2023). It has been shown that estrogen stimulates the release of nitric oxide (NO) resulting in vasodilation in the renal vessels and attenuation of inflammation; NO deficiency can be associated with acceleration of renal injury, based on receptors that impaired vasodilation and endothelial dysfunction; estrogens have anti-apoptotic and anti-fibrotic effects in the kidney, which may partly explain the protective effect of estrogen on the kidney (Farahmand *et al.*, 2021). While other study showed that the majority of T2DM patients are female (77.5%) (Amelia *et al.*, 2021).

There was a highly significant difference in diabetes duration in patients, where the greater percentage of patients had a duration of disease >10 years. These findings are consistent with other results reporting that diabetes duration is greatest in patients with macroalbuminuria. The duration of diabetes has prolonged effect on nephropathy causing kidney failure and severe problems such as cardiac diseases, vascular disease, kidney disease, and blindness if untreated, approximately half of T2DM patients develop diabetic kidney disease, a microvascular complications that is the major cause of ESKD (Kamal *et al.*, 2024). This study revealed that most T2DM patients are obese which confirms the strong relationship between BMI and glucose homeostasis in T2DM patients. Obesity plays a major role in the development of T2DM (Huang *et al.*, 2021). Obesity is defined as excessive accumulation of fat in the adipose tissue throughout the body due to imbalance in the energy intake and expenditure, leading to various cardiovascular and metabolic disorders (Singh & Oladipupo, 2024). Obesity may activate changes to the body's metabolism that cause adipose

