Estimation of Some Biomarkers in Type 2 Diabetic Patients for Detection Early Incidence of Diabetic Nephropathy

Alyaa Kadhim Hliel*, Huda Farhan Ahmed, Hiba Abdul-Hussein

Department of Medical Laboratory Sciences Technology, College of Health and Medical Techniques, Middle Technical University, Baghdad, Iraq

Received: Dec. 2024; Accepted: 11 Apr. 2025

Abstract- Diabetic Nephropathy (DN), is the major microvascular complications of diabetes, affecting 40% of type 2 diabetic patients, is the leading cause of end-stage renal failure. Microalbuminuria has a 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. A Case-control study involved 180 participants aged 40-≥70 year, 60 individuals are healthy, 120 person with type 2 diabetes mellitus (T2DM), they were divided in three groups by urinary albumin/ creatinine ratio (ACR). Group 1:40 patients with normo albuminuria (ACR<30 mg/g). Group 2: 40 patients with micro albuminuria (ACR 30-300 mg/g). Group 3: 40 patients with macro albuminuria (ACR>300 mg/g). Nephrin, transforming growth factor-β (TGF-β) and Wnt inducible signaling pathway protein1 (WISP1) was estimated in serum by an enzyme-linked immunosorbent assay (ELISA); also fasting blood sugar, glycated hemoglobin, blood urea and serum creatinine was determined by enzymatic method, urine albumin/creatinine ratio determined by measurements of albumin and creatinine in urine sample. There was a significant elevation for all parameters in the patients compared to healthy control but eGFR decrease. The prevalence of 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, macro albuminuria, microalbuminuria, and normoalbuminuria groups show a statistically higher S. Nephrin, TGF-β and WISP1 in comparison to control. Nephrin and TGF- β have a strong association with blood urea, serum creatinine and an inverse association with glomerular filtration rate in all diabetic groups. While there were significant positive correlations between the WISP1 with urea. In contrast there was positive correlations with creatinine only in micro- and macro albuminuria groups, while no correlation with eGFR. In Receiver operating characteristic (ROC) curve analysis, nephrin, WISP1 and TGF- β showed high sensitivity and specificity. Elevated levels of Nephrin, WISP1 and TGF-β in type 2 diabetic patients have been reported in the current study. These findings proposed that nephrin, WISP1 and TGF- β could be an early diagnostic marker for DN detection.

Acta Med Iran 2025;63(May-June):180-191.
https://doi.org/10.18502/acta.v63i3.19718

Keywords: Diabetes mellitus type 2; Diabetic nephropathy; Nephrin; Wnt Inducible Signaling Protein-1; Transforming growth factor-β

Introduction

Diabetes Mellitus (DM) is a common disease that includes a complex and heterogeneous group of chronic metabolic diseases characterized by hyperglycemia resulting due to defects in insulin secretion, insulin action, or both. Chronic hyperglycemia is associated with long-term damage, dysfunction, and failure of multiple organs, especially the kidneys, nerves and blood vessels (1-3). Type1 diabetes is a chronic disease that occurs when the pancreas does not produce enough insulin to control blood sugar. Type 2 diabetes is a

Corresponding Author: A.K. Hliel
Department of Medical Laboratory Sciences Technology, College of Health and Medical Techniques, Middle Technical University, Baghdad, Iraq
Tel: 07706468712, E-mail address: EDC4003@mtu.edu.iq

metabolic disease characterized by high blood sugar due to insulin resistance in target tissues and pancreatic cell dysfunction. Type 2 is the common form of diabetes, accounting for 90-95% of all diabetes cases (4-6). Diabetes remains the main form and the cause of the renal disease (7). Diabetic nephropathy, also known as diabetic kidney disease (DKD), is characterized by increased urinary albumin excretion (micro albuminuria) and/or a decreased glomerular filtration rate (GFR) or both (8). The molecular pathophysiology of DN included a complex interaction between hyper glycemia-induced metabolic, hemodynamic and inflammatory factors. These factors change the function and morphology of the walls of blood vessels and interact with adjacent cells causing the renal endothelial dysfunction, which plays a major role in DN development (9). Urea and creatinine are compounds that indicate normal kidney function, urea and creatinine are elevated in diabetic patients during period of hyperglycemia and associated with the degree of renal failure (10). DKD is diagnosed through clinical biomarkers such as serum creatinine, blood urea, estimated glomerular filtration rate, and albuminuria is an important indicator for kidney dysfunction (11). The critical importance of evaluating new markers for the prediction of DN. As it is hard directly to identify podocytes in urine, most studies have focused on the involvement of podocyte-specific proteins as markers of tubular damage, such as nephrin (12).

Nephrin, a 180-KD trans-membrane protein that has been found as an essential biomarker for predicting DKD and the severity of podocyte injury. Nephrin is necessary for the functioning of the renal filtration barrier which forms the main component of slit diaphragm. Nephrin is arranged in a precise pattern that forms pores that allows filtration of blood and prevents albumin and macromolecules from filtration. Decrease in nephrin expression occur due to podocyte loss and progression of kidney disease (13).

Transforming Growth Factor–beta (TGF-β), is a protein that make abundant of cellular functions involving the cell growth control, cell proliferation, and apoptosis, this protein modulates or regulate extra cellular matrix production and stimulate glomerular mesangial and epithelial cells to produce extra cellular matrix proteins. TGF-β, is a growth factor that involved diabetic nephropathy pathogenesis, which cause mesangial extension by promoting glomerular mesangial hyper trophy and by inducing the extracellular matrix expansion (14).

Wnt/beta catenin are a large family of secreted glycoproteins that play a central role in embryonic development, differentiation, cell motility, and cell proliferation (15). Wnt signaling pathways work in a combinatorial with other pathways, including the fibroblast growth factor and transforming growth factor-β pathways. Alterations in Wnt/ β- catenin signaling is involved in congenital defects of the kidney and urinary tract, renal carcinoma, obstructive nephropathy, chronic allograft nephropathy diabetic nephropathy (16). Activation of the Wnt/β-catenin signaling pathway has been shown to correlate with progression of renal injury (17).

