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Evaluation of Correlation Between some physiological and biochemical parameters with incretin hormones in patients with Type 2 Diabetes in AL-Najaf Government

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Abstract

The study evaluated the level of GLP-1, GIP hormones, and some biochemical parameters in patients with type 2 diabetes compared to healthy individuals and determined the relationship between them. The study included the examination of (83) of both genders, 62 of them were type 2 diabetes patients who were receiving care at the (Diabetes –and Endocrine Clinic at Al-Sader Educational Hospital in Najaf province) and 21 were healthy control, underwent blood tests (CBC, N/L) as well as the biochemical and hormonal indicators included (TC, TG, LDL, HDL, VLDL, GIP, GLP-1). The present study's finding indicated an insignificant rise in white blood cells, platelet count, and N/L count ratio, and a decrease in the patient group's average platelet volume, red blood cell count, and hemoglobin compared with the control group. As for fat levels, a significant increase in most types of fats was noted in the patient group compared to the control group, the findings of the present study indicate a significant reduction in concentration levels of hormones GLP-1 and GIP in diabetic patients compared to the control group. Through the results of the current study, we conclude that a decrease in the hormones GIP and GLP-1 are considered predictive indicators of diabetes, and they also play an important role in achieving a state of homeostasis in the level of fat concentration and some blood parameters.

Keywords: Incretin, Type 2 Diabetes, CBC, lipid profile

Introduction

Diabetes is a syndrome that has taken on epidemic names at present, with a steady increase in the proportion of those infected with it, and despite advanced preventive methods, it is now threatening a large percentage in all societies and at all ages, as Researchers believe that 25% of people will develop diabetes in the future, and a large percentage of them will not respond to treatment and will not be able to control its complications [1]. It is also represented as a chronic metabolic condition caused by an inability to regulate blood glucose levels as a result of a lack of insulin secretion from beta cells (type 1 diabetes) or insulin is secreted, but the peripheral tissues resist it (type 2 diabetes) [2]. Although the causes of type 2 diabetes are unknown, its precursors are long-term, known as insulin resistance and the failure of cells to sense it. Recent studies evaluate the relationships between type 2 diabetes and insulin resistance, but the mystery revolves around the relationship between it and other hormones that act as a stimulating factor for insulin secretion in the second type of diabetes, currently known as incretin hormones [3].

The stimulatory procedure includes treatments within pancreatic beta cells that enhance and

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transmit chemical signals, including ion channel activity and the release of insulin-containing granules [4]. Maintaining blood glucose levels is a basic principle for sustaining a person's life. Fluctuations in glucose levels lead to long-term illnesses like diabetes and the resulting vascular conditions of coma, kidney failure, and then death if it is not treated. Diabetes was at the forefront of diseases in the 1920s, before the discovery of insulin Causes sudden death [5,6].

Incretins are hormones secreted from the small intestine shortly after eating a meal, which in turn stimulates beta cells to secrete insulin, leading to lowering the blood sugar level within normal limits. They are of two types (peptide-like glucagon and insulin tropic glucose) [7]. Incretin hormones have a positive effect in improving the functioning of beta cells on the one hand and treating resistance to high blood sugar on the other hand [8].

In this study, we focus primarily on recent trends in understanding the proposed physiological mechanisms in evaluating incretin hormones and their role in stimulating the pancreas to secrete insulin in patients with type 2 diabetes [9].

Material and Methods

Sample study:

This study involved 83 participants (both male and female), comprising 62 individuals with (type 2 diabetes) who attended the Diabetes and Endocrine Clinic at Al-Sadr Educational Hospital in Najaf province, and 21 healthy individuals serving as the control group. The ages of the participants ranged from 30 to 70 years. Samples were obtained during the period starting from December 12, 2022, to 1-5-2023.

Collection of blood samples:

Sterile medical syringes (blood specimen collection syringes) were used to collect blood samples. The sample was taken from a vein in the amount of 6 ml from diabetics and healthy people. 2 ml was placed in anticoagulant tubes (EDTA) (Ethylenediaminetetraacetic acid) for a CBC test. The remaining 4 ml was centrifuged at 3000 r p m for 15 minutes at room temperature, followed by separation of the serum from the samples and then stored at −20C° until analysis for the biochemical test.

Measuring and counting blood components (CBC) Complete blood count WBC, platelet, Hb, MPV, Lymphocytes, Neutrophil and RBC:

Blood components were measured using a Sysmex self-counting device The American Device works Sysmex uses an automated method to measure the percentage and number of blood components after staining them using the device's solutions [10].

Calculating the ratio of Neutrophils to lymphocyte ratio:

The numbers of these cells were checked with a complete blood count, and then the ratio was calculated by dividing the numbers of neutrophil white blood cells into lymphocyte white blood cells.

Lipid Profile Test:

A Fully Automatic Biochemistry Analyser Spin200 was used to detect Lipid Profile levels.