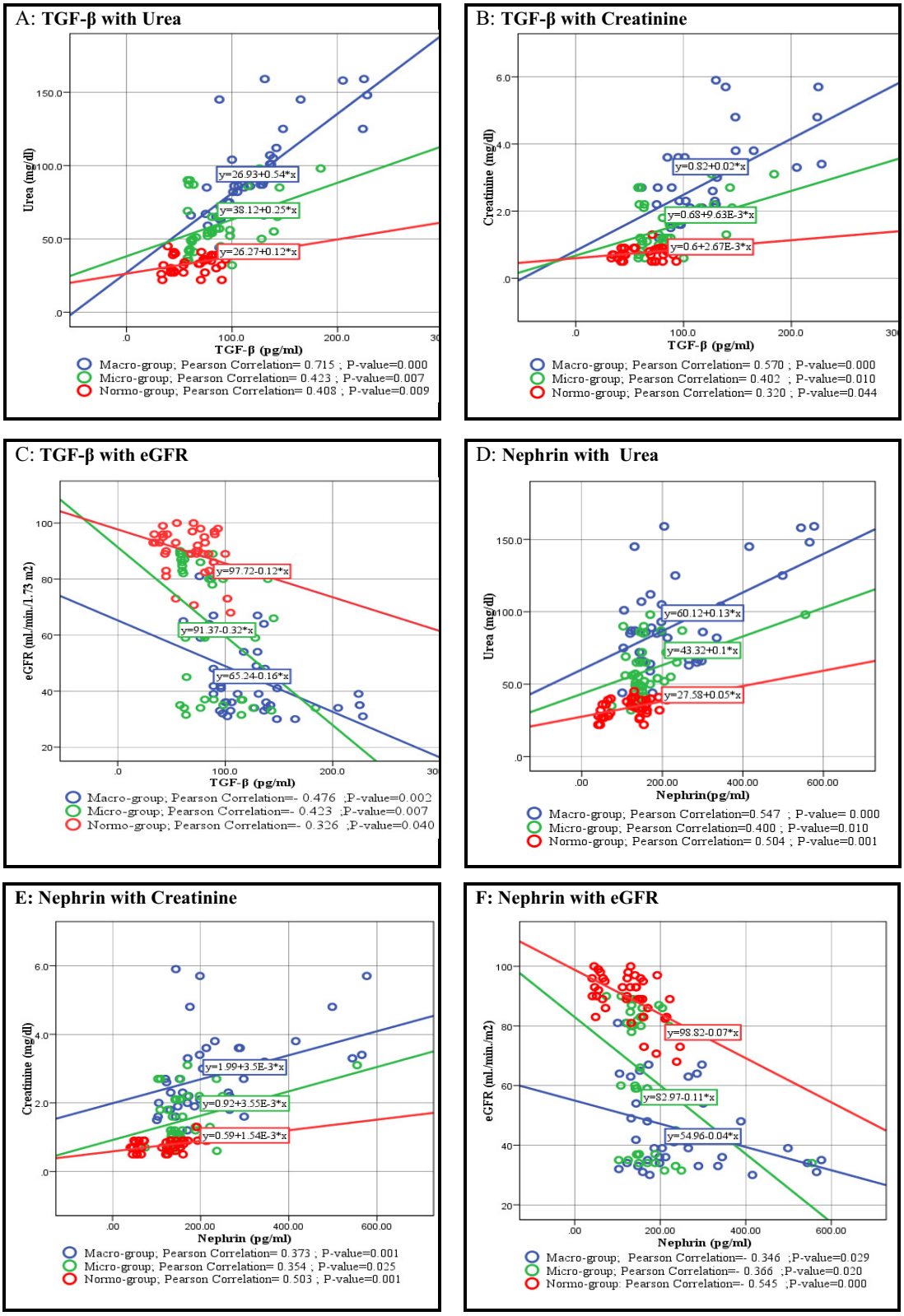

Fig. 1. Correlation between nephrin, TGF-β and RFTs in the diabetic patients.

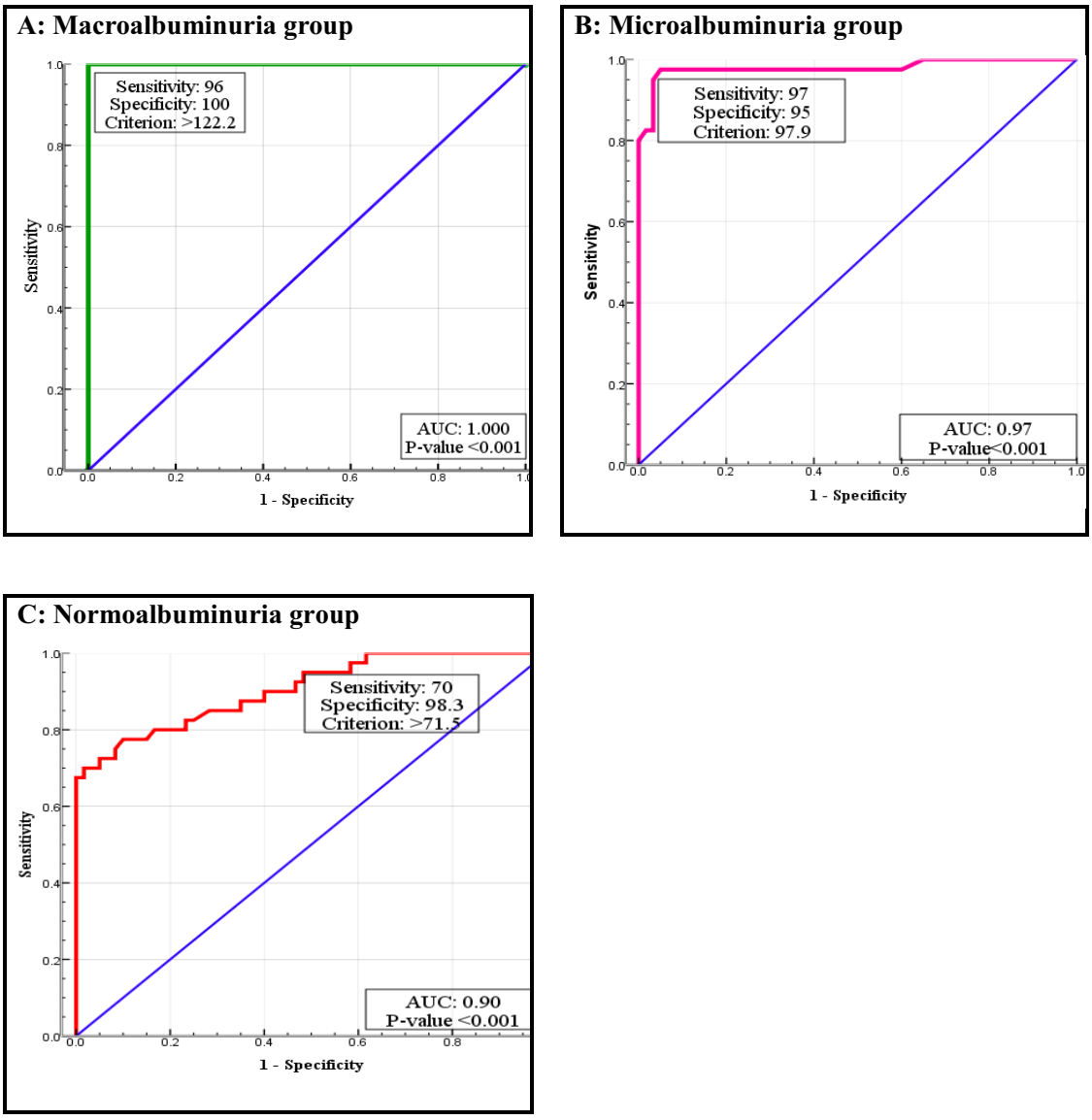

Fig. 2. ROC curve analysis of nephrin level in patients compared to the healthy group.

tissue to release increased amounts of fatty acids, glycerol, hormones, proinflammatory cytokines, and other factors that are involved in the development of insulin resistance (Ibrahim *et al.*, 2023).

