

RESEARCH ARTICLE

Evaluation of Growth-differentiation Factor-15 Level and few Physiological Parameters Patients with Diabetes Mellitus Type 2

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ABSTRACT

The study included 90 subjects and is divided into two groups: The first group is the patient group, which consists of 45 patients with diabetes mellitus type two; 45 patients with diabetes mellitus type two were identified by specialized diabetes doctors by samples collection in outpatients clinic at Diabetes and Endocrine Center in Marjan Medical City in Hilla city, Babylon province from 1 November 2022 till 31 January 2023. The blood samples of this study were obtained from patients and controlled through drug 5 mL of blood by using medical sterile syringes from a brachial vein and placed in a gel tube and ethylenediaminetetraacetic acid (EDTA) tube. Then the gel tube was placed at room temperature for 30 minutes to coagulate the blood, and samples were centrifuged (3000 rpm/min) for 5 minutes to separate the serum from other components of the blood. The serum was withdrawn by micropipette and then placed in the eppendorf tubes in two repeaters and kept frozen at -20°C for the determination of growth differentiation factor 15 (GDF 15), lipid profile and fasting blood glucose. The whole blood in the EDTA tube to determine HbA1c. The mean fasting blood sugar and hemoglobin A1c of diabetes patients is significantly higher at (p -value < 0.001) than that of controls. Diabetes patients had a significantly higher of serum lipid profile than the controls. Therefore, the mean of cholesterol, triglyceride, and low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) was highly significant (p < 0.001). The mean concentration of GDF-15 of patient groups was significantly increased than those matched groups of healthy control at (p < 0.001). In addition, the results also showed a highly significant increase (p < 0.001) in the mean of GDF-15 for the patient's males and females in comparison with the control's males and females. The correlation results indicated a statistically significant positive correlation in diabetic group between GDF-15 vs. FBS ($r = 0.572$, $p = < 0.001$) and HbA1c ($r = 0.576$, $p = < 0.001$). Positive non-significant correlations were demonstrated between GDF-15 vs. cholesterol ($r = 0.131$, $p = > 0.05$), triglyceride (TG) ($r = 0.167$, $p = > 0.05$), LDL ($r = 0.38$, $p = > 0.05$) and VLDL ($r = 0.136$, $p = > 0.05$). Negative non-significant correlation was between GDF-15 and high-density lipoprotein (HDL) ($r = 0.137$, $p = > 0.05$).

Keywords: GDF-15, Type 2 diabetes mellitus, Adipokines.

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INTRODUCTION

Reduced release of insulin by beta-cells in the pancreas and decreased tissues' sensitivity to insulin combine to generate, type 2 diabetes mellitus (T2DM), one of the most prevalent metabolic illnesses worldwide.¹ According to the World Health Organization (WHO), diabetes mellitus is a persistent metabolic disorder marked by elevated blood sugar levels. Over time, organs affected by chronic conditions include the kidneys, eyes, blood vessels, heart, and nerves. More than 90% of diabetes cases are caused by T2DM and is characterized by insulin resistance (IR) in tissue, inadequate balancing insulin secretory response, and inadequate production of insulin by pancreatic islet cells.² In 1997, researchers identified growth

differentiation factor-15 (GDF-15) as a distant relative of the transforming growth factor (TGF) superfamily. Several research groups identified this cytokine under various experimental settings. GDF-15 is hence also known as placental transforming growth factors beta, macrophage inhibitory cytokines-1 (MIC-1), and non-steroidal anti-inflammatory drugs (NSAIDs) activated gene-1 (NAG-1).³ MIC-1, also known as MIC-1 or GDF-15, is a widely expressed stress-inducible cytokine.⁴ T2DM patients have higher circulating GDF-15 concentrations,⁵ and are each reported to be linked to obesity,⁶ liver disease severity,⁷ cardio vascular diseases (CVDs),⁸ and chronic kidney disease (CKD). In addition to genetic variables, environmental factors can have an impact

