



ISSN: 0067-2904

Estimation of Angiopoietin-Like Protein 3 and Fibroblast Growth Factor-23 Levels as Highly Sensitive Early Prognostic Factors in Iraqi Diabetics with Nephropathy

Hiba Haqi Ismael^{1,2*}, Kadhim K. Ghudhaib¹, Ali Abdulmajid Dyab Allawi³

¹Department of Chemistry, College of Science for Women, University of Baghdad, Baghdad, Iraq.

²Scientific Research Commission, Baghdad, Iraq

³Department of Medicine, College of Medicine, University of Baghdad, Baghdad, Iraq.

Received: 1/6/2025

Accepted: 1/3/2026

Published: xx

Abstract

There has been significant interest in the field of diabetes complications, particularly diabetic nephropathy. This includes the use of biochemical factors as early diagnostic markers to prevent the deterioration of patients' health. Accordingly, the current study aimed to identify the levels of angiopoietin-like protein-3 (ANGPTL3) and fibroblast growth factor-23 (FGF23) in Iraqi patients with diabetic nephropathy, which may serve as early indicators for the diagnosis of the disease. The study included 143 individuals aged 35-65 years who were visiting the Medical City of Baghdad Teaching Hospital from August to December 2024. The patients were divided into three groups based on the ACR criteria. The first group was the normoalbuminuria group (ACR < 30 mg/g, 35 patients), and the second was the microalbuminuria group (ACR = 30-300 mg/g, 33 patients). The third group was the macroalbuminuria group (ACR > 300 mg/g, 35 patients). The fourth group, which included 40 healthy individuals, represented the control group. The lipid profile, urea, and creatinine levels were measured using spectrophotometric methods. ELISA was used to measure ANGPTL3 and FGF23 levels. The results showed a significant increase in both ANGPTL3 and FGF23 levels between the patient and healthy groups. The results of the lipid profile, urea, HbA1C, and creatinine were consistent with the results of ANGPTL3 and FGF23, which were found to be significantly increased in the patient groups compared with the control group. Also, the ROC results confirmed that these factors are perfect or excellent for this case. In conclusion, depending on the current outcomes, with ROC results, both ANGPTL3 and FGF23 appear to possess high detection sensitivity toward the targeted case. It may be used as an excellent factor for the early prediction of diabetic nephropathy development.

Keywords: Albumin to creatinine ratio, Angiopoietin-like protein 3, Diabetes, Fibroblast growth factors 23, Nephropathy

تقدير مستويات البروتين الشبيه بالأنجيوبوتين 3 وعامل نمو الخلايا الليفية 23 كعوامل تنبؤية مبكرة
عالية الحساسية لدى مرضى السكري العراقيين المصابين باعتلال الكلى

*Email: heba.haqi2305m@cs.w.uobaghdad.edu.iq



هبة حقي اسماعيل^{1,2,*}, كاظم خضير غضيب¹, علي عبدالمجيد نياي علاوي³

¹قسم الكيمياء، كلية العلوم للبنات، جامعة بغداد، بغداد، العراق

²هيئة البحث العلمي، بغداد، العراق

³قسم الطب، كلية الطب، جامعة بغداد، بغداد، العراق

الخلاصة

شهدت دراسة مضاعفات داء السكري، ولا سيما اعتلال الكلى السكري، اهتمامًا متزايدًا. ويشمل ذلك استخدام المؤشرات الحيوية كعلامات تشخيصية مبكرة للوقاية من تدهور صحة المرضى. وعليه، هدفت هذه الدراسة إلى تحديد مستويات البروتين الشبيه بالأنجيوبوتين-3 (ANGPTL3) وعامل نمو الخلايا الليفية-23 (FGF23) لدى مرضى عراقيين مصابين باعتلال الكلى السكري، والتي قد تُستخدم كمؤشرات مبكرة لتشخيص المرض. شملت الدراسة 143 شخصًا تتراوح أعمارهم بين 35 و65 عامًا، ممن راجعوا مستشفى مدينة بغداد الطبية التعليمي خلال الفترة من أغسطس إلى ديسمبر 2024. قُسم المرضى إلى ثلاث مجموعات بناءً على معايير نسبة الألبومين إلى الكرياتينين في البول (ACR) المجموعة الأولى هي مجموعة الألبومين الطبيعي في البول ($ACR < 30$ ملغم/غرام، 35 مريضًا) والمجموعة الثانية هي مجموعة الألبومين الدقيق في البول ($ACR = 300-3000$ ملغم/غرام، 33 مريضًا). كانت المجموعة الثالثة هي مجموعة البيلة الألبومينية الكبيرة (نسبة الألبومين إلى الكرياتينين > 300 ملغم/غ، 35 مريضًا). أما المجموعة الرابعة، التي ضمت 40 فردًا سليمًا، فكانت تمثل المجموعة الضابطة. تم قياس مستوى الدهون واليوريا والكرياتينين باستخدام طرق قياس الطيف الضوئي. كما استُخدم اختبار ELISA لقياس مستويات ANGPTL3 و FGF23. أظهرت النتائج زيادة ملحوظة في مستويات كلٍّ من ANGPTL3 و FGF23 بين مجموعتي المرضى والأصحاء. وتوافقت نتائج مستوى الدهون واليوريا والهيموغلوبين السكري (HbA1c) والكرياتينين مع نتائج ANGPTL3 و FGF23، حيث وُجد أنها مرتفعة بشكل ملحوظ في مجموعة المرضى مقارنةً بالمجموعة الضابطة. كما أكدت نتائج منحنى ROC أن هذه العوامل مثالية أو ممتازة لهذه الحالة. وختامًا، بناءً على النتائج الحالية، ومع نتائج منحنى ROC، يبدو أن كلاً من ANGPTL3 و FGF23 يتمتعان بحساسية عالية في الكشف عن الحالة المستهدفة. يمكن استخدامه كعامل ممتاز للتنبؤ المبكر بتطور اعتلال الكلى السكري.

