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RESEARCH

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# The potential of fibroblast growth factor-21 and adiponectin as diagnostic biomarkers for type 2 diabetes mellitus: differential levels in response to treatments

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## Abstract

**Background** Diabetes mellitus (DM) is a global epidemic disease affecting millions each year. Recent studies have suggested novel biomarkers that are linked to DM. This study aimed to measure the levels of fibroblast growth factor-21 (FGF-21) and adiponectin in the blood of patients with type 2 DM and to assess the variations in their levels in response to the type of treatments. The possible correlations with several biochemical parameters and the diagnostic potential of FGF-21 and adiponectin as biomarkers for DM were also investigated. Eighty subjects were classified into control, Type 2 DM patients who were treated with metformin, Type 2 DM patients who were treated with metformin + oral hypoglycemic agents (OHAs), and Type 2 DM patients who were treated with insulin + metformin + OHAs.

**Results** The metformin + OHAs group and the insulin + metformin + OHAs group had higher levels of FGF-21 when compared to the control group. The metformin + OHAs also had significantly higher adiponectin levels when compared to the control or metformin groups. The serum levels of FGF-21 in the diabetic subjects were negatively correlated with LDL, direct bilirubin, albumin, and insulin levels and positively correlated with the duration of DM. However, the serum levels of adiponectin in the diabetic subjects were negatively correlated with weight while positively correlated with potassium levels. Remarkably, FGF-21 and adiponectin were effective biomarkers for diagnosing DM with a specificity of 100% and 90% and sensitivity of 52.3% and 64.5%, respectively.

**Conclusion** These findings suggest that FGF-21 and adiponectin play crucial roles in DM diagnosis and prognosis and that their levels change depending on the treatment type.

**Keywords** Diabetes, Biomarker, FGF, ELISA, Adiponectin

## 1 Background

Diabetes mellitus (DM) is a global epidemic disease characterized by a chronically high blood glucose level [1]. The common types of diabetes include Type 1 DM (T1DM), which is characterized by an auto-immune destruction in β-cells of the pancreas that leads to absolute deficiency of insulin secretion. Therefore, the only common choice of medication is insulin therapy. However, Type 2 DM (T2DM) is distinguished by relative insulin deficiency or resistance. So, the first treatment choice for this type is oral medications such as

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metformin. Other oral hypoglycemic agents (OHAs) are added to the treatment regimen according to the patient's response [1].

Chronic hyperglycemia is linked to persistent organ damage, particularly to the kidneys, eyes, heart, nerves, and blood vessels, causing cardiovascular disease, retinopathy, nephropathy, and neuropathy [2]. For diagnosis of nephropathy complications, serum creatinine, urea, sodium, potassium, and chloride are usually considered biomarkers indicating organ damage when their serum levels are elevated above the normal range [3]. Similarly, for diagnosis of liver damage associated with DM complications, the abnormally high serum levels of liver function tests such as alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), albumin, total, direct, and indirect bilirubin are commonly used to indicate hepatocellular injury [4].

The diagnosis of DM can be made using the plasma glucose criteria, which include the fasting blood glucose (FBG) test, the 2-h blood glucose value obtained after 75 g oral glucose intake named the oral glucose tolerance test, and the hemoglobin A1c (HbA1c) criteria [5]. Homeostatic model assessment for insulin resistance (HOMA-IR) and blood insulin level tests could also be used for disease diagnosis [6]. It is currently uncertain which test provides the most accurate diagnosis of DM. Thus, further biomarker validation is needed for a more precise diagnosis [7].

The fibroblast growth factor-21 (FGF-21), a polypeptide consisting of 209 amino acids, belongs to the endocrine subfamily. It is normally expressed in the liver, pancreas, and adipose tissues [8]. To function appropriately, FGF-21 binds to target cells through specific receptors (FGFRs), primarily FGFR-1 and ß-Klotho [9]. Researchers suggest that FGF-21 can regulate lipid and glucose metabolism, maintain energy balance, and have antioxidant, anti-inflammatory, and cardioprotective functions [9, 10]. The FGF-21 has recently been used as a biochemical marker for DM; its serum level changes dynamically in response to DM types, complications, and treatments. For example, the FGF-21 levels are significantly lower in T1DM and higher in T2DM compared to healthy controls [11, 12]. Its level also increases in the metformin-treated patients [13].

Adiponectin, a polypeptide with 247 amino acids, is an adipocyte secretory product. It is produced and released primarily when the white adipose tissue contains less visceral fat [14]. Adiponectin shows many functions, including induction of apoptosis in cancer cells, as well as anti-inflammatory, antioxidant, and vasodilator properties. It also controls body weight, energy, lipid metabolism, and glucose uptake through increasing glycolysis, inhibiting gluconeogenesis and fatty acid oxidation in the liver, enhancing glucose uptake of skeletal muscles, activating fatty acid oxidation, and promoting insulin sensitivity [15]. Studies have shown that in patients with T1DM, the serum levels of adiponectin are significantly higher than healthy individuals [16]. However, other studies indicated that individuals with T2DM, particularly those who are obese, exhibit lower serum levels of adiponectin [17]. In addition, the serum adiponectin levels changed differently in the patients treated with metformin and thiazolidinediones [18].

The current study aims to measure the levels of FGF-21 and adiponectin in the blood of patients with T2DM to assess the variations in their levels in response to DM treatments. Additionally, the study examines the correlations between these biomarkers and the traditional biomarkers, lipid profile, liver and kidney function tests, total protein, and electrolyte levels in the blood. The study also identifies the diagnostic potential of FGF-21 and adiponectin as biomarkers for diabetes mellitus.

## 2 Methods

### 2.1 Subjects

This cross-sectional study was conducted on Jordanian patients diagnosed with T2DM for at least 6 months who regularly visited the Endocrinology outpatient clinics et al. Karak Governmental Hospital.

The inclusion criteria were set as follows:

- Subjects were aged between 30 and 65 years old.
- Subjects had been treated with oral hypoglycemic drugs for at least 6 months.
- Subjects were diagnosed with T2DM with or without complications.

The following subjects were excluded from the study:

- Prediabetic patients and pregnant women with or without gestational diabetes.
- Patients taking fenofibrate medication.
- Individuals taking oral hypoglycemic agents for conditions other than diabetes, such as polycystic ovary syndrome.
- Subjects with chronic inflammatory diseases like hepatitis, pancreatitis, and certain cancers, especially pancreatic cancer.

A total of 80 male and female subjects were enrolled in the study and divided into four groups:

- Control group: non-diabetic subjects (n = 20).
- Metformin group: subjects with T2DM who were treated with metformin (n = 20).

- Metformin + OHAs group: subjects with T2DM who were treated with metformin and oral hypoglycemic agents (n = 20).
- Insulin + metformin + OHAs: subjects with T2DM who were treated with insulin, metformin, and other oral hypoglycemic agents (n = 20).

The oral hypoglycemic agents used by patients were vildagliptin, glimepiride, and gliclazide.