Materials and Methods

A Case- control research involved 180 participants (60 healthy and 120 individuals with T2DM of both gender (40-≥70 years, 68 male and 52 female). Collected during the period between April 2024 and July 2024, the permission for the research obtained from the out patients by the unit of diabetes consultation from Medical City, Baghdad Teaching Hospital/ Baghdad, carried out accordance to ethical standard set forth for the Helsinki declaration. All patients provided written informed consent. The study was approved by the local ethics committee in our institution (MEC-56).

The study groups

A total sample of study be 180, there are 120 samples of T2DM| patients at duration 3->10 year of the diabetes diagnosis divided into groups|by using Albumin to Creatinine Ratio (ACR) as following:

1) Control group: involved 60 subjects without any diseases.

2) Patients groups: involved 120 diabetic patients classified in 3 groups:

- 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

Sample collection

Blood samples

About 5-10 ml of blood| samples were taken| from healthy people and Type 2 diabetic patients. Each blood sample divided into two parts:

A) The first part 3 ml of whole blood retained in EDTA tubes for measuring HbA1c by Tosoh automated G8 HPLC analyzer.

B) The second part of the blood leaves for 30 minute at the temperature of room for clotting in the tube. After coagulation, serum discreted by centrifuge at 3000 rpm to ten minutes. Serum aspirated and divided into Eppendorf tubes for:

Aliquot 1: estimation FBS, S. Creatinine, Urea by automated method by Selectra Pro xl.

Aliquot 2: The rest stored at (<-35 C º) until measure Nephrin, WISP1 and TGF-β by using enzyme-linked immunosorbent assay (ELISA).

Urine sample

5-10 ml urine from each patient with T2DM and healthy subjects. Urinalysis Hybrid FUS-3000 Plus used to measure Albumin/Creatinine Ratio.

Statistical analysis

The data were reviewed, coded and analyzed by Statistical Package of Social Science (SPSS) version 26 software. Descriptive statistics were tabulated as mean and SD. ANOVA test was used to estimate the difference numeric data, Chi square used to estimate the association between qualitative variables. Pearson correlation regression (r) is used to evaluate correlation between Numeric data. Scattered dot diagrams are used to estimate the association between the variables. *P* was <0.05 are a significance.

Results

180 individuals were divided into four groups according to UACR. The patients and control were separated into three age groups, the first group (40-49) years, the second group (50-59) years, and the third group (60-≥60) years, as shown in figure (1). No significant difference between the diabetic patients and control group regarding the gender. As shown in figure (2).

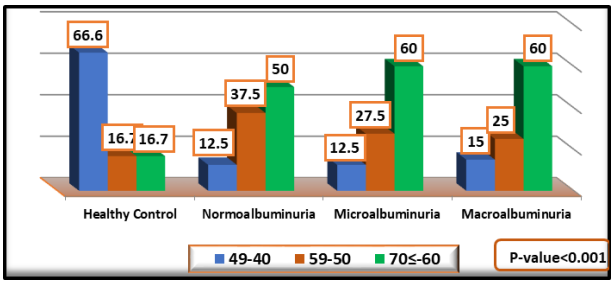


Figure 1. Distribution of the participants according to the age group

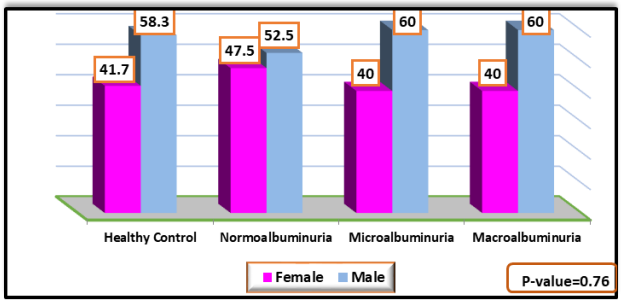


Figure 2. Distribution of the participants according to the gender

The clinical characteristics and biochemical parameters of the patients and control show a significant difference, as observed in tables (1,2).

Table 1. The clinical characteristic of all studied groups

Clinical variables		Control (Non-diabetic) (N=60)	Diabetic patients Normo albuminuria (N=40)	Diabetic patients micro albuminuria (N=40)	Diabetic patients Macro albuminuria (N=40)	*P*
Duration of DM (Years)	<5 yrs.	_____	4 (10%)	1 (2.5%)	_____	0.001 (HS)
	5-10 yrs.		18 (45%)	11(27%)	10 (25%)	
	>10 yrs.		18 (45%)	28 (70%)	30 (75%)	
	Mean±SD	_____	10.40±4.79 (3-23)	15.88±6.31 (4-25)	18.28±6.11(7-26)	
BMI (kg/cm²)	Normal	20 (33.3%)	3 (7.5%)	2 (5%)	2 (5%)	<0.001 (HS)
	Overweight	40 (66.7%)	13 (32.5%)	13 (32.5%)	18 (45%)	
	Obese		24 (60%)	25(62.5%)	20 (50%)	
Mean±SD		25.69±2.65 (21-29.4)	30.23±2.64 (24.5-33.5)	31.42±3.5(24.8-37.7)	30.18±3.29(24.9-36)	

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

Clinical Variables	Control (Non diabetic) (N=60)	Diabetic Patients			*P*
		Normo-albuminuria (N=40)	Micro-albuminuria (N=40)	Macro-albuminuria (N=40)	
eGFR (ml./min./1.73m²)	110.08±10.32 (90-130)	89.4±8.07 (68-100)	64.59±22.36 (31.5-90)	45.22±14.70 (30-81)	<0.001 (HS)
Blood Urea (mg/dl)	26.68±5.76 (20.1-37)	34.3±6.23 (22-45)	59.08±18.79 (32-98)	92.7±31.43.37 (44-159)	<0.001(HS)
S. Creatinine (mg/dl)	0.66±0.17 (0.4-0.9)	0.78±0.18 (0.5-1.3)	1.5±0.73 (0.6-3.1)	2.84±1.21 (1.2-5.9)	<0.001 (HS)
HbA1c %	5.16±0.40 (4.1-5.6)	8.03±1.57 (6.9-12.5)	8.86±1.64 (7-13.4)	8.77±1.99 (6.8-15)	<0.001 (HS)
FBS (mg/dl)	93.28±6.30 (80-100)	196.27±49.23 (130-300)	218±56.77 (141-396)	255.75±97.8 (165-598)	<0.001 (HS)