1. Introduction

Diabetic kidney disease is a disease caused by hyperglycemia, which is characterized by damage in the blood vessels, renal tubules, glomeruli, and fibrosis in the kidneys, leading to a gradual decline in kidney function and kidney failure. Diabetic complications are closely linked to various biochemical parameters, particularly those related to sugar or lipid metabolism and kidney and liver functions. Hyperglycaemia can lead to microvascular complications, resulting in nephropathy [1, 2], retinopathy [3, 4], neuropathy, and an increased risk of heart disease [5, 6]. In diabetics, lipid abnormalities, such as elevated triglyceride and low-density lipoprotein (LDL) levels and decreased high-density lipoprotein (HDL) levels, are also associated with insulin resistance and cardiovascular complications [7]. Furthermore, diabetes complications include the effect of hyperglycemia on periodontitis [8].

A previous study [9] evaluated the levels of matrix metalloproteinases-9 (MMP-9) and tissue inhibitors of matrix metalloproteinases-1 (TIMP-1) in patients with cardiovascular disease and diabetes mellitus. This study showed the possibility of using these factors as markers for the early prediction of cardiovascular disease development in individuals with type II diabetes. In another study, the levels of arginase I and II were evaluated in patients with atherosclerosis and type II diabetes. The study reported that the increasing levels of these isoenzymes can occur as atherosclerosis progresses in patients with type II diabetes mellitus [10]. In addition, numerous other studies in this field have been conducted to explore various important biochemical factors, such as the estimation of FBXW7 and fetuin-A protein ranks in patients with T2DM and the

effect of metformin on their levels [11], in addition to the estimation of pentraxin 3 and nitric oxide levels and the effect of metformin on their ranks in patients with T2DM [12].

In this study, the estimation of ANGPTL3 and FGF23 levels in Iraqi patients with diabetic nephropathy was studied as an excellent early prognostic factor. A complex condition with several underlying causes, type 2 diabetes is characterized by elevated blood sugar levels caused by either insufficient insulin production or activity, or both. A healthy pancreas generates the appropriate amount of insulin to deliver glucose to cells. The cells of the body may not react to the insulin produced by an inefficient pancreas or may produce too little insulin. Blood glucose levels increase and accumulate, leading to the development of diabetes [13].

FGF23 is a bone-derived hormone that plays a central role in phosphate homeostasis, vitamin D metabolism, and bone mineralization. It is a protein that is released from bone cells (osteocytes and osteoblasts). Its purpose is to improve renal phosphate excretion to maintain blood phosphate levels. Fibroblast growth factor 23 (FGF23) regulates calcium and phosphate balance. Its levels are affected by calcium concentrations, as it acts on the kidneys to enhance phosphate secretion and inhibit vitamin D activity, which can have a specific effect on calcium levels. [14-17]. In a recent study [18], FGF23 was associated with proteinuria and musculoskeletal complications in patients with systemic lupus erythematosus. In another study, high levels of FGF23 were positively associated with cardiovascular disease [19].

Angiopoietin-like protein 3 (ANGPTL3) is a 460-amino acid glycoprotein that is primarily produced by renal glomerular cells and hepatocytes. Angiopoietin-like protein 3 is an important regulator of lipid and glucose metabolism. The literature indicates that the level of this factor in the blood may be affected by fasting and nutritional status and is involved in the abnormalities found in obesity [20]. Another study in patients with obstructive sleep apnea found elevated levels of ANGPTL3, which is considered a biomarker for cardiovascular disease risk [21]. Furthermore, DN is characterized by progressive impairment of kidney function due to damage to the glomeruli and tubules [22]. Chronic kidney disease is considered a risk factor for atherosclerosis. Therefore, the progression of diabetic nephropathy causes disturbances in the levels of lipids and lipoproteins due to kidney failure.

Thus, this study aimed to evaluate the levels of ANGPTL3 and FGF23 in patients with diabetic nephropathy to determine the potential of these factors as biomarkers for the early diagnosis of this disorder, which poses serious complications to public health, as well as to determine the relationships between these important factors and other relevant diagnostic factors.

2. Materials and Methods

2.1. Patients and controls

The study included 143 subjects (male and female), aged 35-65 years, among whom 103 patients with diabetes who visited Baghdad Teaching Hospital/Medical City between August 2024 and December 2024 and 40 healthy individuals as a control group. The waist-to-hip ratio (WHR) and body mass index (BMI) were calculated for all groups.

The patient groups with type 2 diabetes and nephropathy complications were divided into three groups depending on the albumin-to-creatinine ratio (ACR) values:

- Normoalbuminuria group (G1): 35 patients with an ACR < 30 mg/g.
- The microalbuminuria group (G2) included 33 patients with an ACR range of 30-299 mg/g.
- Macroalbuminuria group (G3): 35 patients with an ACR of >300 mg/g. As well as
- The control group (G4): It included 40 subjects who appeared to be in good health and did not suffer from any diseases.

2.2. Exclusion Criteria

- Individuals aged >65 years.
- Individuals diagnosed with diabetic neuropathy.

- Individuals diagnosed with DR.
- Individuals with DCM.

We excluded all diabetic complications and kidney diseases (except diabetic nephropathy).

2.3. Sample collection and analysis

Eight milliliters of blood was withdrawn from the cubital vein and divided into two portions. The first portion (6 mL) was clotted in gel tubes at room temperature for 20 min. After 5 min of centrifugation, the serum was separated and stored in Eppendorf tubes. The samples were kept at a temperature -25°C for later analyses of biochemical factors, and 3 mL was used for the evaluation of ANGPTL 3 and FGF 23 by ELISA (My BioSource, USA). The second (3 mL) was used for tests (urea and creatinine) and lipid profile level using auto spectrometry. The anticoagulant test for HbA1c measured by I-chroma in the second portion (6 mL) of EDTA tube1. Urine samples were obtained in a sterile glass tube to ascertain the urine albumin/creatinine ratio, which was quantified using the FUS 3000 urinalysis apparatus [[23].