### 2.2 Patient examination and sample preparation

Patient history, including age, body weight, duration of DM, and BMI ($kg/m^2$) was collected. A physical examination by an endocrinologist was performed to identify any associated physical complications or other diseases. Next, blood samples were withdrawn from each participant after overnight fasting. After allowing the blood to clot at 37 °C for 30 min, the sample was centrifuged at 4000 rpm for 4 min. The resulting supernatant (serum) was separated and subsequently stored at − 80 °C freezer to analyze the biochemical markers.

### 2.3 Biochemical measurements

Utilizing commercial kits and following the manufacturer's instructions, each participant completed the following biochemical analyses:

Haemoglobin-A1c (A1C-3, Roche Diagnostics GmbH, Germany, REF: 05336163190, Lot: 73376801), Fasting blood glucose (GLUC3, Roche Diagnostics GmbH, Germany, REF: 04404483190, LOT: 73288001), Cholesterol (CHOL2, Roche Diagnostics GmbH, Germany, REF: 03039773190, LOT: 73304401), Triglyceride (TRIGL, Roche Diagnostics GmbH, Germany, REF: 20767107322, LOT: 67081601), High-density lipoprotein (HDLC4, Roche Diagnostics GmbH, Germany, REF:07528566190, LOT: 59851601), Low-density lipoprotein (LDLC3, Roche Diagnostics GmbH, Germany, REF:07005717190, LOT: 68807401), Alkaline phosphatase (ALP2, Roche Diagnostics GmbH, Germany, REF: 03333701190, LOT: 71223301), Aspartate aminotransferase (ASTL, Roche Diagnostics GmbH, Germany, REF: 20764949322, LOT: 64235501), Alanine Aminotransferase (ALTL, Roche Diagnostics GmbH, Germany, REF: 20764957322, LOT: 72938001), Albumin (ALB2, Roche Diagnostics GmbH, Germany, REF: 03183688122, LOT: 72570901), Direct bilirubin (BILD2, Roche Diagnostics GmbH, Germany, REF: 05589061190, LOT: 71882501), Total bilirubin (BILT3, Roche Diagnostics GmbH, Germany, REF: 05795397190, LOT: 68886401), Total protein (TP2, Roche Diagnostics GmbH, Germany, REF: 03183734190, LOT: 68769801), Creatinine (CREJ2, Roche Diagnostics GmbH, Germany, REF: 04810716190, LOT: 64726401), Urea (UREAL, Roche Diagnostics GmbH, Germany, REF: 04460715190, LOT: 73051101) Uric acid (UA2, Roche Diagnostics GmbH, Germany, REF: 03183807190, LOT: 71418901), Phosphorous (PHOS2, Roche Diagnostics GmbH, Germany, REF: 03183793122, LOT: 72960101), Calcium (CA2, Roche Diagnostics GmbH, Germany, REF: 05061482190, LOT: 73362201), Sodium, and Potassium (NACL, Roche Diagnostics GmbH, Germany, REF: 04489357190, LOT: 69473301).

### 2.4 Determination of insulin level and HOMA-IR

The insulin levels in the sera samples were measured by the human insulin ELISA Kit (Catalog No: E-EL-H2665), Elabscience, USA. The ELISA kit consists of a microplate, which has been pre-coated with an antibody specific to human insulin. The standards series and samples were added to the wells of the ELISA plate and bound with the specific antibody. Next, a biotin detection antibody specific for human insulin and an avidin-horseradish peroxidase (HRP) conjugate were sequentially added to each well of the microplate. Following the incubation period, the excess substances were rinsed out. The solution of the substrate was added to each well. With the addition of the stop solution, the enzyme–substrate interaction was stopped, resulting in a color change from blue to yellow. At a wavelength of 450 nm, the optical density (OD) was determined using a Multiskan SkyHigh microplate spectrophotometer.

The insulin resistance index (HOMA-IR) was calculated using the homeostasis model assessment (HOMA) according to the following equation [6]:

$$\begin{aligned}\text{HOMA - IR} =& \text{Fasting insulin } (\mu\text{IU/mL}) \\ &\times \text{Fasting blood glucose} \\ &(\text{mg/dL})/405\end{aligned}$$

### 2.5 Measurement of blood FGF-21 and adiponectin concentrations

Human FGF-21 ELISA Kit (Catalog No: EH188RB), ThermoFisher Scientific (Invitrogen), USA was used for the measurement of FGF-21 and human Adiponectin ELISA Kit (Catalog No: ab99968), Abcam, UK was used for the measurement of adiponectin concentration.

In each assay procedure, 100 µL of blank, standards and samples were individually dispensed into their corresponding coated wells. Subsequently, the wells were covered and incubated at room temperature for 2.5 h with gentle shaking. After the completion of incubation, the solution was discarded, and the wells were subjected to four washing steps with a wash buffer. This involved filling each well with 300 µL of wash buffer using a multichannel pipette, followed by complete removal of all liquid. After the last wash, the plate was inverted and

blotted against clean filter papers. Afterward, 100 µL of the biotinylated FGF21 or adiponectin detection antibody was added to each well. The mixture underwent gentle shaking and was incubated for one hour at room temperature. Once again, the solution was discarded, and the wells were washed as described previously. In each well, 100 µL of the Streptavidin-HRP solution was applied, and at room temperature, the plate was incubated for 45 min with gentle shaking. The solution was then discarded, and the wash step was repeated. To develop the color, in each well, 100 µL of tetramethylbenzidine (TMB) substrate was added in the dark and at room temperature; the plate was incubated for 30 min with gentle shaking, initiating a color change as the substrate turned blue. After 30 min, 50 µL of stop solution was added to each well, turning the color from blue to yellow. Finally, the color absorbance in each well was measured spectrophotometrically using a Multiskan SkyHigh Microplate Spectrophotometer at a wavelength of 450 nm.

### 2.6 Statistical analysis

The Statistical Package for the Social Sciences (SPSS) version 28 (IBM Corp., Armonk, NY, USA) was used to input and code the data. Frequencies (number of instances) and relative frequencies (percentages) were used to represent categorical variables. Mean and standard deviation (SD) were used to express quantitative variables. An analysis of variance (ANOVA) with multiple comparisons post hoc test was utilized to compare groups in normally distributed quantitative data. A chi-square test was conducted to compare categorical data, with the exact test being employed when the anticipated frequency was below 5. The Spearman correlation coefficient was used to ascertain the correlation between quantitative variables. The ideal cut-off value of FGF-21 and adiponectin for DM detection has been determined by constructing a receiver operating characteristic (ROC) curve and analyzing the area under the curve. A $p$ value below 0.05 was accepted as statistically significant.

## 3 Results

### 3.1 Demographic and biochemical characteristics of the subjects

The diabetic cases group showed significantly higher age, weight, height, HbA1c, FBG, HOMA-IR, total protein, ALP, and phosphorus levels but significantly lower albumin level, compared to the control group (Table 1).