A significant difference in mean±SD of nephrin between the groups, macroalbuminuria shows higher serum nephrin (244.61±129.2 pg/ml) in comparison to microalbuminuria (165.83±73.71 pg/ml) and normoalbuminuria(127.16±59.39 pg/ml), normoalbuminuria had higher mean±SD in compare to control (46.86±11.8). A significant difference in mean±SD of TGF-β, macro albuminuria revealed increased TGF-β (121.71±41.44pg/ml) in comparison to micro albuminuria (86.31±30.8pg/ml) and normoalbuminuria (68.71±21.77 pg/ml), also normoalbuminuria group had higher mean±SD in compare to control (25.91±8.09pg/ml). A significant difference in mean±SD of WISP1, macroalbuminuria revealed increased WISP1 (125.83±41.44 pg/ml) in comparison to micro albuminuria (94.58±26.9 pg/ml) and normoalbuminuria (59.34±21.3 pg/ml), also normoalbuminuria group had higher mean±SD in compare to control group (24.64±7.6pg pg/ml) respectively, *P*<0.001. as presented in figures 3,4, and 5.

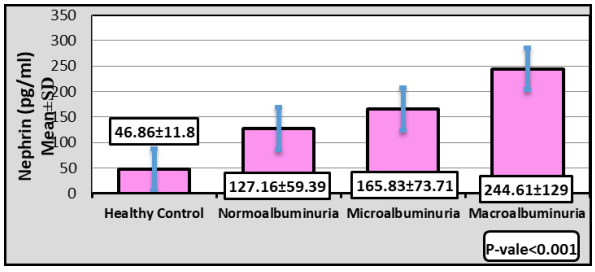


Figure 3. Distribution of the Nephrin according to the study groups

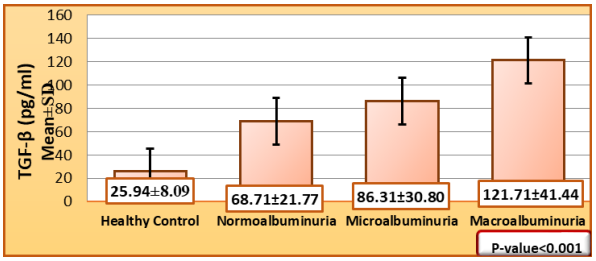


Figure 4. Distribution of the TGF-β according to the study groups

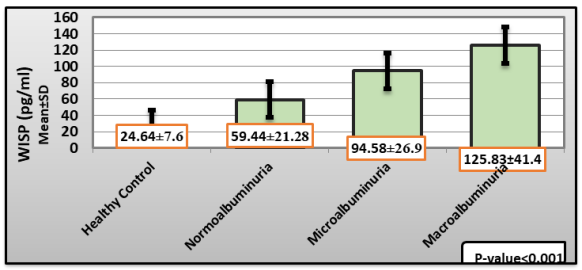


Figure 5. Distribution of the WISP1 according to the study groups

Regarding macroalbuminuria and microalbuminuria groups, S. nephrin and TGF-β have a significant positive correlation with urea and creatinine and negative correlation with eGFR, while WISP1 have correlation with urea and creatinine but not have correlation with eGFR, as presented in (table 3 and table 4). Regarding normoalbuminuria group, S. nephrin and TGF-β have a significant positive correlation with urea and creatinine and negative correlation with eGFR, while WISP1 have correlation only with urea, as presented in (table 5).

Table 3. Correlation between nephrin, WISP1 and TGF-β with RFTs in macroalbuminuria group

			Nephrin	TGF-β	WISP1
Macroalbuminuria	Urea	Pearson Correlation	0.547	0.715	.0.460
		P	0.000	.000	0.003
	Creatinine	Pearson Correlation	0.373	0.570	0.306
		P	0.001	.000	0.05
	eGFR	Pearson Correlation	-0.346	-0.476	-0.258
		P	0.029	.002	0.108

In ROC curve analysis, cut-off points of Nephrin were ≥71.5, ≥97.9 and ≥122.2 pg/ml in normo, micro, and macroalbuminuria respectively. Sensitivity and specificity in normogroup were 70%, 94%, in micro group were 97%, 95%, and in macroalbuminuria 96%,100% (Table 6 and figure 6). TGF-β revealed the best cut-off points were >38, >52.7 and >73.5 pg/ml in normo, micro, and macroalbuminuria groups respectively. SN and SP were 92.5%, 91.7% in normo, 100%, 95% in micro, and 97.5%, 100% in macro albuminria (Table 7 and Figure 7). WISP1 revealed the best cut-off points were >25, >34.3 and >60 pg/ml in normo, micro, and macroalbuminuria groups respectively. SN and SP of WISP1 were 80%, 51% in normo albuminuria, 50%, 96% in micro albuminuria, and 97%, 83% in macroalbuminria (Table 8 and figure 8).