The current study revealed that the eGFR in the healthy group was greater than that in patients, which is similar to the findings of other studies (Kamal *et al.*, 2024)، which revealed differences in the eGFR between healthy individuals and patients. Other studies also revealed that patients in the microalbuminuria and macroalbuminuria groups had lower eGFR than normoalbuminuria patients and controls (Hamid *et al.*, 2021, Ibrahim *et al.*, 2023). The GFR can be used as marker for the prediction of nephropathy to prevent end-stage kidney disease progression (Ibrahim *et al.*, 2023). The decrease in eGFR in type 2 diabetic nephropathy patients is because the glomeruli become damaged, impairing the kidneys' ability to filter waste products from the blood (Kamal & Taher, 2024). Elevated creatinine levels in diabetic patients are due to poor kidney filtering ability causing the accumulation of nitrogenous waste and decreased nephron function (Hamad & Abdulrahman, 2024). An increase in urea level is occur when there is damage to the kidney or the kidney is not functioning properly, variations in urea and creatinine levels in T2DM patients is related to the duration of disease as their level is higher in a patient with longer disease duration. Increment of blood urea and creatinine levels with the increment of blood sugar

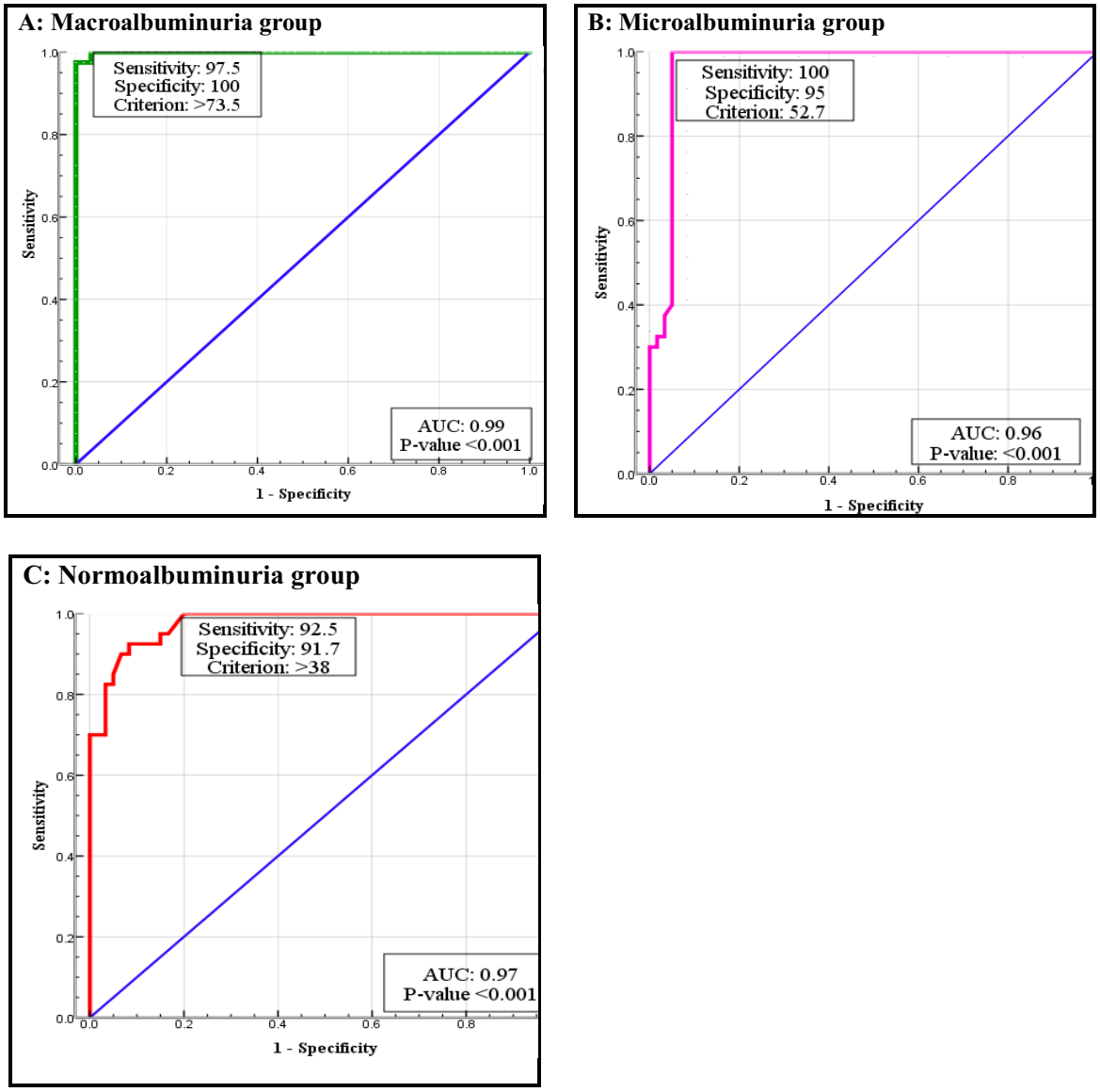

Fig. 3. ROC curve analysis of TGF-β level in patients compared to healthy group.

level clearly indicates that the hyperglycemia cause damage to the kidney and kidney fail to eliminate creatinine (Ibrahim *et al.*, 2023).

The results of a study identified the relationship between glycemic control and the development of microalbumin in T2DM patients. HbA1c is used to predict the development of ESRD in T2DM patients (Hu & Zhang, 2020). The poor glycemic management used as predictors for the diagnosis of micro albuminuria in diabetic patients**.** Prolonged exposure to hyperglycemia caused renal endothelial cells to malfunction (Kamal *et al.*, 2024). In diabetes, glycosylated hemoglobin used as the gold standard for glycemic control. For every 1% increase in HbA1c, the risk of having microalbuminuria increased by 23% (Salh *et al.*, 2024). Several factors contributed to elevated in glucose and HbA1c in DN patients. One possible explanation is insulin resistance, which is a common feature of T2DM (Raheem *et al.*, 2023).

The study revealed a significant differences in level of serum nephrin between the diabetic patients and controls groups, macroalbuminuria group show higher serum nephrin compared with microalbuminuria and normoalbuminuria, this findings show that serum nephrin started increasing even in the stage of normoalbuminuria. Nephrinuria was present in 100% of patients with macroalbuminuria and microalbuminuria, as well as 50% of patients with normoalbuminuria (Mizdrak *et al.*, 2022). A nother study presented that nephrin loss significantly and redistribution in the glomeruli of diabetic patients with microalbuminuria, and also revealed that

patients with diabetes and nephropathy have structural changes to the glomerular filtration unit, such as increased width of podocyte foot processes and filtration slits (Hassan *et al.*, 2024). Nephrin is a component of the slit-diaphragm in glomeruli that is essential for maintaining the permeability of urinary proteins, and is an integral part of podocytes and along with the basement membrane, and endothelial cells, forms the glomerular filtration barrier, podocyte injury lead to presence of nephrin in urine (Surya *et al.*, 2021). However, nephrinuria may provide an early indicator of renal damage and the presence of nephrin protein in the T2DM patients with normoalbuminuria, demonstrating that nephrinuria may precede microalbuminuria (Aljorani *et al.*, 2023).

Nephrin presented positive correlation with urea and creatinine levels and negative correlation with the eGFR, this study shown that it is possible to enhance the prediction of eGFR decline by using this biomarker. The present results as the same with those of another study, which indicating that nephrin is a marker of disordered of renal function. Nephrinuria is a better indicator of renal insufficiency in T2DM (Kondapi *et al.*, 2021; Shyamala Rajendran *et al.*, 2023). The level of urinary nephrin correlated negatively with GFR, indicating that nephrinuria is a marker of disordered renal function (Kostovska *et al.*, 2020). Podocyte destruction may occur in T2DM patients prior to the development of microalbuminuria (Aljorani *et al.*, 2023).