The diabetic cases group was further divided into three sub-groups according to the type of treatments they received: metformin, metformin + OHAs, and insulin + metformin + OHAs. The results showed no significant differences between the sub-groups regarding sex, age, weight, height, BMI, LDL, total cholesterol, ALT, AST, direct bilirubin, total bilirubin, uric acid, potassium, calcium, phosphorus, or insulin levels. There were no significant differences between the subgroups regarding retinopathy and macrovascular complications. However, there was a significant difference in terms of neuropathy complications in which 90% of the insulin + metformin + OHAs group, 50% of the metformin + OHAs group, and 45% of the metformin group had neuropathy complications (Table 2).

HbA1c levels were significantly higher in all subgroups when compared to the control group. Also, the HbA1c level was significantly different among the sub-groups, where the highest value was measured in the insulin + metformin + OHAs group. The FBG levels were significantly higher in the metformin + OHAs

**Table 1** The demographic and biochemical characteristics of subjects

| Biochemical and clinical characteristics | Control (N = 20) | Diabetic cases (N = 60) | *P* value |
|---|---|---|---|
| Sex (N) | M:9 | M:27 | 0.83 |
| | F:11 | F:33 | |
| Age (year) | 48.5 ± 7.86 | 54.02 ± 8.19 | **0.01*** |
| Weight (kg) | 75 ± 16.71 | 84.96 ± 15.53 | **0.017*** |
| Height (m) | 1.62 ± 0.08 | 1.67 ± 0.09 | **0.031*** |
| BMI | 28.46 ± 5.68 | 30.55 ± 5.74 | 0.162 |
| HbA1c (%) | 5.31 ± 0.48 | 8.57 ± 2.07 | **< 0.001*** |
| FBG (mg/dL) | 86.66 ± 6.58 | 199.95 ± 93.5 | **< 0.001*** |
| LDL (mg/dL) | 126.38 ± 31.45 | 111.36 ± 33.39 | 0.081 |
| TRG (mg/dL) | 159.59 ± 138.59 | 215.18 ± 128.46 | 0.105 |
| Total cholesterol (mg/dL) | 194.2142.73 | 180.05 ± 41.24 | 0.191 |
| HDL (mg/dL) | 44.91 ± 11.4 | 43.82 ± 10.02 | 0.691 |
| ALT (U/L) | 18.37 ± 7.8 | 22.72 ± 16.81 | 0.44 |
| AST (U/L) | 19.28 ± 5.05 | 19.67 ± 10.84 | 0.339 |
| Direct bilirubin (mg/dL) | 0.17 ± 0.06 | 0.18 ± 0.09 | 0.824 |
| Total bilirubin (mg/dL) | 0.47 ± 0.18 | 0.53 ± 0.65 | 0.358 |
| Total protein (g/dL) | 7.27 ± 0.25 | 7.57 ± 0.37 | **0.001*** |
| ALP (U/L) | 79.25 ± 16.06 | 104.15 ± 39.13 | **< 0.001*** |
| Albumin (g/dL) | 4.62 ± 0.25 | 4.4 ± 0.33 | **0.015*** |
| BUN (mg/dL) | 27.96 ± 7.61 | 33.26 ± 14.51 | 0.123 |
| Uric acid (mg/dL) | 5.41 ± 1.57 | 4.88 ± 1.5 | 0.185 |
| Creatinine (mg/dL) | 0.8 ± 0.17 | 0.9 ± 0.41 | 0.271 |
| Calcium (mg/dL) | 9.57 ± 0.5 | 9.79 ± 0.45 | 0.084 |
| Potassium (mmol/L) | 4.37 ± 0.42 | 4.58 ± 0.41 | 0.051 |
| Sodium (mmol/L) | 139.05 ± 2.5 | 138.33 ± 4.33 | 0.496 |
| Phosphorus (mg/dL) | 3.33 ± 0.42 | 3.63 ± 0.61 | **0.049*** |
| HOMA-IR | 2.53 ± 2 | 7.8 ± 10.13 | **0.011*** |
| Insulin (µIU/mL) | 12.07 ± 10.02 | 15.72 ± 13.16 | 0.433 |
| Duration of DM (months) | – | 71.63 ± 50.49 | – |