Table 4. Correlation between nephrin, WISP1 and TGF-β with RFTs in microalbuminuria group

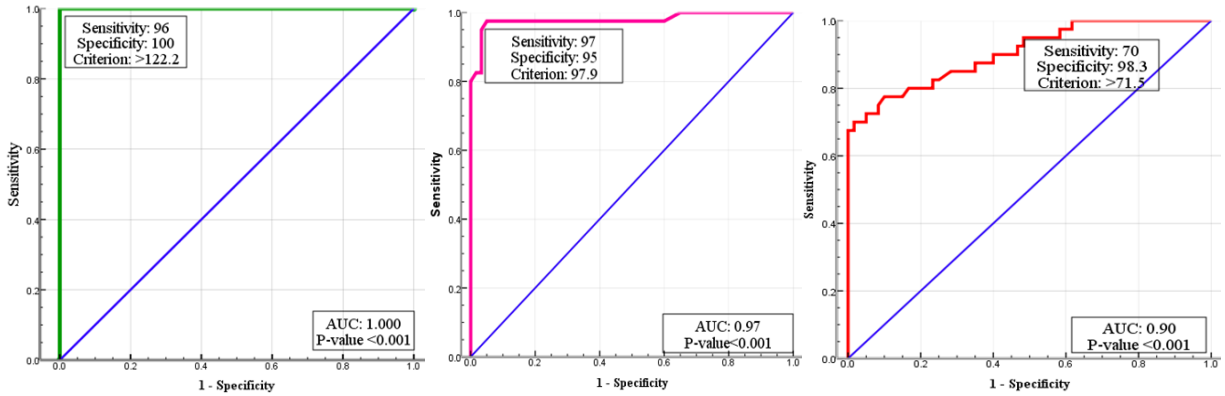
			Nephrin	TGF-β	WISP1
Microalbuminuria	Urea	Pearson Correlation	0.400	0.423	0.459
		P	0.010	.000	0.003
	Creatinine	Pearson Correlation	0.354	.402	0.285
		P	0.025	0.010	0.046
	eGFR	Pearson Correlation	-0.366	-0.423	-0.227
		P	0.020	.007	.160

Table 5. Correlation between Nephrin, WISP1 and TGF-β with RFTs in normoalbuminuria group

			Nephrin	TGF-β	WISP1
Normoalbuminuria	Urea	Pearson Correlation	0.504	0.408	0.492
		P	0.001	.009	0.001
	Creatinine	Pearson Correlation	0.503	.320	0.317
		P	0.001	.044	0.074
	eGFR	Pearson Correlation	-0.545	-0.326	-0.304
		P	0.000	.040	0.056

Table 6. ROC curve analysis of nephrin in all diabetic patients groups

Groups	AUC	Cut off value	*P*	Sensitivity	Specificity
Normo-albuminuria	0.90	71.5	<0.001	70%	94%
Micro-albuminuria	0.97	>97.9	<0.001	97%	95%
Macro-albuminuria	1.00	>122.2	<0.001	96%	100%

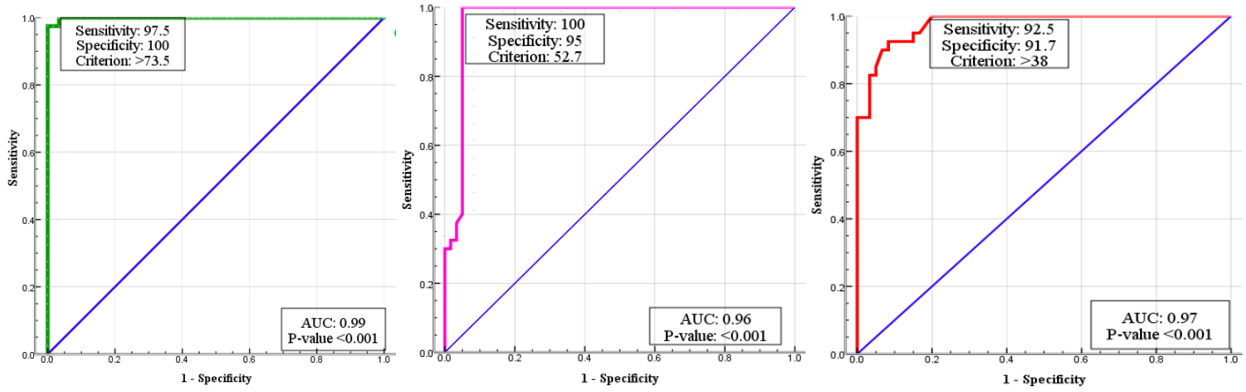


A b c

Figure 6. ROC curve of Nephrin in; (a) Macroalbuminuria; (b) Microalbuminuria; (c) Normoalbuminuria

Table 7. ROC curve analysis for TGF-β in all diabetic patients groups

Groups	Area	Cut off value	*P*	Sensitivity	Specificity
Normo-albuminuria	0.97	>38	<0.001	92.5%	91.7
Micro-albuminuria	0.96	>52.7	<0.001	100%	95%
Macro-albuminuria	0.99	>73.5	<0.001	97.5	100



a b c

Figure 7. ROC curve of TGF- β; (a) Macroalbuminuria; (b) Microalbuminuria; and (c) Normoalbuminuria

Table 8. ROC curve analysis of WISP1 in all diabetic patients groups

Groups	Area	Cut off value	*P*	Sensitivity	Specificity
Normo-albuminuria	0.70	>25	0.001	80%	51%
Micro-albuminuria	0.71	>34.3	<0.001	50%	96%
Macro-albuminuria	0.87	>60	<0.001	97%	83%

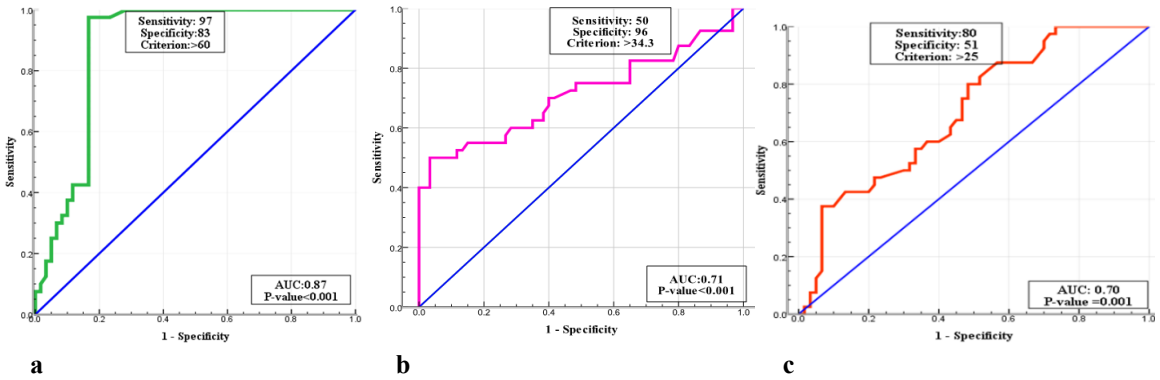


Figure 8. ROC curve of WISP1; (A) Macroalbuminuria; (B) Microalbuminuria and (C) Normoalbuminuria