2.4. Statistical Analysis

The data were statistically analyzed using Statistical Package for the Social Sciences version 29, and the variable means were given. One-way analysis of variance was employed to ensure that the means of the four independent groups under investigation (control group and patient groups) differed statistically [24]. Receiver operating characteristic curve analysis was performed to ascertain the optimal diagnostic value, sensitivity, and specificity of the biomarkers.

3. Results

Table 1 shows the results of age, BMI, and WHR in the patient groups (G1, G2, G3) and control group (G4) in the current study. In the case of age, the results revealed significant differences between the groups of (G1-G3) and (G3-G4), while no significant differences between (G1-G2) and (G1-G4), as recorded in Table 1. The results of BMI indicated a significant difference between G2 and G3 only, while no significant differences were observed between the other groups, which may be attributed to good glycemic control in macroalbuminuria (G3). The WHR results showed significant differences between the patient and control groups. This can be attributed to the high visceral fat level within the abdominal area.

Table 1: Results of age, BMI, and WHR in the patient and control groups

Groups					
Parameters	G1 n=35	G2 n=33	G3 n=35	G4 n=40	P Value
Age (years)	51.24±1.4 ^b	53.91±1.5	56.64±1.31 ^{b, f}	49.23±1.3	0.0012
BMI Kg/m ²	31.93± 0.9	34.24± 1.3 ^d	28.52±0.96 ^d	30.45±0.99	0.0034
WHR	0.93±0.01 ^c	0.92±0.01 ^{d, c}	0.96±0.01 ^{d, f, c}	0.83±0.003	0.000

G1: Normoalbuminuria group, G2: Microalbuminuria group, G3: Macroalbuminuria group, G4: Control group, WHR: waist-to-hip ratio, significant differences $p \leq 0.05$, $p > 0.05$, significant differences

^a Significant difference between G1 and G2, ^d Significant difference between G2 and G3

^b Significant difference between G1 and G3, ^e Significant difference between G2 and G4

^c Significant difference between G1 and G4, ^f Significant difference between G3 and G4

Table 2 lists the results of creatinine and urea of all the studied groups. Creatinine results revealed highly significant differences among all the studied groups (patients and healthy groups). Significant differences were found between G1 and G3 and between G2 and G3.

Significant differences were recorded between G3 and G1. The results of urea showed that there were highly significant differences among all the studied groups of patients and controls. Significant differences in the urea case can be explained in detail. A significant difference was found between G1 and G3, similarly between G2 and G3. Significant differences were noted between G3 and G1, as reported in Table 2. These results confirmed the kidney disorders that occurred in terms of urea and creatinine due to hyperglycemia compared with the control group. The results of albumin-to-creatinine ratio (ACR) for the patient groups (G1-G3) were showed significant differences among patient groups and represented the three classified groups, including normoalbuminuria, microalbuminuria, and macroalbuminuria. Furthermore, HbA1C showed highly significant differences among patients and control groups, and these results were associated with hyperglycemia condition compared with normal cases, as shown in Table 2.

Table 2: Results of creatinine, urea, ACR, and hemoglobin A1C in the patient and control groups

Groups Parameters	G1 n=35	G2 n=33	G3 n=35	G4 n=40	P Value
Creatinine (mg/dL)	0.696±0.04 ^b	1.319±0.3 ^d	6.629±0.6 ^{b, d, f}	0.5688±0.02	0.000
Urea (mg/dL)	30.65±1.6 ^b	39.98±6.8 ^d	105.9±10 ^{b, d, f}	35.78±1.6	0.000
ACR (mg/g)	3.83±2.5 ^{a, b}	74.03±24.2 ^{a, d}	363.4±63.0 ^{b, d}	-	0.001
HbA1C	8.962±0.3 ^{b, c}	8.852±0.3 ^{d, e}	6.653±0.2 ^{b, d, f}	4.613±0.08	0.000

G1: Normoalbuminuria group, G2: Microalbuminuria group, G3: Macroalbuminuria group, G4: Control group, significant differences $p \leq 0.05$, $p > 0.05$.

^a Significant difference between G1 and G2, ^d Significant difference between G2 and G3

^b Significant difference between G1 and G3, ^e Significant difference between G2 and G4

^c Significant difference between G1 and G4, ^f Significant difference between G3 and G4

The results of the lipid profile in the patient and healthy groups in the current study are shown in Table 3. The cholesterol and LDL results revealed a highly significant increase in the patient groups than in the control group. In contrast, the results of HDL showed a significant decrease in the patient groups (G1 and G2) than in the control group. Also, both TG and VLDL results were significantly increased in G2 than in G3. These results can be attributed to the lipid disturbances of diabetes and its complications.

Table 3: Results of lipid profile in all the studied groups

Groups Parameters	G1 n=35	G2 n=33	G3 n=35	G4 n=40	P Value
Cholesterol (mg/dL)	187.2±7.8 ^{b, c}	195.3±7.6 ^{d, e}	147.6±6.2 ^{b, d}	161.7±4.1	0.0000
TG (mg/dL)	173.5±15	205.1±20 ^d	127.5±10 ^d	174.3±8.7	0.0029
VLDL (mg/dL)	34.71±3.1	41.01±4.1 ^d	25.50±2.1 ^d	34.85±1.7	0.0029
HDL (mg/dL)	42.44±1.4 ^{b, c}	40.06±1.6 ^{d, e}	48.06±1.3 ^{b, d}	48.38±0.7	0.000
LDL (mg/dL)	110.0±7.5 ^{b, c}	114.2±7.6 ^{d, e}	73.99±6.2 ^{b, d}	78.48±5.2	0.000

G1: Normoalbuminuria group, G2: Microalbuminuria group, G3: Macroalbuminuria group, G4: Control group, significant differences $p \leq 0.05$, $p > 0.05$.