The data is presented as mean ± SD

*Statistically significant *P* value

**Table 2** Demographic and biochemical characteristics of the sub-groups

| Characteristics | Groups | | | | *P* value |
|---|---|---|---|---|---|
| | Control | Metformin | Metformin + OHAs | Insulin + metformin + OHAs | |
| Sex (N) | M:9 | M:7 | M:7 | M:13 | 0.183 |
| | F:11 | F:13 | F:13 | F:7 | |
| Retinopathy | | | | | |
| Yes | 0 (0%) | 9 (45%) | 8 (40%) | 11 (55%) | 0.626 |
| No | 20 (100%) | 11 (55%) | 12 (60%) | 9 (45%) | |
| Neuropathy | | | | | |
| Yes | 0 (0%) | 9 (45%) | 10 (50%) | 18 (90%) | **0.006*** |
| No | 20 (100%) | 11 (55%) | 10 (50%) | 2 (10%) | |
| Macrovascular complications | | | | | |
| Yes | 0 (0%) | 9 (45%) | 8 (40%) | 7 (35%) | 0.812 |
| No | 20 (100%) | 11 (55%) | 12 (60%) | 13 (65%) | |
| Age (yr) | 48.5 ± 7.86 | 52.8 ± 8.81 | 54.35 ± 7.73 | 54.9 ± 8.27 | 0.065 |
| Weight (Kg) | 75 ± 16.71 | 84.8 ± 10.63 | 86.03 ± 19.61 | 84.06 ± 15.8 | 0.123 |
| Height (m) | 1.62 ± 0.08 | 1.66 ± 0.08 | 1.66 ± 0.09 | 1.69 ± 0.11 | 0.102 |
| BMI | 28.46 ± 5.68 | 30.73 ± 4.27 | 31.29 ± 6.55 | 29.63 ± 6.31 | 0.426 |
| HbA1c (%) | 5.31 ± 0.48 | 7.16 ± 1.02 | 8.53 ± 2.07 | 10.02 ± 1.91 | **< 0.001*** |
| FBG (mg/dL) | 86.66 ± 6.58 | 137.38 ± 42.37 | 184.75 ± 71.81 | 277.71 ± 97.3 | **< 0.001*** |
| LDL-cholesterol (mg/dL) | 126.38 ± 31.45 | 101.89 ± 27.35 | 106.75 ± 36.62 | 125.45 ± 32.34 | 0.052 |
| TRG (mg/dL) | 159.59 ± 138.59 | 204.22 ± 106.62 | 171.71 ± 77.42 | 267.43 ± 168.64 | **0.044*** |
| Total cholesterol (mg/dL) | 194.21 ± 42.73 | 171.33 ± 36.87 | 173.12 ± 39.1 | 195.69 ± 44.79 | 0.113 |
| HDL-cholesterol (mg/dL) | 44.91 ± 11.4 | 46.91 ± 7.75 | 45.96 ± 9.65 | 38.69 ± 10.76 | **0.048*** |
| ALT (U/L) | 18.37 ± 7.8 | 25.62 ± 25.08 | 22.67 ± 12.92 | 19.86 ± 8.04 | 0.869 |
| AST (U/L) | 19.28 ± 5.05 | 22.4 ± 16.49 | 19.46 ± 6.35 | 17.16 ± 6.2 | 0.469 |
| Direct bilirubin (mg/dL) | 0.17 ± 0.06 | 0.19 ± 0.1 | 0.17 ± 0.09 | 0.17 ± 0.09 | 0.569 |
| Total bilirubin (mg/dL) | 0.47 ± 0.18 | 0.53 ± 0.34 | 0.68 ± 1.04 | 0.38 ± 0.22 | 0.11 |
| Total protein (g/dL) | 7.27 ± 0.25 | 7.61 ± 0.36 | 7.63 ± 0.44 | 7.48 ± 0.29 | **0.007*** |
| ALP (U/L) | 79.25 ± 16.06 | 92.85 ± 22.11 | 107.8 ± 52.76 | 111.8 ± 35.67 | **0.015*** |
| Albumin (g/dL) | 4.62 ± 0.25 | 4.49 ± 0.4 | 4.48 ± 0.17 | 4.28 ± 0.31 | **0.014*** |
| BUN (mg/dL) | 27.96 ± 7.61 | 28.49 ± 8.01 | 26.63 ± 5.08 | 44.08 ± 18.87 | **< 0.001*** |
| Uric acid (mg/dL) | 5.41 ± 1.57 | 5.59 ± 1.47 | 4.5 ± 1.17 | 4.59 ± 1.67 | 0.054 |
| Creatinine (mg/dL) | 0.8 ± 0.17 | 0.83 ± 0.23 | 0.76 ± 0.25 | 1.12 ± 0.56 | **0.005*** |
| Sodium (mmol/L) | 139.05 ± 2.5 | 139.89 ± 2.4 | 138.63 ± 4.83 | 136.47 ± 4.79 | **0.048*** |
| Potassium (mmol/L) | 4.37 ± 0.42 | 4.49 ± 0.24 | 4.68 ± 0.44 | 4.57 ± 0.5 | 0.118 |
| Calcium (mg/dL) | 9.57 ± 0.5 | 9.86 ± 0.41 | 9.67 ± 0.45 | 9.83 ± 0.48 | 0.183 |
| Phosphorus (mg/dL) | 3.33 ± 0.42 | 3.59 ± 0.65 | 3.82 ± 0.71 | 3.5 ± 0.41 | 0.067 |
| HOMA-IR | 2.53 ± 2 | 4.36 ± 4.32 | 7.74 ± 8.61 | 16.46 ± 13.47 | **0.01*** |
| Insulin (μlU/mL) | 12.07 ± 10.02 | 12.19 ± 9.18 | 15.5 ± 13.89 | 20.99 ± 16.15 | 0.562 |
| Duration of DM (months) | – | 46.6 ± 26.67 | 78.3 ± 52.79 | 90 ± 57.94 | **0.033*** |

The data is presented as mean ± SD

*Statistically significant *P* value

group and the insulin + metformin + OHAs group when compared to the control group. However, the metformin group and metformin + OHAs had significantly lower FBG levels, when compared to the insulin + metformin + OHAs group (Tables 2, 3).

The analysis of the other biochemical parameters showed that the metformin group and the metformin + OHAs group had significantly higher total protein levels, when compared to the control group. The insulin + metformin + OHAs group had significantly higher levels of TRG, ALP, BUN, creatinine, and

**Table 3** Post hoc pairwise comparison between each two sub-groups

| | Control versus Metformin | Control versus Metformin + OHAs | Control versus Insulin + metformin + OHAs | Metformin versus Metformin + OHAs | Insulin + metformin + OHAs versus Metformin | Insulin + metformin + OHAs versus Metformin + OHAs |
|---|---|---|---|---|---|---|
| HbA1c (%) | 0.001* | < 0.001* | < 0.001* | 0.034* | < 0.001* | 0.016* |
| FBG (mg/dL) | 0.087 | < 0.001* | < 0.001* | 0.133 | < 0.001* | < 0.001* |
| TRG (mg/dL) | 1 | 1 | 0.047* | 1 | 0.736 | 0.134 |
| HDL (mg/dL) | 1 | 1 | 0.332 | 1 | 0.046* | 0.155 |
| Total protein (g/dL) | 0.021* | 0.012* | 0.327 | 1 | 1 | 1 |
| ALP (U/L) | 1 | 0.066 | 0.024* | 1 | 0.527 | 1 |
| Albumin (g/dL) | 1 | 1 | 0.008* | 1 | 0.286 | 0.553 |
| BUN (mg/dL) | 1 | 1 | < 0.001* | 1 | < 0.001* | < 0.001* |
| Creatinine (mg/dL) | 1 | 1 | 0.025* | 1 | 0.055 | 0.009* |
| Sodium (mmol/L) | 1 | 1 | 0.246 | 1 | 0.044* | 0.515 |
| HOMA-IR | 1 | 0.434 | 0.001* | 1 | 0.026* | 0.149 |
| Duration of DM (months) | | | | 0.078 | 0.011* | 0.435 |

*$P$ value < 0.05 is statistically significant

HOMA-IR but lower levels of albumin, compared to the control group. Furthermore, the metformin group had significantly lower levels of BUN and HOMA-IR while having higher HDL and Sodium levels, compared to the insulin + metformin + OHAs group. The insulin + metformin + OHAs showed significantly higher levels of BUN and creatinine when compared to the metformin + OHAs and significantly longer disease duration, compared to the metformin group (Tables 2, 3). The correlations among the biochemical parameters in each individual group are found in supplementary Tables S1-S4.

### 3.2 The expression levels of FGF-21 and adiponectin among the study groups

The study found that the control group had FGF-21 and adiponectin values of 0.86 ng/mL and 14.3 ng/mL, respectively. The diabetic cases group had significantly higher serum levels of FGF-21 by 32.9% and adiponectin by 10.7% compared to the control group, as shown in Fig. 1A. Interestingly, the levels of FGF-21 in the insulin + metformin + OHAs group increased significantly by 37.6% compared to the control group. Also, the metformin + OHAs group showed increased FGF21 levels by 34.1% compared to the control group (Fig. 1B).