Discussion

DN is the major cause of end-stage kidney disease (ESKD). Albumin in urine measurement is the gold test in diagnosing nephropathy (18). Age is also a risk factor for the onset of DN and the development of macro albuminuria. In this study, significant differences were found when comparing the age of patients and control and indicating that older people are more likely to develop T2DM. These results are like other studies showing a significant difference between diabetic patients and control. T2DM is directly related to age. As people get older many body function change and the body can no longer control metabolism and organs secretion (19). Also previous findings showed that glucose intolerance prevalence [pre-diabetes and, T2DM] increased in patients that aged 45 and older (20). Other study also found that most of DM patients in age group 46-55 years (21).

The present findings shown no differences in gender of all the studied groups, .which is in agreement with the other findings that demonstrated there was no differences in gender between the patients and control (22). Additionally, there were no significant differences in the diabetic patients with and without nephropathy when compared to the control in their gender (23). Other study found that the most of DM patients are female (21).

A high significant difference in diabetes duration in patients with micro, macro, and normoalbuminuria, where the higher percentage was in duration of disease >10 years. This findings consistent with other results that reported there was significant difference in diabetes duration in patients with micro, macro and normo albuminuria, duration is a highest in patients with macro albuminuria (24). Diabetes duration has an effect on nephropathy in prolonged time cause kidney failure and causes severe problems involved cardiac diseases, vascular disease, kidney disease, and blindness. T2DM is the common cause of end stage kidney disease and cause cirrhosis and impair the function of nephron (24).

This study found most T2DM patients are obese which confirm the strong relationship between elevated BMI and associated with glucose homeostasis in T2DM cases. Obesity plays a major role in the development of T2DM (25). Other findings also demonstrated there was a significant differences in BMI between the three groups of the diabetic patients and the control (26). While other findings demonstrated there was no significant differences in the diabetes duration between three groups of the diabetic patients (18).

The current study revealed the eGFR in healthy group elevated (due to normal kidney function), there were increased in urea and creatinine levels in diabetic patients, this findings is similar to other study (24), that show differences in eGFR level between the healthy and patients, other studies also show that micro and macro albuminuria groups showed decline in eGFR compared to normo albuminuria patients and control (27,28). GFR can used as markers for prediction of nephropathy to prevent end stage kidney disease progression (28). Urea and creatinine were elevated in macro and micro groups in comparison to normo albuminuria and healthy individuals. An elevated urea occurs when there is damage to the kidney or the kidney not properly functioning. Increment of blood urea with the blood sugar indicates that the increase blood sugar cause damage to the kidney, these results agreed with other findings, which found significant increases in level of urea and creatinine in the micro, macro groups comparison to patients with normo albuminuria and control (27,28). While other studies show no significant difference in serum and urinary creatinine, blood urea and eGFR between patients with nephropathy (micro

and macro) and without nephropathy (normo albuminuria), blood urea elevated only when more than sixty percent of renal cells are no longer working (29).

FBS and HbA1c levels showed a significant difference in patients compared to the control, however, DN is associated with poor glycemic control. 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 (24). In diabetes, glycosylated hemoglobin is used as the gold standard for glycemic control. For every 1% increase in HbA1c, the risk of having microalbuminuria increased by 23% (30). Several factors contributed to elevation in glucose and HbA1c in DN patients. One possible explanation is insulin resistance, which is a common feature of T2DM. Insulin resistance reduces the ability of cells to respond to insulin, leading to elevated glucose, in addition, impaired insulin secretion by the pancreas may also contribute to hyper glycemia in DN (31). While other study reported no significant difference in glucose and HbA1c between the diabetic patients with nephropathy (micro and macroalbuminuria) and without nephropathy (normoalbuminuria) (32). Also, no significant difference between diabetic nephropathy groups and HbA1c, the HbA1c level may give error result in patients with CKD due to several conditions such as iron deficiency anemia (due to reduced erythrocyte life span or iron deficiency), the management of erythropoietin (29).

Our study revealed a statistically significant difference in level of serum nephrin between the groups, macro group showed higher serum nephrin in comparison to micro and normo groups. Also, normo group had higher levels of nephrin in compare to control, these findings show that serum nephrin started increasing even in the stage of normo albuminuria. Nephrinuria was present in 100% of patients with macro albuminuria, in 88% of micro albuminuria patients, as well as 82% of normo albuminuria patients. Podocyte protein nephrin are considered as earlier and more specific markers for diagnosis of DN compared to micro albuminuria (33). Elevated level of nephrin in T2DM patients with normo albuminuria and increased levels of more than two times in T2DM patients with micro albuminuria showed disease progression toward end-stage kidney disease (34). Nephrinuria was found in a high proportion of diabetic patients with normo albuminuria; thus, given that hyperglycemia damage to renal vasculature and glomerular filtration barrier over time (12). Nephrin is the best biomarker for predicting diabetic kidney disease as well as the severity of the damage to podocytes (35).

There were 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 and cumulative sugar in the 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 (36). A high glucose environment lead toTGF-β expression and activation, and cause push podocytes into the apoptosis, which impairs filtration barrier and renal function (37). In DN patients, TGF-β overexpression by mesangial tubular or infiltrating renal cells associated with hyperglycemia (38). TGF-β is the main cytokines in the pathogenesis of renal inflammation and fibrosis (39). The molecular weight of TGF-β1 is about 25 kD, one-third of albumin. The glomerular basement membrane can permeate its passage (40). TGF-β 1 has been considered as one of the major cytokines in the pathogenesis of renal inflammation and fibrosis (39).