^a Significant difference between G1 and G2, ^d Significant difference between G2 and G3

^b Significant difference between G1 and G3, ^e Significant difference between G2 and G4

^e Significant difference between G1 and G4, ^f Significant difference between G3 and G4

Table 4 shows the results of ANGPTL3 and FGF23 for all the studied groups. The ANGPTL3 level was significantly increased between the patient groups compared with the control group. Furthermore, these results revealed a highly significant increase among the patient groups (G1, G2, and G3). Similarly, the results of FGF23 showed a highly significant increase in the patient groups compared with the control group. Moreover, a highly significant increase in FGF23 results was recorded among patient groups (G1, G2, and G3), as shown in Table 4.

Table 4: Results of ANGPTL3 and FGF-23 in the patients and control groups

Groups Parameters	G1 n=35	G2 n=33	G3 n=35	G4 n=40	P Value
ANGPTL3 (pg/mL)	807.8±35 ^{a, b, c}	1170±16.8 ^{a, e}	1236±35 ^{b, f}	192.0±6.6	0.000
FGF-23 (pg/mL)	243.5±12.7 ^{a, b, c}	439.8±22.8 ^{a, d, e}	554.5±24.1 ^{b, d, f}	100.3±1.9	0.000

G1: Normoalbuminuria group, G2: Microalbuminuria group, G3: Macroalbuminuria group, G4: Control group, significant differences $p \leq 0.05$, $p > 0.05$.

^a Significant difference between G1 and G2, ^d Significant difference between G2 and G3

^b Significant difference between G1 and G3, ^e Significant difference between G2 and G4

^c Significant difference between G1 and G4, ^f Significant difference between G3 and G4

Receiver operating characteristic curve

The receiver operating characteristic (ROC) is a statistical analysis used to find the appropriate specificity and sensitivity of a diagnostic test by drawing a graph that relates the relationship between sensitivity and specificity. The ROC test for ANGPTL3 markers had a very clear cut-off value (> 353.7) with 100% sensitivity and 100% specificity, with the area under the curve (AUC) at a high value (100), which is equal to a value of (1), indicating that ANGPTL3 is a perfect diagnostic marker for the normoalbuminuria group (Table 5 and Figure 1). Furthermore, the ROC data of FGF23 had a very clear cut-off value (> 124.9) with a sensitivity of 97% and a specificity of 97%, with the AUC at a high value (96), which is equal to a value of 0.96, indicating that FGF23 is an excellent diagnostic marker, as shown in Table 5 of the normoalbuminuria group and illustrated in Figure 2.

Table 5: ROC curves of ANGPTL3 and FGF23 in the normoalbuminuria group

Parameters	AUC	Sensitivity%	Specificity%	Cut off
ANGPTL3	1	100	100	> 353.7
FGF23	0.96	97	97	> 124.9

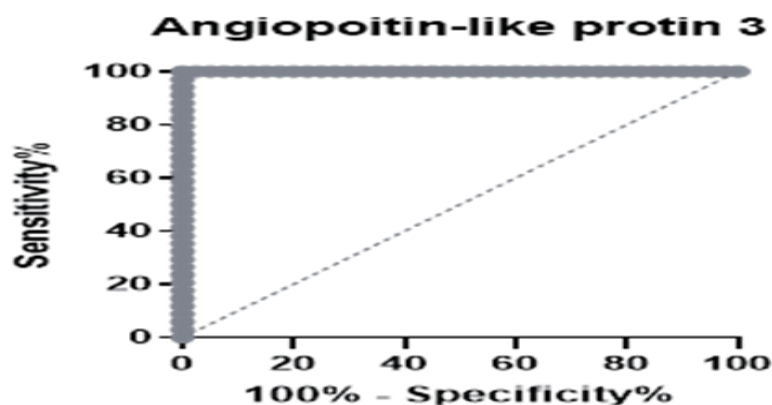
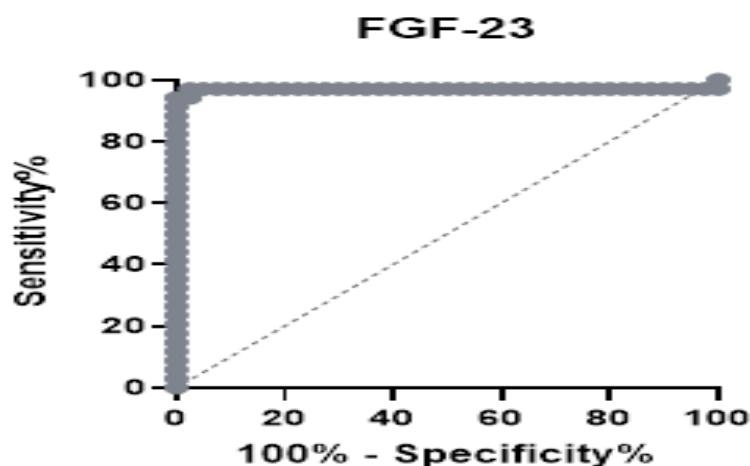
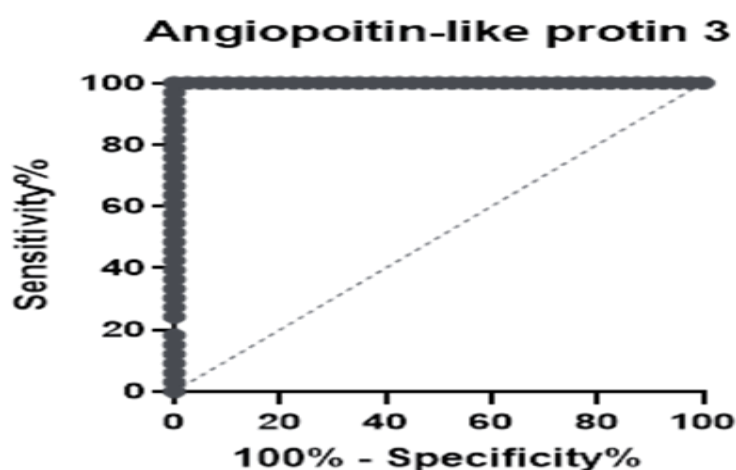


Figure 1: ROC curve analysis of ANGPTL3 expression in normoalbuminuria**Figure 2:** ROC curve analysis of FGF-23 expression in normoalbuminuria

The ROC analysis results for both ANGPTL3 and FGF23 in microalbuminuria are recorded in Table 6. ANGPTL3 has a very clear cut-off value (> 680.3) with 100% sensitivity and 100% specificity, with the AUC at a high value (100), which is equal to a value of 1, indicating that ANGPTL3 is a perfect diagnostic marker for the microalbuminuria group (Table 6 and Figure 3). Furthermore, the ROC data of FGF23 had a very clear cut-off value (> 189.8) with a sensitivity of 96% and a specificity of 100%, with an AUC of 96, which is equal to a value of 0.96, indicating that FGF23 is an excellent diagnostic marker of microalbuminuria (Table 6 and Figure 4).