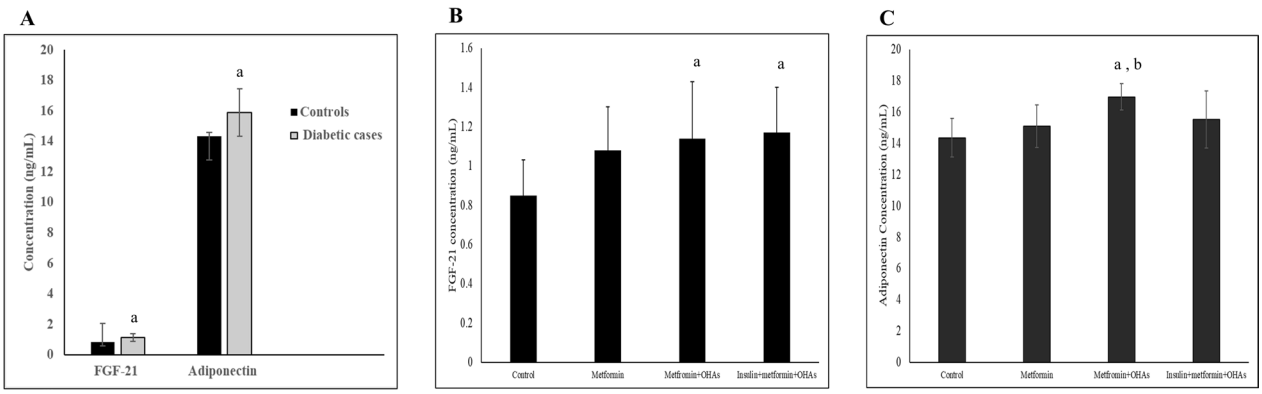



**Fig. 1** The expression levels of FGF-21 and adiponectin among the study groups. **A**: Column figure shows the serum FGF-21 and adiponectin levels between the controls and diabetic cases. **B**: Column figure shows the FGF-21 level among the sub-groups. **C**: Column figure shows the adiponectin level among the sub-groups. The data is presented as mean ± SD. "a" indicates a significant difference compared to the control group. "b" indicates a significant difference compared to the metformin group

The results of adiponectin level showed that the metformin + OHAs group had significantly higher adiponectin level by 18.11% compared to the control group and 12.32% compared to the metformin group (Fig. 1C).

### 3.3 Significant correlations of serum FGF-21 level with the biochemical parameters in the sub-groups

In the control group, the serum level of FGF-21 was positively correlated with age and phosphorus levels. In the metformin group, FGF-21 levels showed a negative correlation with LDL, total cholesterol, total protein, insulin levels, and HOMA-IR but a positive correlation with uric acid levels. In the metformin + OHAs group, FGF-21 concentration was positively correlated with TRG levels but negatively correlated with BUN levels. Finally, in the insulin + metformin + OHAs group, there were significant negative correlations between FGF-21 with ALT, AST, direct bilirubin, and total bilirubin levels. In contrast, positive correlations were found between FGF-21 and the sodium, potassium levels, and duration of DM (Table 4). Supplementary Table S5 shows the correlations of FGF-21 with biochemical parameters in the diabetic cases.

### 3.4 Significant correlations of serum adiponectin level with the biochemical parameters in the sub-groups

In the control group, the serum adiponectin level was negatively correlated with ALP levels. In the metformin group, the serum concentration of adiponectin was positively correlated with HDL and potassium levels. In the metformin + OHAs group, the adiponectin concentration was negatively correlated with weight, BMI, TRG, and uric acid levels while positively correlated with sodium and potassium levels. Finally, in the insulin + metformin + OHAs group, the adiponectin level was positively correlated with total protein levels (Table 5). Supplementary Table S5 shows the correlations of adiponectin with biochemical parameters in the diabetic cases.

### 3.5 FGF-21 and adiponectin as biomarkers for DM diagnosis

To determine the best diagnostic thresholds, a receiver operating characteristic (ROC) curve analysis was conducted; this analysis allowed us to pinpoint the cut-off values that optimize the sensitivity and specificity for distinguishing between the control and diabetic case groups. For FGF-21, the identified optimal threshold was 1.155 ng/mL, and for adiponectin, it was 15.45 ng/mL. The sensitivity and specificity of FGF-21 in detecting the DM cases were 52.3% and 100%, respectively with an AUC of 0.804. The sensitivity and specificity of adiponectin were 64.5% and 90%, respectively with an AUC = 0.766, as shown in Fig. 2.

## 4 Discussion

The current study investigated the levels of two biomarkers, FGF-21 and adiponectin, and identified the differences and correlations in their levels amongst T2DM patients treated with different anti-diabetic drugs. Our study is the first to provide insights into the change in the FGF-21 and adiponectin levels based on the type of DM treatments in Jordanian patients.

Remarkably, studies show that obesity, testosterone hormone, and aging inhibit the secretion of FGF-21 from the liver. At the same time, prolonged fasting, exercise, peroxisome proliferator-activated receptor-α (PPAR-α) activation, ketogenic diet, hyperglycemia, protein restriction, glucose or fructose ingestion, and alcohol stimulate FGF-21 secretion. This activation leads to increased insulin sensitivity, hepatic fatty acid oxidation, and decreased hepatic steatosis. FGF-21 also increases adipose tissue lipoprotein catabolism, reduces renal albuminuria, decreases plasma blood glucose, decreases food intake, increases energy expenditure, and reduces body weight [14].

On the other hand, insulin inhibits the secretion of adiponectin, while factors such as reduction in adipose tissue mass, activation of PPAR-γ, aging, severe insulin resistance, and inflammation stimulate adiponectin secretion. Consequently, several metabolic pathways are affected, including increased insulin sensitivity, fatty acid oxidation in muscles, triglyceride storage in subcutaneous adipose tissue, higher nitric oxide and endothelium-dependent vasodilation levels, and increased HDL plasma levels. Additionally, adiponectin decreases hepatic glucose production, lipogenesis, triglyceride storage, fibrosis, endothelial cells' oxidative stress, and plasma triglyceride levels [14].

The current results demonstrated that the diabetic cases had significantly higher serum levels of FGF-21, especially in the metformin + OHAs and insulin + metformin + OHAs groups compared to the control group. Many studies have shown increased FGF-21 levels in T2DM patients treated with metformin, vildagliptin, glimepiride, and insulin [13, 19, 20]. The increase in FGF-21 level is mainly induced through various mechanisms depending on the medication used. For example, in the liver cells, metformin activates adenosine monophosphate-activated protein kinase (AMPK) and inhibits mitochondrial complex I, leading to the stimulation of the activating transcription factor-4 (ATF4) protein, which increases FGF-21 expression [13]. Vildagliptin drug previously induced FGF-21 expression