There was a significant elevation in the level of WISP1 in T2DM patients compared to its level in healthy groups. This result was consistent with the findings of other results that reported there was increased in the WISP1 level in patients with diabetes as compared to control group, and the higher plasma concentration of WISP1 is associated with insulin resistance (41). Activation of the Wnt/β-catenin signaling pathway has been shown to correlate with progression of renal injury (17). The related proteins in Wnt/β-catenin signaling pathway were elevated in diabetic nephropathy, high glucose-induced tubulointerstitial fibrosis is related to Wnt/β-catenin signaling pathway (42). Wnt/β-catenin signaling plays a role in podocyte dysfunction and proteinuria and is activated in a number of proteinuria kidney diseases (43).

Nephrin showed positive association with urea and creatinine and negative association with eGFR, this study showed that it is possible to considerably enhance the prediction of eGFR decline using this biomarker. The current study agreement with previous study, Nephrin showed positive association with serum creatinine and negative correlation with eGFR. Nephrinuria is a better indicator of renal insufficiency in T2DM (44,45). Other study found that urinary nephrin was positively correlated with urea, creatinine, and negative correlated with eGFR. Nephrin is a protein located in the podocytes of GBM of the kidney and it is play a vital role in podocytes and slit diaphragm to

prevent excretion of protein in urine, but minimal structural alterations in podocytes lead to excretion of nephrin in urine prior to proteinuria (34). Nephrinuria is a marker of disordered renal function (33). While no significant correlation observed between urinary nephrin with creatinine and eGFR in diabetic nephropathy patients (46).

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 compatible 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 (47,48). Also other study found that TGF-β1 level had a negative association with eGFR and positive association with blood urea and serum creatinine (39). While other study give different results found no significant association between urinary TGF-β1 with serum creatinine and eGFR among DN patients (40). Other results also revealed a significant inverse correlation between eGFR and no significant association with urea and creatinine (38).

The present study shows a positive correlation between WISP-1 with urea in all diabetic groups, and positive correlation with creatinine only in micro- and macroalbuminuria groups, while there was no correlation with eGFR. WISP1 is an extracellular matrix protein associated with fibrogenesis in the kidney and was elevated in patients with diabetic nephropathy (49). Other result also found that serum WISP1 level was positive related with serum creatinine (50). WISP1 can affect the proliferation and differentiation of kidney cells by affecting Wnt/β-catenin, which can lead to kidney disease. WISP1 expression can be increased in renal injury, which may promote the development of renal fibrosis and accelerate the process of CKD (51).

The ROC curve of serum nephrin can discriminate healthy subjects from diabetic patients. The diagnostic accuracy of serum nephrin to predict nephropathy in the current investigation revealed extremely strong diagnostic sensitivity and specificity, indicating that serum nephrin may be a potential biomarker of glomerular damage. Another results for the diagnostic sensitivity and specificity of serum nephrin for nephropathy indicating that nephrin may be a potential biomarker of glomerular damage (12). Further research on podocyte metabolism might confirm that nephrinuria to be a biomarker of pre-clinical DN (44). Nephrin has a total predicted probability of 96% in subjects with DN. This means high discriminatory power between healthy subjects and patients with DN; also this result means high sensitivity and specificity of nephrin as a biomarker in early detection of DN (33). The diagnostic utility of nephrin for the detection of early DN suggest that nephrin are strongly and positively associated with nephropathy in type 2 diabetic patients and it has a greater potential to be an early predictable marker of nephropathy than urinary albumin creatinine ratio (34).

The objective of this research was to assess the use of TGF-β1 as a biomarker for diabetic nephropathy in individuals with T2DM, So, the present study found that S.TGF-β1 had more sensitivity and specificity. So, these results made S. TGF-β1as a good prognostic marker in the early detection of DN, and these results matched with the other study that found the S. TGF-β used as a biomarker for DN in individuals with T2DM (48). The diagnostic accuracy of TGF-β markers determined by indicated high sensitivity and specificity values in screening of patients with DKD (52). TGF-β1 is a good diagnostic marker for early detection of DN with 80% sensitivity and 95% specificity (9).

Serum WISP1 has an area under curve of 0.87 in macro-, and 0.71 in micro-, 0.70 in normo albuminuria groups. These results were followed by determining the cutoff point 60, 34.3, 25 in three diabetic groups. The Wnt canonical signaling is being activated during kidney injury while it is relatively inactive in normal adult kidney and has been implicated as a dominant regulator in the development of DN (15). Wnt/B-catenin signaling is reactivated in a wide range of chronic kidney diseases, such as diabetic nephropathy and its activation is one of the most relevant mechanisms of cellular senescence in diabetic nephropathy (53).

These biomarkers correlate with the developing of renal impairment via changing different concentrations according to the degree of the nephron damage. Owing to its low molecular weight, most of these biomarkers produced are filtered through the glomerulus and reabsorbed at the proximal tubule. If the renal tubule is damaged, biomarkers reabsorption decreases while its production from epithelial cells increases, which subsequently leads to increases their levels in the blood and urine; Therefore, these biomarkers are a promising biomarker to detect DN in the earliest stages.

Increased levels of nephrin, WISP1 and TGF-β play a role in the pathogenesis of diabetic nephropathy, increase serum levels of these biomarkers in type 2 diabetes mellitus patients might be used for early diagnosis and progression of nephropathy.