Table 6: ROC curves of ANGPTL3 and FGF23 in the microalbuminuria group

Parameters	AUC	Sensitivity%	Specificity%	Cut off
ANGPTL3	1	100	100	> 680.3
FGF-23	0.96	96.9	100	> 189.8

**Figure 3:** ROC curve analysis of ANGPTL3 expression in the microalbuminuria group

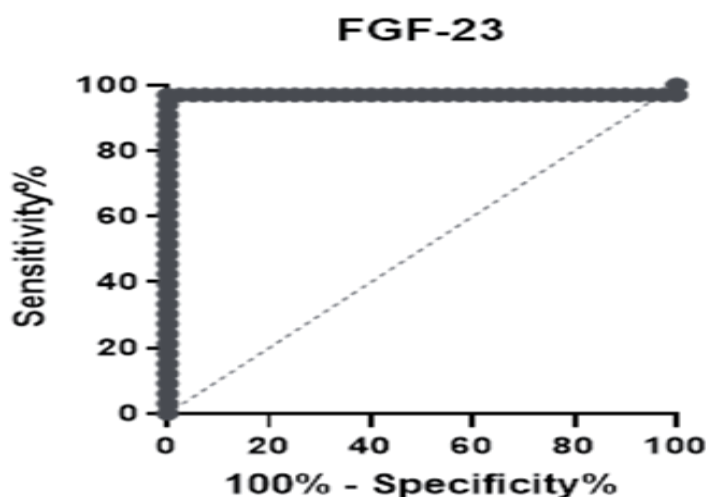


Figure 4: ROC curve analysis of FGF-23 in the microalbuminuria group

In addition, the ROC analysis for ANGPTL3 and FGF23 in macroalbuminuria is documented in Table 7. The results of ANGPTL3 showed that it has a very clear cut-off value (> 391.0) with 100% sensitivity and 100% specificity, with the AUC at a high value (100), which is equal to a value of 1, indicating that ANGPTL3 is a perfect diagnostic marker of macroalbuminuria (Table 7 and Figure 5). Furthermore, the ROC data of FGF23 had a very clear cut-off value (> 241.5) with a sensitivity of 100% and a specificity of 100%, with an AUC of 1 indicating that FGF23 is a perfect diagnostic marker of macroalbuminuria, as shown in Table 7 and Figure 6.

Table 7: ROC curves of ANGPTL3 and FGF-23 in the macroalbuminuria group

	AUC	sensitivity%	specificity%	Cut off
ANGPTL3	1	100	100	> 391.0
FGF-23	1	100	100	> 241.5

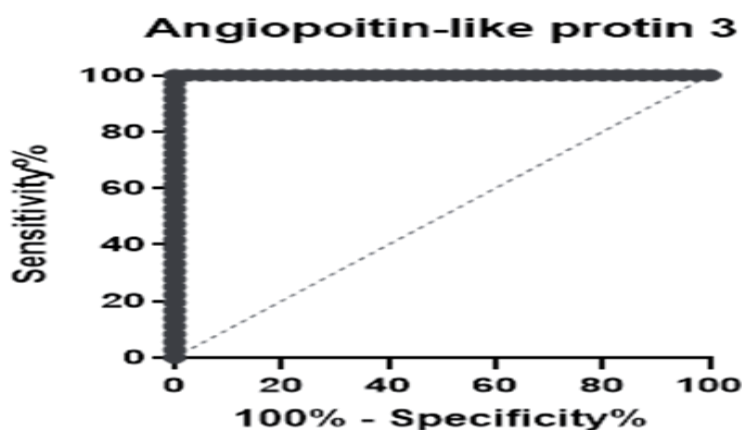


Figure 5: ROC curve analysis of ANGPTL3 expression in the macroalbuminuria group

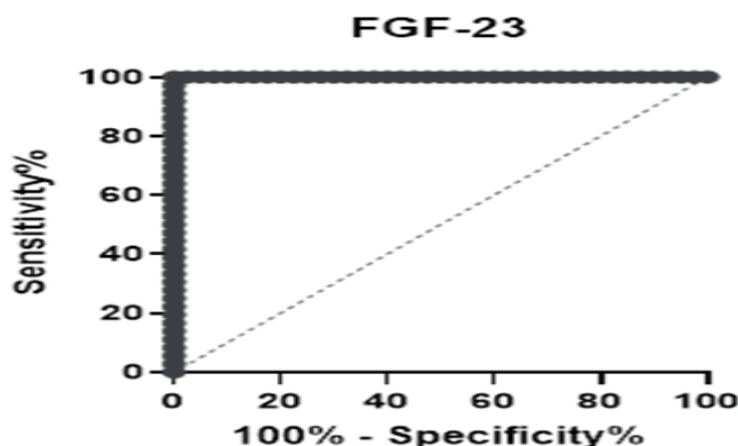


Figure 6: ROC curve analysis of FGF-23 in the macroalbuminuria group

4. Discussion

Diabetic nephropathy (DN) is a complication of diabetes that causes progressive kidney damage and possible kidney failure. It is characterized by persistent urine protein (albuminuria). It is a leading cause of end-stage kidney disease (ESKD). Treatment focuses on blood sugar and blood pressure control. Chronic high blood sugar damages the kidney tubules and glomeruli, making it difficult to prevent the disease and identify the risks associated with diabetic kidney disease [25] studies have been conducted to identify the etiology of diabetic nephropathy, which includes tubular and glomerular damage and fibrosis, to predict these conditions earlier by measuring the related biochemical factors [26-28]. The current study included determining some biochemical levels such as ANGPTL3 and FGF23 in patients with diabetes and the complications of diabetic effect on the kidneys. The three groups of patients were classified based on ACR, which is an important criterion for determining the degree of kidney damage resulting from the effects of blood sugar [29, 30]. There was also a fourth control group within the age ranges of all groups. Along with additional variables, such as creatinine and urea, which validate the criteria results, the above indicators indicate the emergence of problems caused by diabetes. Risk factors for diabetic kidney disease (DKD) include inflammation, fibrosis, and high blood sugar [31].