**Table 4** Correlations of FGF-21 with the biochemical and clinical characteristics in the study sub-groups

| Biochemical and clinical characteristics | FGF-21 concentration (ng/mL) | | | |
|---|---|---|---|---|
| | Control group | Metformin group | Metformin + OHAs group | Insulin + metformin + OHAs group |
| Age (yr) | | | | |
| r | 0.621 | 0.266 | −0.473 | −0.115 |
| *P* value | **0.013*** | 0.337 | 0.075 | 0.695 |
| Weight (Kg) | | | | |
| r | 0.235 | 0.234 | −0.009 | −0.221 |
| *P* value | 0.398 | 0.401 | 0.975 | 0.449 |
| Height (m) | | | | |
| r | 0.13 | −0.072 | −0.558 | −0.082 |
| *P* value | 0.644 | 0.799 | **0.031*** | 0.781 |
| BMI | | | | |
| r | 0.359 | 0.184 | 0.121 | −0.011 |
| *P* value | 0.188 | 0.512 | 0.666 | 0.97 |
| HbA1c (%) | | | | |
| r | 0.444 | −0.113 | 0.075 | −0.062 |
| *P* value | 0.098 | 0.688 | 0.79 | 0.834 |
| FBG (mg/dL) | | | | |
| r | 0.134 | −0.041 | −0.021 | −0.224 |
| *P* value | 0.634 | 0.884 | 0.94 | 0.441 |
| LDL (mg/dL) | | | | |
| r | 0.266 | −0.596 | −0.107 | −0.422 |
| *P* value | 0.337 | **0.019*** | 0.704 | 0.132 |
| TRG (mg/dL) | | | | |
| r | 0 | −0.118 | 0.661 | 0.475 |
| *P* value | 1 | 0.675 | **0.007*** | 0.086 |
| Total Cholesterol (mg/dL) | | | | |
| r | 0.291 | −0.644 | −0.082 | −0.139 |
| *P* value | 0.292 | **0.01*** | 0.771 | 0.637 |
| HDL (mg/dL) | | | | |
| r | 0.317 | 0.193 | −0.22 | −0.255 |
| *P* value | 0.27 | 0.49 | 0.45 | 0.379 |
| ALT (U/L) | | | | |
| r | 0.241 | 0.331 | 0.171 | −0.631 |
| *P* value | 0.386 | 0.228 | 0.541 | **0.015*** |
| AST (U/L) | | | | |
| r | 0.009 | 0.15 | 0.474 | −0.601 |
| *P* value | 0.975 | 0.593 | 0.075 | **0.023*** |
| Direct Bilirubin (mg/dL) | | | | |
| r | −0.022 | −0.145 | −0.19 | −0.748 |
| *P* value | 0.937 | 0.606 | 0.498 | **0.002*** |
| Total bilirubin (mg/dL) | | | | |
| r | 0.057 | −0.004 | 0.029 | −0.674 |
| *P* value | 0.84 | 0.988 | 0.919 | **0.008*** |
| Total protein (g/dL) | | | | |
| r | 0.329 | −0.678 | −0.027 | 0.128 |
| *P* value | 0.251 | **0.011*** | 0.928 | 0.677 |
| ALP (U/L) | | | | |
| r | 0.089 | −0.358 | 0.114 | −0.21 |

**Table 4** (continued)

| Biochemical and clinical characteristics | FGF-21 concentration (ng/mL) | | | |
|---|---|---|---|---|
| | Control group | Metformin group | Metformin + OHAs group | Insulin + metformin + OHAs group |
| *P* value | 0.753 | 0.19 | 0.685 | 0.47 |
| Albumin (g/dL) | | | | |
| r | −0.492 | −0.36 | −0.392 | −0.377 |
| *P* value | 0.104 | 0.227 | 0.297 | 0.204 |
| BUN (mg/dL) | | | | |
| r | 0.147 | 0.416 | −0.565 | 0.396 |
| *P* value | 0.602 | 0.139 | **0.035*** | 0.161 |
| Uric acid (mg/dL) | | | | |
| r | 0.099 | 0.851 | −0.182 | 0.298 |
| *P* value | 0.726 | **< 0.001*** | 0.516 | 0.347 |
| Creatinine (mg/dL) | | | | |
| r | 0.227 | 0.262 | −0.473 | 0.205 |
| *P* value | 0.415 | 0.345 | 0.088 | 0.483 |
| Sodium (mmol/L) | | | | |
| r | 0.213 | −0.397 | 0.296 | 0.604 |
| *P* value | 0.465 | 0.16 | 0.285 | **0.029*** |
| Potassium (mmol/L) | | | | |
| r | 0.526 | 0.518 | −0.304 | 0.702 |
| *P* value | 0.053 | 0.058 | 0.271 | **0.008*** |
| Calcium (mg/dL) | | | | |
| r | 0.225 | −0.392 | 0.143 | 0.261 |
| *P* value | 0.44 | 0.166 | 0.626 | 0.368 |
| Phosphorus (mg/dL) | | | | |
| r | 0.542 | −0.111 | 0.15 | 0.497 |
| *P* value | **0.045*** | 0.694 | 0.594 | 0.07 |
| HOMA-IR | | | | |
| r | 0.536 | −0.677 | −0.183 | −0.643 |
| *P* value | 0.059 | **0.016*** | 0.637 | 0.086 |
| Insulin (μIU/Ml) | | | | |
| r | 0.512 | −0.681 | −0.15 | −0.69 |
| *P* value | 0.074 | **0.015*** | 0.7 | 0.058 |
| Duration of DM (months) | | | | |
| r | | 0.298 | −0.237 | 0.824 |
| *P* value | | 0.281 | 0.396 | **< 0.001*** |

*Statistically significant *P* value

r: correlation coefficient

through sirtuin-1 signaling in cardiac fibroblasts [19]. A study demonstrated that subcutaneously administrated basal and bolus insulin injections significantly increased FGF-21 levels. This effect suggests that insulin can preserve FGF-21 secretion in diabetic patients, while pancreatic β-cells failure reduces the baseline levels of FGF-21 [21]. In addition, a recent study demonstrated that combination therapy of metformin, glipizide, and basal insulin glargine increased FGF-21 levels but not to significant values compared to the pretreatment baseline levels [22]. Not enough data are available to reveal the association between sulfonylurea drugs, especially glimepiride or gliclazide, and the serum levels of FGF-21.

Similarly, the diabetic cases, especially the metformin + OHAs group, showed significantly higher serum adiponectin levels, compared to the controls or the metformin group. Many studies have demonstrated that adiponectin concentrations in T2DM patients treated with metformin for more than 6 months, glimepiride,