References

1. Jasim HS, Altaie AF, Saloum WT, Ali AH. Comparative study of new biomarkers in Iraqi DM2 with and without complications. Rep Biochem Mol Biol 2022;11:400.
2. Swapna B, Fathima K, Muwayyad H, Khanam M, Salma S, Harsha S, et al. A study to assess the associated risk of developing cardiovascular diseases in chronic kidney disease. Cell Mol Biomed Rep 2024;4:150-8.
3. Talebi Mehrdar M, Ebadi G. Increased levels of serum adenosine deaminase (ADA) enzyme and increased risk of T cell activation markers in type 2 diabetes. Cell Mol Biomed Rep 2024;4:159-67.
4. Mohammed KK, Sabbar AG, Hmeash HH. The comparison and correlation of serum levels of insulin and Na+/K+ ATPase in type 2 diabetes mellitus (T2DM) and non-diabetes mellitus individuals. J Techniques. 2022;4(3):34-9.
5. Kakaei M, Rehman FU, Fazeli F. The effect of chickpeas metabolites on human diseases and the application of their valuable nutritional compounds suitable for human consumption. Cell Mol Biomed Rep 2024;4:30-42.
6. Shaker Al-Awady M, Mohammed Ali B. Effect of vitamin D supplementation on glycemic control in type 1 diabetes mellitus. Cell Mol Biomed Rep 2024;4:189-98.
7. Salman MN, Hamzah AS. Determination of cystatin C level in a sample of patients with chronic kidney disease. J Techniques 2022;4:7-11.
8. Azagew AW, Beko ZW, Mekonnen CK. Determinants of diabetic nephropathy among diabetic patients in Ethiopia: Systematic review and meta-analysis. PLoS One 2024;19:e0297082.
9. Abbas HS, Nada SZ, Fadhel AA. Assessment of transforming growth factor-beta (TGF-β) and albumin to creatinine ratio (ACR) in patients with type 2 diabetic nephropathy. World Journal of Pharmacy and Pharmaceutical Sciences 2022;1188-103.
10. Akpotaire P, Seriki S. Assessment and correlation of serum urea and creatinine levels in normal, hypertensive, and diabetic persons in Auchi, Nigeria. Clin Res 2023;7:7-16.
11. Devarajan P, Chertow GM, Susztak K, Levin A, Agarwal R, Stenvinkel P, et al. Emerging role of clinical genetics in CKD. Kidney Med 2022;4:100435.
12. Aljorani RH, Saleh ES, Al Mohammadawi KGH. Correlation of kidney injury molecule-1 and nephrin levels in Iraqi patients with diabetic nephropathy. Al-Rafidain J Med Sci 2023;5:99-104.
13. Devanath A, Jayakumari S, Sankar S, Prakash SN. Nephrin a biomarker of early glomerular injury in newly diagnosed untreated hypertensive subjects. Biomedicine. 2022;42:484-8.
14. Wang L, Wang HL, Lan HY. TGF-β signaling in diabetic nephropathy: An update. Diabet Nephropathy 2022;2:7-16.
15. Wang H, Zhang R, Wu X, Chen Y, Ji W, Wang J, et al. The Wnt signaling pathway in diabetic nephropathy. Front Cell Dev Biol 2022;9:701547.
16. Al-Hakeim HK, Asad HN, Maes M. Wnt/β-catenin pathway proteins in end-stage renal disease. Biomark Med 2021;15:1423-34.
17. Wang B, Ding C, Ding X, Tesch G, Zheng J, Tian P, et al. WNT1-inducible signaling pathway protein 1 regulates kidney inflammation through the NF-κB pathway. Clin Sci (Lond) 2022;136:29-44.
18. Surya M, Rajappa M, Vadivelan M. Utility of urinary nephrin in patients with and without diabetic nephropathy and its correlation with albuminuria. Cureus 2021;13:e20567.
19. Bohan AH. Assessment of ferritin level and some biochemical variables in Iraqi anemic diabetic mellitus in both gender. J Al-Farabi Med Sci 2024;1:19-25.
20. Chachan TAKA, Farhan H, Hamed S. Determination of irisin, body mass index, and other biochemical parameters in a sample of Iraqi type II diabetic patients. J Techniques 2022;4:53-9.
21. Amelia R, Sari DK, Muzasti RA, Fujiati II, Wijaya H. Early detection of diabetic nephropathy based on albumin creatinine ratio (ACR) in type 2 diabetes mellitus patients in Medan, Indonesia. Fam Med Prim Care Rev 2021;23:233-9.
22. Shaban A, Abbas SA-R, Abed BA. Estimation of tenascin-C levels in Iraqi patients with diabetic nephropathy. Al-Rafidain J Med Sci 2023;5:S8-13.
23. Abdulrahman SB, Saleh ES, Saeedi SM. Assessing the reliability of serum macrophage migration inhibitory factor as a marker for diabetic nephropathy prediction in type 2 diabetes patients and the effect of ACE inhibitors on its level. Iraqi J Pharm Sci 2023;32:83-90.
24. Kamal A, Adem S, El-Yassin HD. Role of beta trace protein and cystatin C as a potential biomarker for early detection of type 2 diabetic nephropathy. Al-Kindy Coll Med J 2024;20:53-8.
25. Huang J, Peng X, Dong K, Tao J, Yang Y. The association between insulin resistance, leptin, and resistin and diabetic nephropathy in type 2 diabetes mellitus patients with different body mass indexes. Diabetes Metab Syndr Obes 2021;14:2357-65.
26. Kamal A, Taher AY. A comparison between beta trace protein, cystatin C and creatinine for the estimation of eGFR in diabetic nephropathy. J Sci Med Life 2024;2:126-37.