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on GDF-15 levels. One investigation found that In contrast to other markers of mortality risk, such as age, body mass index (BMI), smoking history, IL-6, CRP, and telomere length, GDF-15 levels predict all-cause death in the general population in a novel and efficient manner.⁹ GDF-15 functions as an adipokine similar to adiponectin and leptin,¹⁰ and thus has also been termed as cardiokine.¹¹ Adipokines control food intake and body weight, improve insulin sensitivity, control lipid and glucose metabolism, and defend adipose tissue against ongoing inflammation.¹² GDF-15, according to Macia *et al.*, improves sensitivity to glucose in both regular and obesity-promoting diets by reducing calorie intake, weight, and obesity.¹³

MATERIALS AND METHODS

Subject of Study

The 90 study participants were split into two groups. The patient group comprised the first group, which comprises 45 people with type 2 diabetes. A diabetes specialist examined samples from November 1, 2022, to December 31, 2023, to identify the 45 individuals with type 2 diabetes in the outpatient clinic at the Marjan Medical City in Hilla city, Babylon Province’s Diabetes and Endocrine Center.

Exclusion Criteria

- Patients suffer from other autoimmune and chronic diseases such as rheumatoid arthritis, malignancies, severe liver disease and kidney disease.
- The normal control group should not be diabetics without a family history.
- All patients not clinically classified as type 2 diabetic were excluded.

Sample Collection

The blood samples for this investigation were drawn from patients and controls using medical sterile syringes to draw 5 mL of blood from the brachial vein, which was then deposited in a gel tube and an EDTA tube. The serum was separated from the other blood components by centrifuging the samples for 5 minutes at a speed of 3000 rpm after the blood had been permitted to clot for 30 minutes at standard room temperature. For the measurement of GDF 15, lipid profile, and fasting blood glucose, the serum was removed using a micropipette, deposited in two repeaters of Eppendorf tubes, and kept

frozen at -20°C. Using whole blood in an EDTA tube, one can calculate HbA1c.

Biochemical assay

Fasting blood sugar, HbA1c, cholesterol, triglycerides (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very low-density lipoproteins (VLDL) biochemistry assays were carried out utilizing the protocol of a fully automated biochemistry analyzer (Beckman Coulter).

Immunological Assays

Growth differentiation factor 15 was calculated using an ELISA kit, a biochemical tool utilized in a study conducted by the US Business Elabscience.

Statistical Analysis

To enter, maintain, and analyze data from study participants, type 2 diabetes mellitus patients, and controls, Microsoft Windows version 25 of IBM’s Statistical Package for Social Sciences (SPSS), IBM, US, 2017 was used. Before beginning the study, all variables were reviewed for mistakes or inconsistencies. The statistical normality of the distribution of continuous variables such as cholesterol, TG, HDL, LDL, VLDL, FBS, HbA1c, and GDF-15 was examined using histograms and normal distribution curves. and it appeared that they all followed the statistical normal distribution. The T-independent test was utilized to compare the average levels of GDF-15 and the study parameter with the control. A *p-value* of 0.05 or below is considered a significant significance level. Final data and conclusions were presented using the Microsoft Word 2010 for Windows application in the appropriate tables and/or figures.

RESULTS AND DISCUSSION

Comparing the Serum Lipid Profiles of Diabetes Patients with the Control Group

Diabetes patients had blood lipid profiles significantly greater than the controls. Consequently, as seen in Table 1, the mean of triglycerides, low-density, and very low-density lipoproteins was extremely significant, with a *p-value* of 0.001. The findings revealed that, whereas serum levels of total cholesterol, TG, LDL, and VLDL are statistically significantly higher in type 2 diabetes mellitus patients, the level of HDL in patients was statistically lower in contrast to controls. Patients with diabetes

Table 1: Serum lipid profile levels in diabetes patients in comparison with control group