The results of the current study indicated disturbances in lipid levels in patients with diabetes compared to healthy individuals. These disorders are characterized by a decrease in high-density lipoprotein levels and an increase in low-density lipoprotein, triglyceride, and cholesterol levels. The disturbance in lipids is associated with type 2 diabetes and obesity, as the breakdown of fats into glycerol and free fatty acids leads to increased inflammation as a result of the metabolic changes that can occur as a consequence of obesity development [32][32]. Furthermore, the current study recorded a significant increase in urea and creatinine levels in the patient groups compared with their normal levels. High glucose levels are associated with elevated urea and creatinine levels [33][33]. High blood glucose affects the glomeruli, epithelial cells, fibroblasts, and vascular endothelium, which explains the elevated blood urea and creatinine levels associated with CHG [34].

The current study relied on the ACR to classify patient groups based on the severity of kidney disease in patients with diabetes. This criterion is important because it confirms the presence of DKD in its primary and advanced forms, based on the presence of urine protein [35].

Fibroblast growth factor 23 is a protein that is produced in bone cells. It is considered an essential factor for regulating the body's phosphate levels. Its main effect is on mineral

metabolism and its role in maintaining phosphate levels and inhibiting the synthesis of active vitamin D₃ in the kidneys. FGF23 is produced in osteocytes when phosphate levels rise. It is released into the kidneys and binds to its klotho receptor. This binding prevents the reabsorption of phosphate from the kidneys, resulting in more excretion in the urine, which helps lower the blood phosphate level. In addition, it works to reduce the synthesis of the active vitamin D, which leads to excessive calcium absorption and bone mineralization, by inhibiting 25-hydroxyvitamin D 1 α -hydroxylase in the kidneys.[36, 37] . Mutations in FGF23 make its protein resistant to proteolysis and increase phosphate loss, which leads to rickets due to a lack of phosphate in the body. Excessive FGF23 production, as occurs in some tumors, leads to tumor-related OM. FGF23 overexpression has also been linked to cardiovascular disease, chronic kidney disease, vascular calcification, and endothelial disorders [38]. Therefore, it is considered a biomarker for determining the risk of developing chronic and acute diseases and appears early in CKD [39].

ANGPTL3 regulates lipid metabolism by synergizing with ANGPTL4 and ANGPTL8 to form protein-protein complexes. It also inhibits the lipase enzyme in lipoprotein and liver, and studies have indicated its association with lower levels of triglycerides and low-density lipoprotein cholesterol. ANGPTL3 activity was found to be high when it binds to low-density lipoprotein particles [40, 41]. Angiopoietin-like protein 3 is associated with heart disease, kidney disease, and lipid metabolism [42].

More recently, ANGPTL3 has been identified as an important mediator of kidney injury in diabetic nephropathy through numerous mechanisms, including glomerular damage, dyslipidemia, and increased inflammation, making it a promising therapeutic target. The level of INGPTL3 in the blood serum increases in the case of diabetic nephropathy and increases as the severity of the condition increases and dyslipidemia occurs. This result is consistent with the results of the current study [43, 44]. Similarly, FGF23 plays an important role in diabetic nephropathy, as it is associated with fibrosis, inflammation, and disease progression, and regulates phosphate levels. It has been reported that blocking its action reduces kidney damage in animal models, suggesting that it could be a potential target for treating the disease [45].

According to the results of ANGPTL3 and FGF23 in the current study, these factors have a high considerable increase among all the studied groups. These results were supported by the ROC findings, which confirmed the sensitivity of both factors toward this case of kidney disorders. Moreover, the ROC outcomes are in agreement with several previous studies. In these studies, markers such as connective tissue growth factor (CTGF), angiotensin-converting enzyme 2 (ACE2), and ATPase were found to be good or perfect markers in their studied cases. Similarly, ANGPTL3 and FGF23 were found to be perfect or excellent markers for the studied case [1, 2].

Conclusion

Based on the findings reported in this study, ANGPTL3 was found to be a perfect predictor of diabetic nephropathy and the diagnosis of renal function. Additionally, FGF23 is an excellent indicator for monitoring the development of DKD complications. According to the ROC curve analysis results, they are excellent markers for the early prediction of diabetic nephropathy in all disease stages.

Acknowledgment

We thank and appreciate the staff of Baghdad Medical City Hospital for their great efforts and facilities that helped in completing this research. We thank and appreciate all participants, both patients and healthy individuals, for their consent to take blood samples for this study.