27. Hamid GS, Allawi AA, Ghudhaib KK. Correlation of pentosidine with kidney diseases in Iraqi patients with diabetic nephropathy. Iraqi J Sci 2021;62:3436-42.
28. Ibrahim RK, Ghudhaib KK, Allawi AAD. Early prediction of nephropathy in Iraqi patient with diabetes type II by evaluating some relevant biochemical factors. Baghdad Sci J 2023;20:89-95.
29. Hataem D, Alasady RA, Khudhair SA. Evaluation of urinary monocyte chemoattractant protein-1 and serum cystatin C as markers for detection of diabetic nephropathy in comparison to albumin to creatinine ratio (ACR) 2020.
30. Salh HJH, Aziz TA, Ahmed ZA, Mahwi TO. Association between albuminuria, glycated hemoglobin with comorbidities in type 2 diabetes patients: Experience in Sulaimani City, Iraq. Al-Rafidain J Med Sci 2024;6:1-8.
31. Raheem R, Al-Qaisi AHJ, Mohammed S. Assessment of serum apelin level in Iraqi type 2 diabetic nephropathy patients. Al-Nahrain J Sci 2023;26:18-23.
32. AlRheem SZ, Altareehee SWMA, Hassan HMM. Serum extracellular superoxide dismutase concentration in type 2 diabetic patients with nephropathy: An investigation of the relationship, 2024.
33. Kostovska I, Tosheska-Trajkovska K, Topuzovska S, Cekovska S, Spasovski G, Kostovski O, et al. Urinary nephrin is earlier, more sensitive and specific marker of diabetic nephropathy than microalbuminuria. J Med Biochem 2020;39:83-90.
34. Veluri G, Mannangatti M. Urinary nephrin is a sensitive marker to predict early onset of nephropathy in type 2 diabetes mellitus. J Lab Physicians 2022;14:497-504.
35. El Nagar AMT, Metwaly OEA, Nasrallah AAEM, Kamel LMM. Role of nephrinuria in early diagnosis of type 2 diabetic nephropathy at Zagazig University Hospitals. Egypt J Hosp Med 2022;87:2113-8.
36. Hassan AM, Kata FS. The role of the biomarkers interleukin-18 and transforming growth factor-beta-1 in predicting and detecting diabetic nephropathy in patients with type 2 diabetes. J Pharm Negat Results 2022;13:243-9.
37. Chen J, Liu Q, He J, Li Y. Immune responses in diabetic nephropathy: Pathogenic mechanisms and therapeutic target. Front Immunol 2022;13:958790.
38. Kadhim SH, Khakzad MR, Eshaghi A. Evaluation of the relationship between TGF-β expression and clinical symptoms in patients with diabetic nephropathy. J Pharm Negat Results 2023;14:211-26.
39. Van Nguyen T, Ngo KD, Thi MH, Dam LTP, Huynh TQ. Evaluating the serum transforming growth factor-beta 1 level in chronic kidney disease caused by glomerulonephritis. Nephro Urol Mon 2021;13:e117806.
40. Kulkarni AR, Bale CB, Wakhare PS, Shinde NS, Chavan AS, Dighe TA, et al. Study of the urinary TGF-β1 profile in diabetic nephropathy: A single-center experience from India. Cureus 2023;15:e45231.
41. Klimontov VV, Bulumbaeva DM, Fazullina ON, Lykov AP, Bgatova NP, Orlov NB, et al. Circulating Wnt1-inducible signaling pathway protein-1 (WISP-1/CCN4) is a novel biomarker of adiposity in subjects with type 2 diabetes. J Cell Commun Signal 2020;14:101-9.
42. Zhang RD, Shi M. Occurrence and development of diabetic nephropathy caused by CD63 by inhibiting Wnt-β-catenin signaling pathway. Eur Rev Med Pharmacol Sci 2020;24:284-94.
43. Woodhams L, Sim TF, Chalmers L, Yeap B, Green D, Schlaich M, et al. Diabetic kidney disease in type 2 diabetes: A review of pathogenic mechanisms, patient-related factors and therapeutic options. PeerJ 2021;9:e11070.
44. Kondapi K, Kumar NL, Moorthy S, Silambanan S. A study of association of urinary nephrin with albuminuria in patients with diabetic nephropathy. Indian J Nephrol. 2021;31:142-8.
45. Shyamala Rajendran DSP, Natesan S, Rajendran S. Study to determine urinary nephrin as an earlier marker in diabetic nephropathy than microalbuminuria. Int J Life Sci Biotechnol Pharm Res 2023;4:1490-4
46. Elhammady AM, ELShafie HS, El Fallah AA, Khalil MA. Serum suPAR and urinary nephrin as novel sensitive and specific markers for diabetic nephropathy in patients with type 2 DM. Egypt J Hosp Med 2023;91:4373-9.
47. Hadad NS, Albrahimi N. Serum and urinary TGF-β1 in diabetic nephropathy patients. Med J Basrah Univ 2023;41:177-87.
48. Jasem AH, Haddad NS, Yaseen NT, Alrufaie MM. Association of TGF-β1 with cystatin-C in patients with diabetic nephropathy. Anaesth Pain Intensive Care 2024;28:151-4.
49. Rupprecht H, Catanese L, Amann K, Hengel FE, Huber TB, Latosinska A, et al. Assessment and risk prediction of chronic kidney disease and kidney fibrosis using non-invasive biomarkers. Int J Mol Sci 2024;25:3678.
50. Cheng Y, Du X, Zhang B, Zhang J. Increased serum WISP1 levels are associated with lower extremity atherosclerotic disease in patients with type 2 diabetes mellitus. Exp Clin Endocrinol Diabetes 2022;130:248-53.
51. Chen Y, Li P, Lin M, Jiang Y, Tan G, Huang L, et al. Silencing of METTL3 prevents the proliferation, migration, epithelial-mesenchymal transition, and renal fibrosis of high glucose-induced HK2 cells by mediating WISP1 in m6A-dependent manner. Aging (Albany NY). 2024;16:1237-48.

52. Mezher MA, Al-Radeef MY, Salih KS. Roles of interleukins (interleukin-18 and interleukin-17a) and transforming growth factor-beta markers in the pathogenesis of diabetic kidney diseases. J Adv Pharm Technol Res 2023;14:229-34.

53. Sanabria-de la Torre R, García-Fontana C, González-Salvatierra S, Andújar-Vera F, Martínez-Heredia L, García-Fontana B, et al. The contribution of Wnt signaling to vascular complications in type 2 diabetes mellitus. Int J Mol Sci 2022;23:6995.