Variables	Groups		Statistical test	p-value
	Diabetic patients No. (45) Mean ± SD*	Healthy control No. (45) Mean ± SD		
Cholesterol (mg/dl)	48.3 ± 189.7	30.1 ± 166.6	t:2.7	<0.001 H.S
Triglyceride (mg/dl)	117.03 ± 181.3	50.5 ± 120.1	t:3.2	<0.001 H.S
HDL (mg/dl)	10.6 ± 38.8	7.6 ± 45.08	t:3.1	<0.001 H.S
LDL (mg/dl)	38.1 ± 115.2	35.4 ± 100.8	t:1.8	<0.001 H.S
VLDL (mg/dl)	24.1 ± 34.5	10 ± 24.02	t:2.6	<0.001 H.S

*standard deviation; t: independent t-test; HDL: high density lipoprotein; LDL: low density lipoprotein; VLDL: very low density lipoprotein.

Table 2: Level of FBS and HbA1c among diabetes patients in comparison with control

Parameter	Subject	Mean ± SD*	p-value
Fasting blood sugar (mg/dl)	Diabetic patients No. (45)	55.3 ± 252.5	<0.001 H.S
	Healthy control No. (45)	9.8 ± 98.4	
HbA1c (%)	Diabetic patients No. (45)	1.2 ± 8.6	<0.001 H.S
	Healthy control No. (45)	0.54 ± 5.07	

*standard deviation; HbA1c: Hemoglobin A1c; significance at p<0.05.

generally have abnormal amounts of lipids and lipoprotein due to insulin’s decreased ability to activate certain metabolic enzymes. Normal serum insulin levels have been connected to the start of atherosclerosis problems in persons with diabetes mellitus, while abnormal serum insulin levels have been linked to abnormal insulin sensitivity, glucose tolerance, and blood lipoprotein concentrations.¹⁴ Due to the close connection between the metabolism of carbohydrates and lipids, there are numerous factors that can affect blood lipid levels in people with diabetes mellitus. As a result, any flaw in lipid metabolism also leads to flaws in sugar metabolism. Insulin resistance has a good prognostic value for type 2 diabetes because it works as a major defect for future development when combined with hyperinsulinemia.¹⁵ Numerous studies have shown that insulin influences the liver’s ability to produce apolipoprotein and controls the enzymatic activity of lipoprotein lipase and cholesterol ester transport protein, resulting in lipid and lipoprotein metabolic pathway type 2 diabetic mellitus anomalies.¹⁶

Levels of Fasting Blood Sugar (mg/dl) and Hemoglobin A1c (%) among Diabetes Mellitus Type 2 in Comparison with Control

The mean fasting blood sugar and hemoglobinA1c of diabetes patients are significantly greater than those of controls, as indicated in Table 2 (p-value 0.001). The study’s findings revealed that, as compared to controls, type 2 diabetes patients’ blood glucose levels dramatically rose. This condition, which often appears around the age of 40, is hypothesized to include flimsy cells, insufficient insulin synthesis and/or activity, and an increase in insulin resistance as potential reasons. These findings are consistent with the research described by,¹⁷ who discovered that people with type 2 diabetes had much greater serum glucose levels than normal, healthy controls.

Serum Growth Differentiation Factor 15 (pg/mL) Concentration in Diabetes Patients and Healthy Control Groups

The mean concentration of GDF-15 in patient groups was significantly higher than that in matched groups of healthy controls, as shown in Table 3 and Figure 1 (p0.001). In a nested× case-control research with 552 people,¹⁸ found that type 2 diabetes patients had considerably higher serum GDF-15 levels than healthy controls and discovered that GDF-15 was positively related to body weight in their study of 54 obese adults (with or without diabetes). Furthermore, when compared to the control’s male and female GDF-15 means, the patient’s male and female GDF-15 means increased significantly (p0.001). This result is consistent with findings from.¹⁹⁻²²