Measurement of GIP (Glucose-Dependent Insulinotropic) hormone concentration:

The concentration of the GIP hormone in the serum was estimated using the kit prepared by Elabscienceb. This kit works with the enzyme-linked immune sorbent assay (ELISA). The plate prepared with the kit is coated with antibodies to the human hormone GIP. When working, the serum (sample) is added, which will bind to the antibodies coated to each plate [11].

Measuring the concentration of the hormone GLP-1 (Glucagon_like peptide 1):

The concentration of the GLP 1 hormone in the serum was estimated using the kit prepared by Elabscienceb. This kit works using the enzyme-linked immune sorbent assay (ELISA). The plate prepared with the kit is coated with antibodies to the human hormone GLP-1. When working, the serum

(sample) is added, this will bind to the antibodies Replacement for the drilling plate [11].

Statistical analyses:

The Lilliefors-corrected Kolmogorov-Smirnov test was used to examine the distribution types of the results group. The results of the normally distributed variable were presented as (mean± standard deviation). The comparisons between normally dispersed variables were made by independent samples t-test. The relationship between parameters was assessed by computing Pearson's correlation coefficients for the normally distributed variables. The statistically significant difference or correlation was considered when p<0 05 [12].

Results

1. Comparison of hematological parameters between T2DM patients and controls:

The results of the current study showed that there were significant differences between the control group and type 2 diabetes patients in both the number of red blood cells and hemoglobin, while a non-significant increase was observed in each of the white blood cells, platelet count, and N/L count ratio, and a decrease in the average platelet volume in the Patients group compared with the control group as in Table 1.

2. Comparison of lipid profile parameters between T2DM patients and controls:

The results of the current study showed that there were significant differences between the control groups and type 2 diabetes patients in the levels of triglycerides, very low-density lipoprotein, total cholesterol, and low-density lipoprotein at the probability level ($p < 0.001$), ($p < 0.001$), ($p < 0.033$) and ($p < 0.059$) (respectively, while no significant differences appeared in the level of high-density lipoprotein at the probability level of ($p<0.236$) as shown in Table 2.

3. Comparison of GLP-1 and GIP levels between T2DM patients and controls:

Table 3 showed a significant decrease in the level of GLP-1 hormone concentration in the patient group and at the probability level ($p<0.001$) compared to the control. The GLP-1 hormone concentration in the patients was 21.234 pg/ml, as compared to its concentration in healthy people, 88.847 pg/ml. The results of the study also showed that there was a significant decrease in the probability level ($p<0.004$) in the concentration of the GIP hormone in patients with type 2 diabetes (331.117 pg/ml) compared to the control group (540.973 pg/ml).

4. Correlation between GIP and GLP-1 with hematological parameters

The results of the current study showed a positive correlation between physiological blood parameters (white blood cells, red blood cells, Platelet count, platelet volume rate) and GLP-1 hormone 0.151) (0.070) (0.131) (0.249), respectively, while it showed a negative correlation with hemoglobin (0.010), but it did not reach significance, with a very high negative correlation between N/L and the GLP-1 hormone level at a significance level of $p<0.01$.

The study also showed a positive correlation between the GIP hormone and white blood cells, hemoglobin, and N/L ratio (0.067) (0.176) (0.028) respectively, while a negative relationship appeared with red blood cells, Platelet count, and platelet volume (-0.150) (-0.124) (0.069), as in Table 4.

5. Correlation between GIP and GLP-1 with lipid biomarkers:

The results of the current study showed a positive correlation between total cholesterol and low-density lipoprotein with the hormone GLP-1, but it did not reach the level of significance. It was also observed that there was a significant positive relationship for triglycerides ($p<0.05$), high-density lipoprotein ($p <0.01$), and very low-density lipoprotein ($p<0.05$) with the GLP-1 hormone.

The results of the study also showed the correlation between fats and hormones GIP, A positive correlation was found between triglycerides

and very low-density lipoprotein with GIP, while a negative correlation appeared between total cholesterol and low-density lipoprotein with GIP, while high-density lipoprotein was associated with a significant negative correlation at the probability $p<0.05$ level as in Table 5.

6. GLP-1 and GIP as predictive tools for T2DM

GLP-1 concentration indicates that the sensitivity and specificity rate for diabetes is 66.7% at a cut-off level of less than (46.065 pg/ml) and at a probability level of $p < 0.001$. We also note that the sensitivity rate is 61.9 % and a specific rate of 62.9 % of patients suspected of having type 2 diabetes have type 2 diabetes if the GIP concentration was less than the cut-off level (451.334 pg/ml) and at a probability level of $p < 0.004$) Table 6 and figure 1.