References

- [1] R. K. Ibrahim, K. K. Ghudhaib and A. A. D. Allawi, "Early Prediction of Nephropathy in Iraqi Patient with Diabetes Type II by Evaluating Some Relevant Biochemical Factors," *Baghdad Science Journal*, vol. 21, no. 5, pp. 1549-1560, 2024.
- [2] R. k. Ibrahim, K. K. Ghudhaib and A. A. D. Allawi, "ATPase level in Iraqi Diabetic Patients with and without Diabetic Kidney Disease," *Baghdad Science Journal*, vol. 22, no. 6, pp. 1750-1756, 2025.
- [3] P. Li, H. Wang, G. Tian .et al., "Identification of key biomarkers for early warning of diabetic retinopathy using BP neural network algorithm and hierarchical clustering analysis," *Scientific Reports*, vol. 14, no. 1, pp. 15108, 2024.
- [4] P. Khanorkar, B. Agrawal and K. K. B. Sodavadiya, "Role of biochemical markers in type 2 diabetic patients of cataract and retinopathy attending Index Medical College," *HospitalBiochemistry Research Centre, Indore International Journal of Clinical Research*, vol. 5, no. 1, pp. 17-22, 2018.
- [5] R. Guttikonda and U. K. Pothu, "Role of biochemical parameters in prediction of diabetic peripheral neuropathy," *Journal of Research in Applied Basic Medical Sciences*, vol. 10, no. 2, pp. 169-177, 2024.
- [6] K. Kim, S. N. Lee, Y. B. Ahn .et al., "Associations of polyneuropathy with risk of all-cause and cardiovascular mortality, cardiovascular disease events stratified by diabetes status," *Journal of Diabetes Investigation*, vol. 14, no. 11, pp. 1279-1288, 2023.
- [7] H. T. AlAni and M. Q. Al-Lami, "Evaluation of some Biochemical and Hematological Parameters in Patients with Chronic Kidney Disease," *Journal of the Faculty of Medicine Baghdad*, vol. 66, no. 2, pp. 154-161, 2024.
- [8] A. Trullenque-Eriksson, C. Tomasi, K. Eeg-Olofsson .et al., "Periodontitis in patients with diabetes and its association with diabetes-related complications. A register-based cohort study," *BMJ open*, vol. 14, no. 7, pp. e087557, 2024.
- [9] S. A. Wadood, R. Al Shawk and R. Hashem, "Variants of MMP-9 and TIMP-1 levels could be a predictor of an early development of cardiovascular diseases in type 2 diabetes among Iraqi patients," *Iraqi Journal of Science*, vol. 56, no. 1C, pp. 622-632, 2015.
- [10] A. Ibrahim Suhad, A. Zainulabdeen Jwan and M. Jasim Hameed, "Levels of Arginase Isoenzymes in Sera of Iraqi Patients with Atherosclerosis and Type 2 Diabetes Mellitus," *Research Journal of Biotechnology*, vol. 14, no. 3, pp. 201-207, 2019.
- [11] S. K. Mohammed, E. M. Taha and S. A. Muhi, "A case-control study to determination FBXW7 and Fetuin-A levels in patients with type 2 diabetes in Iraq," *Journal of Diabetes Metabolic Disorders*, vol. 20, no. 1, pp. 237-243, 2021.
- [12] S. K. Mohammed, E. M. Taha, S. A. Muhi .et al., "Pentraxin3 and Nitric Oxide-Associated with an Atherogenic Index and Type II Diabetes Mellitus " *Biochemical Cellular Archives*, vol. 20, no. 1, pp. S488, 2020.
- [13] S. Thipsawat, "Early detection of diabetic nephropathy in patient with type 2 diabetes mellitus: A review of the literature," *Diabetes Vascular Disease Research*, vol. 18, no. 6, pp. 14791641211058856, 2021.
- [14] S. Seiler, G. H. Heine and D. Fliser, "Clinical relevance of FGF-23 in chronic kidney disease," *Kidney International*, vol. 76, pp. S34-S42, 2009.
- [15] M. E. Rodriguez-Ortiz, I. Lopez, J. R. Muñoz-Castañeda .et al., "Calcium deficiency reduces circulating levels of FGF23," *Journal of the American Society of Nephrology*, vol. 23, no. 7, pp. 1190-1197, 2012.
- [16] S. Rayego-Mateos, N. Doladé, A. García-Carrasco .et al., "The Increase in FGF23 Induced by Calcium Is Partially Dependent on Vitamin D Signaling," *Nutrients*, vol. 14, no. 13, pp. 2576, 2022.
- [17] S. Vermeulen, M. E. A. Scheffer-Rath, M. T. P. Besouw .et al., "Fibroblast growth factor 23 and calcium-phosphate metabolism in relation to cardiovascular risk factors in patients with type 1 diabetes," *Journal of Diabetes*, vol. 16, no. 6, pp. e13500, 2024.
- [18] Y. Fernández-Cladera, F. Gómez-Bernal, M. García-González .et al., "Relationship of Fibroblast Growth Factor 23 Serum Levels with Disease Characteristics in Systemic Lupus Erythematosus Patients," *Biomolecules*, vol. 13, no. 8, pp. 1222, 2023.
- [19] M. Liu, P. Xia, Z. Tan .et al., "Fibroblast growth factor-23 and the risk of cardiovascular diseases and mortality in the general population: A systematic review and dose-response meta-analysis," (in