**Table 5** Correlations of adiponectin with the biochemical and clinical characteristics in the study sub-groups

| Biochemical and clinical characteristics | Adiponectin concentration (ng/mL) | | | |
|---|---|---|---|---|
| | Control group | Metformin group | Metformin + OHAs group | Insulin + metformin + OHAs group |
| Age (yr) | | | | |
| r | −0.030 | −0.396 | 0.508 | −0.287 |
| *P* value | 0.934 | 0.257 | 0.111 | 0.422 |
| Weight (kg) | | | | |
| r | 0.434 | −0.301 | −0.850 | −0.140– |
| *P* value | 0.21 | 0.399 | **< 0.001*** | 0.7 |
| Height (m) | | | | |
| r | 0.697 | −0.110 | 0.064 | 0.006 |
| *P* value | **0.025*** | 0.763 | 0.852 | 0.987 |
| BMI | | | | |
| r | 0.079 | −0.340 | −0.645 | −0.249 |
| *P* value | 0.829 | 0.336 | **0.032*** | 0.487 |
| HbA1c (%) | | | | |
| r | 0.396 | −0.117 | 0.1 | 0.28 |
| *P* value | 0.257 | 0.747 | 0.77 | 0.432 |
| FBG (mg/dL) | | | | |
| r | 0.285 | −0.273 | 0.2 | −0.055- |
| *P* value | 0.425 | 0.446 | 0.555 | 0.881 |
| LDL (mg/dL) | | | | |
| r | 0.188 | −0.236 | −0.319 | −0.370- |
| *P* value | 0.603 | 0.511 | 0.339 | 0.293 |
| TRG (mg/dL) | | | | |
| r | −0.091 | −0.195 | −0.745 | 0.552 |
| *P* value | 0.803 | 0.59 | **0.008*** | 0.098 |
| Total cholesterol (mg/dL) | | | | |
| r | 0.055 | −0.164 | −0.400 | −0.091 |
| *P* value | 0.881 | 0.651 | 0.223 | 0.803 |
| HDL (mg/dL) | | | | |
| r | −0.200 | 0.784 | −0.305 | −0.394 |
| *P* value | 0.58 | **0.007*** | 0.361 | 0.26 |
| ALT (U/L) | | | | |
| r | −0.285 | 0.152 | −0.473 | −0.139- |
| *P* value | 0.425 | 0.676 | 0.142 | 0.701 |
| AST (U/L) | | | | |
| r | −0.236 | −0.200 | 0 | −0.333- |
| *P* value | 0.511 | 0.58 | 1 | 0.347 |
| Direct bilirubin (mg/dL) | | | | |
| r | 0.03 | −0.261 | −0.556 | −0.152- |
| *P* value | 0.934 | 0.467 | 0.076 | 0.676 |
| Total bilirubin (mg/dL) | | | | |
| r | 0.03 | −0.600 | −0.200 | −0.201 |
| *P* value | 0.934 | 0.088 | 0.555 | 0.577 |
| Total protein (g/dL) | | | | |
| r | 0.183 | 0.048 | −0.271 | 0.722 |
| *P* value | 0.614 | 0.909 | 0.449 | **0.018*** |
| ALP (U/L) | | | | |
| r | −0.699 | −0.188 | −0.164- | −0.042- |

**Table 5** (continued)

| Biochemical and clinical characteristics | Adiponectin concentration (ng/mL) | | | |
|---|---|---|---|---|
| | Control group | Metformin group | Metformin + OHAs group | Insulin + metformin + OHAs group |
| *P* value | **0.024*** | 0.603 | 0.631 | 0.907 |
| Albumin (g/dL) | | | | |
| r | −0.243 | 0.464 | 0.47 | 0.024 |
| *P* value | 0.529 | 0.294 | 0.24 | 0.947 |
| BUN (mg/dL) | | | | |
| r | 0.248 | −0.633 | 0.018 | −0.115- |
| *P* value | 0.489 | 0.067 | 0.96 | 0.751 |
| Uric acid (mg/dL) | | | | |
| r | −0.116 | −0.524 | −0.736- | 0.329 |
| *P* value | 0.751 | 0.183 | **0.01*** | 0.353 |
| Creatinine (mg/dL) | | | | |
| r | 0.285 | −0.150 | 0.1 | −0.539- |
| *P* value | 0.425 | 0.7 | 0.769 | 0.108 |
| Sodium (mmol/L) | | | | |
| r | −0.331 | −0.346 | 0.695 | 0.146 |
| *P* value | 0.35 | 0.362 | **0.026*** | 0.687 |
| Potassium (mmol/L) | | | | |
| r | 0.292 | 0.733 | 0.661 | 0.212 |
| *P* value | 0.413 | **0.025*** | **0.038*** | 0.556 |
| Calcium (mg/dL) | | | | |
| r | −0.043 | 0.1 | −0.055 | 0.042 |
| *P* value | 0.907 | 0.798 | 0.881 | 0.907 |
| Phosphorus (mg/dL) | | | | |
| r | −0.079 | 0.018 | 0.009 | 0.188 |
| *P* value | 0.829 | 0.96 | 0.979 | 0.603 |
| HOMA-IR | | | | |
| r | 0.217 | −0.491 | −0.167 | −0.071 |
| *P* value | 0.576 | 0.15 | 0.668 | 0.879 |
| Insulin (µlU/Ml) | | | | |
| r | 0.25 | −0.455 | −0.317 | 0.107 |
| *P* value | 0.516 | 0.187 | 0.406 | 0.819 |
| Duration of DM (months) | | | | |
| r | | −0.117 | 0.366 | 0.55 |
| *P* value | | 0.747 | 0.268 | 0.099 |

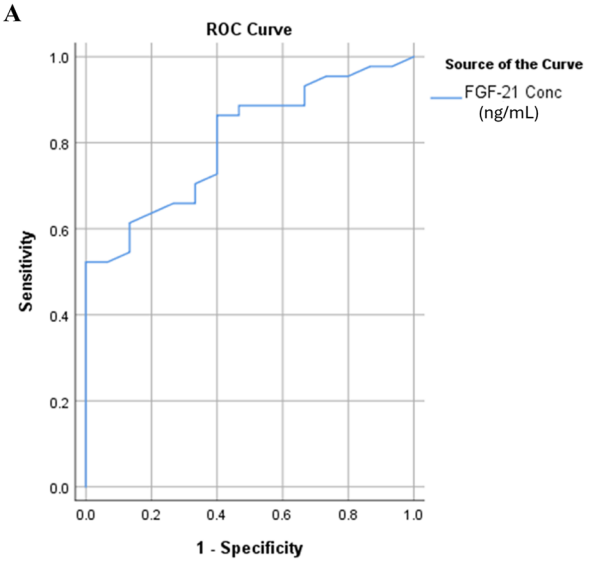
*Statistically significant *P* value

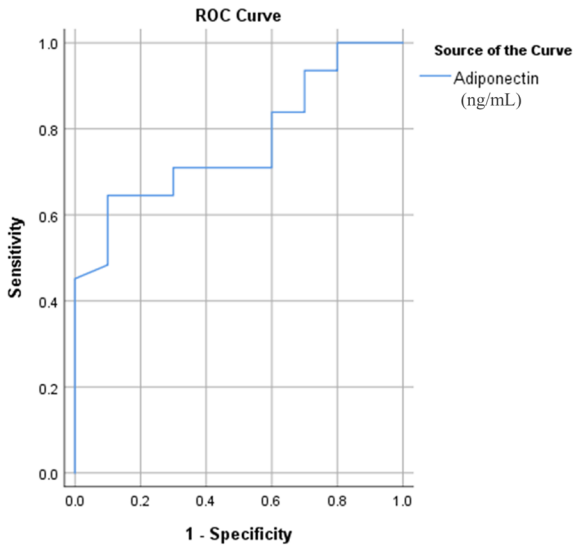
r: correlation coefficient

vildagliptin, and insulin were significantly increased [18, 23, 24]. Metformin could increase in vitro and in vivo adiponectin expression, mainly from the adipose tissue, through AMPK activation [18]. Vildagliptin may promote adiponectin secretion by increasing the synthesis of active glucagon-like peptide 1 (GLP-1) [25]. Furthermore, adiponectin levels were considerably higher in the patients treated with vildagliptin plus metformin than in those treated with glimepiride plus metformin [25] or high doses of metformin alone [24]. Another study demonstrated that glimepiride increases the serum levels of adiponectin after 3 months of treatment [26]. Not enough data has shown the effect of gliclazide on the serum adiponectin concentrations.