Elevated in the TGF-β in T2DM patients compared to its level in healthy group. High levels of TGF-β in diabetic patients may be attributed to the high glucose in blood, as elevated glucose stimulates and activates the synthesis of di acylglycerol, then leads to the activation of protein kinase, which elevated TGF-β synthesis in mesenchymal and tubular cells (Hassan & Kata, 2022). High levels of TGF-β in diabetic patients may be involved in the development of DN (Hassan & Kata, 2022).In the kidney, three isoforms of TGF-β are expressed: TGF-β1, TGF-β2, and TGF-β3. Of these three, the predominant one is TGF-β1.In the human kidney, TGF-β2 and TGF-β3 are mainly expressed in podocyte and TGF-β1 in tubules. However TGF-β1was detected in mesengial cells in DN patients (Kulkarni *et al.*, 2023). A high glucose environment induces TGF-β expression and activation, and causes podocytes to undergo the apoptosis, which impairs the filtration barrier and renal function (Chen *et al.*, 2022). The molecular weight of TGF-β1 is around 25 kD, almost one-third that of albumin. The glomerular basement membrane can permeate its passage (Kulkarni *et al.*, 2023).

There was positive association between TGF-β with urea and creatinine and negative association with eGFR in three diabetic groups. This finding is supported by other studies that found TGF-β1 associated positively with creatinine, an increased level of serum TGF-β1in type 2 diabetic patients may be associated with nephropathy (Hadad & Albrahimi, 2023, Jasem *et al.*, 2024). Hyperglycemia may trigger the activation of transforming growth factor-beta which in turn mediates progressive renal damage in type 2 DM. Increased serum transforming growth factor-beta may be useful as a marker of diabetic renal disease as it shows a close association with the parameters of renal injury in type 2diabetes mellitus (Abbas *et al.*, 2022).

ROC curve analysis was done to find the predictive value of nephrin in T2DM patients, serum nephrin can discriminate healthy subjects from diabetic patients. The diagnostic accuracy of serum nephrin to predict nephropathy in the current investigation revealed strong diagnostic sensitivity and specificity, indicating that serum nephrin may be a potential biomarker of glomerular damage (Aljorani *et al.*, 2023). Nephrin is a very good parameter to discriminate healthy subjects from diabetic patients with sensitivity and specificity of 100% (El Nagar *et al.*, 2022). Nephrin can be found in the systemic circulation or secreted by podocytes while passing through the nephron can be reabsorbed in the renal tubular system and discovered in the serum. Nephrin, a protein exclusive to podocytes found in serum, indicates only podocyte injury. Because podocyte damage is assumed to exist before to the onset of microalbuminuria and proteinuria, podocyte proteins, including nephrin, are regarded as more accurate and early indicators of diabetic kidney disease (Hassan *et al.*, 2024).

The objective of this research was to assess the use of TGF-β1 as a biomarker for diabetic nephropathy in individuals with T2DM,. Therefore, the present study revealed that TGF-β1 had greater sensitivity and specificity in the micro-, and macro- and normoalbuminuria groups. These results suggest that TGF-β1 is a good prognostic marker for the early detection of DN in patients with T2DM, and these results are consistent with those of another study in which TGF-β was used as a biomarker for DN in individuals with T2DM (Jasem *et al.*, 2024). The diagnostic accuracy of TGF-β markers indicated high sensitivity and specificity values in screening the patients with DKD (Mezher *et al.*, 2023). TGF-β1 is a good diagnostic marker for the early detection of DN, with 80% sensitivity and 95% specificity (Abbas *et al.*, 2022).

5. Conclusion

Increased levels of nephrin and TGF-β in type 2 diabetic patients play a role in the pathogenesis of diabetic nephropathy, the current study demonstrates

there were positive correlation between nephrin and TGF-β in type 2 diabetic patients, increase serum nephrin and TGF-β levels in type 2 diabetes mellitus patients might be used for early diagnosis and progression of nephropathy.

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Author contributions

Alyaa Kadhim Hliel: the research article proposal, experiment design, explaining the findings, and article writing. Huda Farhan Ahmed: Supervision, Data analysis, proof editing, Review and editing. Dr. Hiba Abid Al-Hussein Hassan: Supervision, methodology, Review and editing.

Competing interests

The authors declared that there were no conflicts of interest.

Abbreviations

ACR:	Albumin creatinine ratio
BMI:	Body mass index
DM:	Diabetes mellitus
DN:	Diabetic nephropathy
DM:	Diabetes mellitus
DKD:	Diabetic Kidney disease
eGFR:	Estimated glomerular filtration rate
ELISA:	enzyme-linked immunosorbent assay
ESRD:	End stage renal disease
RFT:	Renal function test
SD:	Standard deviation
TGF-β:	Transforming growth factor beta
T2DM:	Type 2 diabetes mellitus
UACR:	Urinary albumin creatinine ratio

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