- English), *Frontiers in Cardiovascular Medicine*, Systematic Review vol. Volume 9 - 2022, pp. 1-18, 2022.
- [20] T.-C. Wu, B.-G. Hsu, C.-H. Kuo .et al., "Serum Angiopoietin-like Protein 3 Levels Are Associated with Endothelial Function in Patients with Maintenance Hemodialysis," *Life*, vol. 14, no. 1, pp. 18, 2024.
- [21] J. Li, Y. Yang, X. Jiao .et al., "The clinical role of angiopoietin-like protein 3 in evaluating coronary artery disease in patients with obstructive sleep apnea," *Cardiovascular Drugs Therapy*, vol. 34, no. 6, pp. 773-780, 2020.
- [22] A. Panduranga, K. Chaturvedy, M. Chaturvedy .et al., "Study of renal parenchymal changes by diffusion tensor imaging in diabetic nephropathy," *Polish Journal of Radiology*, vol. 87, no. 1, pp. 163-171, 2022.
- [23] L. Kennedy, "Hypertension: when to treat," in *Problem Solving in Hypertension*, Oxford, UK: Clinical Publishing, 2009. [Online]. Available: http://www.clinicalpublishing.co.uk/samples/09/PS_Hypertension%20ebook%20chp%201.pdf
- [24] S. W. Lee, "Methods for testing statistical differences between groups in medical research: statistical standard and guideline of Life Cycle Committee," *Life Cycle*, vol. 2, pp. e1, 2022.
- [25] E. Mohammed, I. Al Salmi, D. Al Riyami .et al., "Non-Diabetic Kidney Disease in Type 2 Diabetes Mellitus: A Study of 82 Patients and Review of the Literatures," *Open Journal of Nephrology*, vol. 12, no. 1, pp. 169-186, 2022.
- [26] J. Ahmad, "Management of diabetic nephropathy: Recent progress and future perspective," *Diabetes & Metabolic Syndrome: Clinical Research & Reviews*, vol. 9, no. 4, pp. 343-358, 2015.
- [27] W. Y. Cho, S. Y. Lim, J. H. Yang .et al., "Urinary tissue inhibitor of metalloproteinase-2 and insulin-like growth factor-binding protein 7 as biomarkers of patients with established acute kidney injury," *The Korean Journal of Internal Medicine*, vol. 35, no. 3, pp. 662, 2019.
- [28] C. Delrue and M. M. Speeckaert, "Tissue Inhibitor of Metalloproteinases-2 (TIMP-2) as a Prognostic Biomarker in Acute Kidney Injury: A Narrative Review," *Diagnostics*, vol. 14, no. 13, 2024.
- [29] N. M. Selby and M. W. Taal, "An updated overview of diabetic nephropathy: Diagnosis, prognosis, treatment goals and latest guidelines," *Diabetes, Obesity and Metabolism*, vol. 22, no. S1, pp. 3-15, 2020.
- [30] D. Garg, C. Naugler, V. Bhella .et al., "Chronic kidney disease in type 2 diabetes," *Canadian Family Physician*, vol. 64, no. 10, pp. e446, 2018.
- [31] J. Halminen, N. Sattar, A. Rawshani .et al., "Range of Risk Factor Levels, Risk Control, and Temporal Trends for Nephropathy and End-stage Kidney Disease in Patients With Type 1 and Type 2 Diabetes," *Diabetes Care*, vol. 45, no. 10, pp. 2326-2335, 2022.
- [32] T. Hirano, "Abnormal lipoprotein metabolism in diabetic nephropathy," *Clinical and Experimental Nephrology*, vol. 18, no. 2, pp. 206-209, 2014.
- [33] M. A. Aderibigbe, T. O. Obafemi, M. T. Olaleye .et al., "Effects of gender, age and treatment duration on lipid profile and renal function indices in diabetic patients attending a teaching hospital in South-Western Nigeria," *African health sciences*, vol. 18, no. 4, pp. 900-908, 2018.
- [34] Y. Yamamoto, K. Hanai, T. Mori .et al., "Kidney outcomes and all-cause mortality in people with type 2 diabetes exhibiting non-albuminuric kidney insufficiency," *Diabetologia*, vol. 65, no. 1, pp. 234-245, 2022.
- [35] E. A. Christofides and N. Desai, "Optimal Early Diagnosis and Monitoring of Diabetic Kidney Disease in Type 2 Diabetes Mellitus: Addressing the Barriers to Albuminuria Testing," *Journal of Primary Care & Community Health*, vol. 12, pp. 21501327211003683, 2021.
- [36] H. Diniz and J. M. Frazão, "The role of Fibroblast Growth Factor 23 in chronic kidney disease-mineral and bone disorder," *Nefrología (English Edition)*, 10.3265/Nefrologia.pre2013.Jul.12091 vol. 33, no. 6, pp. 835-844, 2013.
- [37] R. G. Erben, "Physiological actions of fibroblast growth factor-23," *Frontiers in endocrinology*, vol. 9, pp. 267, 2018.
- [38] S. S. Beck-Nielsen, Z. Mughal, D. Haffner .et al., "FGF23 and its role in X-linked hypophosphatemia-related morbidity," *Orphanet Journal of Rare Diseases*, vol. 14, no. 1, pp. 58, 2019.

- [39] D. Liu, S. Yu, Y. Zhang .et al., "Fibroblast growth factor 23 predicts incident diabetic kidney disease: A 4.6-year prospective study," *Diabetes, Obesity and Metabolism*, vol. 27, no. 4, pp. 2232-2241, 2025.
- [40] C. E. Kosmas, M. D. Bousvarou, A. Sourlas .et al., "Angiopoietin-like protein 3 (ANGPTL3) inhibitors in the management of refractory hypercholesterolemia," *Clinical Pharmacology: Advance*, vol. 14, pp. 49-59, 2022.
- [41] J. M. Kraaijenhof, T. R. Tromp, N. S. Nurmohamed .et al., "ANGPTL3 (Angiopoietin-Like 3) preferentially resides on High-density lipoprotein in the human circulation, affecting its activity," *Journal of the American Heart Association*, vol. 12, no. 21, pp. e030476, 2023.
- [42] S. M. Azadi, R. Fadaei, R. Omid-Shafaat .et al., "Elevated angiopoietin-like protein 3 serum levels in diabetic nephropathy patients and its association with renal function and lipid profile," *BMC nephrology*, vol. 24, no. 1, pp. 172, 2023.
- [43] Y. Ma, Y. Chen, H. Xu .et al., "The influence of angiopoietin-like protein 3 on macrophages polarization and its effect on the podocyte EMT in diabetic nephropathy," *Frontiers in Immunology*, vol. 14, pp. 1228399, 2023.
- [44] S. S. T. Zadeh, P. P. Toth, M. D. Shapiro .et al., "ANGPTL3 vital role in different kidney diseases. Current knowledge and future perspectives," *Biomedicine Pharmacotherapy*, vol. 188, pp. 118189, 2025.
- [45] H. A. Abdel-Azeez, S. A. M. M. Eldeeb, D. M. Atef .et al., "Fibroblast Growth Factor 21 and 23 as Biomarkers for Early Stage Diabetic Nephropathy in Type 2 Diabetic Patients," *Zagazig University Medical Journal*, vol. 30, no. 6, pp. 2465-2474, 2024.