In the control group, the serum level of FGF-21 was positively correlated with age. A study on a healthy population suggests that FGF-21 levels increase with age and independently with body composition [27]. In the metformin group, a positive correlation between FGF-21 and uric acid levels was noticed. Another study demonstrated

**A**




**C**

| | Area Under the Curve | P value | 95% Confidence Interval | | Cut off | Sensitivity % | Specificity % |
|---|---|---|---|---|---|---|---|
| | | | Lower Bound | Upper Bound | | | |
| FGF-21 (ng/mL) | 0.804 | < 0.001* | 0.69 | 0.918 | 1.155 | 52.3 | 100 |
| Adiponectin (ng/mL) | 0.766 | < 0.001* | 0.617 | 0.915 | 15.45 | 64.5 | 90 |

**Fig. 2** The sensitivity and specificity of FGF-21 and adiponectin for DM detection. **A** The ROC of the FGF21 biomarker. **B** The ROC of the adiponectin biomarker. **C** A table shows the area under the curve, 95% confidence intervals, *P* values, sensitivity %, and specificity % of FGF21 and adiponectin biomarkers for detecting DM cases

a consistent correlation between uric acid and FGF-21 in individuals with and without metabolic abnormalities [28]. Metformin might offer a protective effect on lipid levels by enhancing the expression of FGF-21, which may explain the observed negative correlation between FGF-21 and LDL or total cholesterol levels [20]. In the metformin + OHAs group, we found that serum FGF-21 concentration was positively correlated with TRG levels. A previous study found that in diabetic patients treated with metformin, pioglitazone, and exenatide (a triple therapy), serum FGF-21 levels were positively correlated with serum TRG levels, compatible with our results [22].

In the control group, the serum adiponectin level was negatively correlated with ALP level, which is a biomarker of liver disease. Other studies found that reduced serum adiponectin levels could be a significant biomarker for detecting non-alcoholic fatty liver disease in healthy individuals. This consistent finding could be attributed to the effect of adiponectin on regulating the inflammation processes, thus maintaining normal liver function [29, 30]. For patients in the metformin group, adiponectin concentration was positively correlated with HDL and potassium levels, which was compatible with a previous study [30]. Metformin improved the lipid profile in patients with T2DM by increasing HDL levels [31]. In the metformin + OHAs group, adiponectin level was negatively correlated with weight, BMI, TRG, and uric acid levels. The same correlations were observed in a study on T2DM patients treated with OHAs [32]. An earlier study has demonstrated that adiponectin levels increase with weight loss [30]. Furthermore, our study found that adiponectin levels were positively correlated with Sodium and Potassium levels. A previous study revealed that adiponectin was negatively linked with urinary sodium excretion [33]. This decrease in sodium excretion may be explained by the role of adiponectin in enhancing the renal activity and expression of G-protein coupled receptor kinase-4 [34].

This study revealed that FGF-21 and adiponectin are highly effective at differentiating between individuals with and without DM. In accordance with our results, a study demonstrated that FGF-21 is a highly effective biomarker for detecting DM compared to other biomarkers [35]. Similar results observed that adiponectin

levels were considerably higher in T2DM participants with good blood glucose control than in individuals with poor control, indicating that adiponectin may be a helpful biomarker for T2DM monitoring [35]. In this study, high levels of FGF-21 were observed in the insulin + metformin + OHAs group, which comprised the highest percentage of diabetic neuropathy (90% of cases). Therefore, FGF-21 can be used as a prognostic biomarker for this complication. FGF21 levels were previously found to be higher in patients who developed microvascular disease, nephropathy, or neuropathy compared to those who did not experience any of these conditions [36].

The clinical implications of FGF-21 and adiponectin biomarkers in patients with diabetes were previously highlighted. According to a human-based study, the administration of FGF-21 analog named LY2405319 to obese patients with T2DM for 28 days resulted in the normalization of FBG level and a reduction in body weight [37]. Furthermore, an effective FGF-21 delivery nanocarrier was established using ultrasound-assisted nanobubbles to improve the cardiac delivery of FGF-21 and prevent diabetic cardiomyopathy [38]. On the other hand, adiponectin has been extensively proposed as a marker for metabolic dysregulation and therapy effectiveness [39]. Several studies have found a positive correlation between serum adiponectin concentration and the severity of diabetic nephropathy and retinopathy, suggesting that adiponectin can serve as a biomarker for diabetes complications [40–42]. Therefore, FGF-21 and adiponectin biomarkers may be used to detect disease complications and tailor personalized treatment to potentially improve patient outcomes.

The current study suffered from demographic limitations and a small number of subjects whereas a larger number of subjects from diverse populations should have been examined to firmly generalize the findings. Also, other confounding variables, such as dietary habits, exercise, and other medications may have influenced the biomarkers measured in the current study. Nevertheless, our findings provide preliminary insights that can guide more comprehensive future research on this research topic.

## 5 Conclusion

The study found that serum FGF-21 and adiponectin levels were significantly increased in T2DM patients and that their levels changed in response to the type of treatment, particularly in patients receiving metformin plus oral hypoglycemic agents. The levels of FGF21 and adiponectin were correlated with several biochemical parameters and disease duration. Remarkably, FGF21 and adiponectin can serve as significant diagnostic biomarkers for T2DM with high specificity and sensitivity.

**Abbreviations**

| | |
|---|---|
| ALP | Alkaline phosphatase |
| ALT | Alanine transaminase |
| AST | Aspartate transaminase |
| BMI | Body mass index |
| BUN | Blood urea nitrogen |
| DM | Diabetes mellitus |
| FBG | Fasting blood glucose |
| FGF-21 | Fibroblast growth factor-21 |
| HbA1c | Glycosylated hemoglobin |
| HDL | High-density lipoprotein |
| HOMA-IR | Homeostasis model assessment-insulin resistance |
| LDL | Low-density lipoprotein |
| OHAs | Oral hypoglycemic agents |
| r | Correlation coefficient. |
| TRG | Triglycerides |

## Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s43088-024-00571-0.

Additional file1 (PDF 709 kb)

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**Author contributions**
MNA-Q: data curation; investigation; methodology; funding acquisition; writing—original draft. RD: investigation; methodology; supervision. RMH: conceptualization; project administration; supervision; validation; visualization; writing—review and editing.

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**Availability of data and materials**
All data analyzed during this study are included in this published article and its supplementary information files.

## Declarations

**Ethics approval and consent to participate**
The experimental procedures of the current study were reviewed and approved by the Ethics Review Committee of the Faculty of Pharmacy at Mutah University (approval no: 2023-5) and the Research Ethics Committee of the Jordanian Ministry of Health (approval no: MOH/REC/2024/194). All subjects provided written informed consent before participation in the study.

**Consent for publication**
Not applicable.

**Competing interests**
The authors declare that they have no competing interests.

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