Greater serum GDF-15 levels were found in obese patients with type 2 diabetes compared to obese patients without type 2 diabetes. According to a proteomics investigation, problems from both microvascular and macrovascular diseases were substantially connected with type 2 diabetes patients’ high blood GDF-15 levels.²³ In multiple human studies, it was discovered that the serum level of GDF-15 was associated with body mass index, obesity, and blood sugar. For instance, obese patients’ blood levels of GDF-15 were 344 to 626 ng/mL compared to healthy participants’ levels of 275 to 11 ng/mL. In a cohort research with 118 obese patients and 30 healthy controls, it was discovered that the serum level of GDF-15 was higher in obese patients than in controls.²⁴ Within the first 7 days of experimental streptozotocin-induced× diabetes mellitus in mice, the serum level of GDF-15 rose²⁵ GDF-15 serum levels were higher in patients with type 2 diabetes mellitus and impaired fasting glucose and were correlated with insulin resistance, BMI, and age, 75 people with type 2 diabetes and 29 people with impaired fasting glucose participated

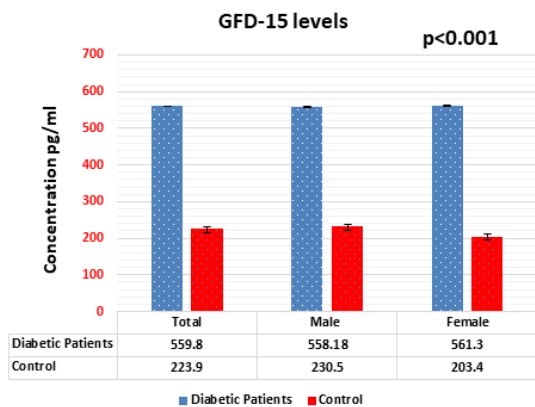


Figure 1: Serum GDF-15 among diabetes patients and control

Table 3: Serum GDF-15 (pg/mL) concentration in diabetes patients and healthy control groups

Parameter		Diabetic patients No. (45) Mean ± SD	Healthy control No. (45) Mean ± SD*	p-value
GDF 15 (pg/mL)	Total mean	148.2 ± 559.8	62.1 ± 223.9	<0.001
	Male	265.4 ± 558.18	59.7 ± 230.5	<0.001
	Female	237.6 ± 561.3	67.7 ± 203.4	<0.001

Significant at p < 0.001. Standard deviation; two independent t-test;*

Table 4: Correlation between study parameter

Parameters	Correlation coefficient R	p-value
GFD15 vs. FBS	0.572	<0.001
GFD15 vs. HbA1c	0.576	<0.001
GFD15 vs. Cholesterol	0.131	>0.05
GFD15 vs. TG	0.167	>0.05
GFD15 vs. HDL	-0.137	>0.05
GFD15 vs. LDL	0.83	>0.05
VLDL vs. GFD15	0.136	>0.05

TG ($r = 0.167$, $p = >0.05$), LDL ($r = 0.38$, $p = >0.05$) and VLDL ($r = 0.136$, $p = >0.05$).

in a case-controlled study compared to 137 healthy control volunteers. Plasma GDF-15 levels and insulin resistance were positively correlated, according to (4) research. The correlation analysis revealed a statistically significant positive association between GDF-15 Vs. FBS and HbA1c in the diabetic group ($r = 0.572$, $p = 0.001$) and GDF-15 Vs. FBS and HbA1c in the diabetic group ($r = 0.576$, $p = 0.001$). GDF-15 and cholesterol showed positive non-significant associations ($r = 0.131$, $p > 0.05$),

Table 4 shows a weak, negative non-significant connection between GDF-15 and HDL ($r = 0.137$, $p > 0.05$).

CONCLUSION

Compared to healthy controls, the GDF-15 levels in people with type 2 diabetes mellitus were considerably higher. Additionally, elevated lipid profile levels were linked to higher GDF-15 levels, indicating that GDF-15 is crucial in the prevention of hyperlipidemia.

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