Discussion

1. Comparison of hematological parameters:

The results of the study revealed marked a decrease in patients with type 2 diabetes compared with the control group in both the number of red blood cells and hemoglobin, this study agreed with many studies [13]. The reason for this may be attributed to the decline in the ability of the bone marrow to produce red blood cells in patients with type 2 diabetes [14] The reason for the decrease in the number of red blood cells may be a result of a disturbance in the hematopoietic environment, such as fluctuation in blood sugar levels and hyperosmolarity. These disorders can cause an increase in internal viscosity and an increase in the stiffness of the membrane surrounding the red blood cell, causing a decrease in the number of red blood cells [15] or might be due to inadequate management of blood glucose leading to an increase in inflammatory cytokines altering the erythropoietin response and promote programmed death of red blood cells, thus reducing their number, followed by a decrease in the level of hemoglobin. [16] Chronic high blood sugar is a major cause of the production of reactive factors that promote the breakdown of blood cells and accelerate the development of type 2 diabetes complications [17].

The risk of reactive factors increases when a balance is not achieved between the generation of reactive factors and the generation of antioxidants, which are either manufactured internally in the body or can be obtained from external sources. This leads to the occurrence of internal conditions known as oxidative stress, which in turn damages the lining of blood vessels, causing a general imbalance. For hematological and immunological parameters [18,19].

As for the level of hemoglobin, the study found a significant decrease in patients with type 2 diabetes compared to the control group, and this result agreed with[20,21]The reason for this is due to the relationship between the concentration of hemoglobin in the blood and the number of red blood cells, as the average lifespan of a red blood cell decreases in diabetic patients due to the alteration in the permeability of the red blood Barrier [22] As for the ratio of the number of white blood cells, neutrophils to lymphocytes, in diabetic patients, there was a significant increase compared to healthy people, and this result was consistent with many studies [23,24]. It is known that diabetes is associated with many chronic inflammatory diseases [25] A non-significant increase in the number of platelets was observed, and this result did not agree with what was found [20] while it agreed with [26] The reason for this may be attributed to the association of diabetes with blood clots and atherosclerosis, as the change in osmotic pressure in the blood due to high blood glucose stimulates platelets to aggregate, and these atherosclerosis begin to form aggregated platelets resulting from the interaction of platelets and white blood cells with the endothelium of blood vessel [17,27].

2. Difference assessment of lipid profile criteria between T 2 D M patients and control:

The results of the current study showed that there were significant differences between the control

groups and type 2 diabetes patients in the levels of triglycerides, very low-density lipoprotein, total cholesterol, and low-density lipoprotein, this study agreed with [28] While no significant differences appeared in the level of high-density lipoprotein and found all fats are higher than in patients with type 2 diabetes, except for beneficial fats [29]. The reason is that any defect in carbohydrate metabolism is followed by a defect in fat metabolism and distribution [30]. The reason may be the increase in fatty deposition in triglycerides and not using them as an energy source again due to nutritional imbalance in patients. Type 2 diabetes leads to an increase in central obesity in patients as shown in Table 2.

3. Comparison of hormonal levels of GLP-1 and GIP levels between T2DM patients and controls:

Table 3 shows a significant decrease in the level of GLP-1 hormone concentration in the patient group compared to the control. The results of the study also showed that there was a significant decrease in the concentration of the GIP hormone in patients with type 2 diabetes compared to the control group affected by diabetes a lot with a lack of organization of receptors GIP, GLP-1) and this is confirmed by the study [31] and the current study did not agree with the study of [32] who revealed that the concentration of the hormones incretin does not differ between patients with type II diabetes and healthy people, which differs is the decline in the patient's response to their effect is the result of type II diabetes and not a characteristic and one of the determinants on which their high or low concentration depends is the speed of decay by the enzyme DPP-4 [33].

We note from the study that the two incretin hormones vary in their concentration for one group. Despite the similarity between their insulin-adjuvant effects, one of them differs from the other biologically. The gene transcription factor 7-like 2 (TCF7L2) is also greatly affected by diabetes, with a lack of regulation of GIP and GLP-1 receptors, and this was confirmed by a study [34].

4. Correlation between GIP and GLP-1 with hematological parameters:

The observations of the study revealed a positive correlation between physiological blood parameters (white blood cells, red blood cells, platelet count and platelet volume) and the GLP-1 hormone, while it showed a negative correlation with hemoglobin, but it did not reach significance, with a very high negative correlation between N/L and the GLP-1 hormone level. The study revealed a positive correlation between the GIP hormone and white blood cells, hemoglobin, and the N/L ratio, while a negative relationship appeared with red blood cells, platelet count, and platelet volume rate as in Table 4.

A study found [35] that the decrease in the number of platelets compared to the increase in the concentration of the GLP-1 hormone is due to the effect of the hormone on reducing the size of blood clots while a study he conducted showed [36] the GIP hormone reduces both blood cell aggregation and oxidative stress. There was also a very high inverse correlation between N/L and the level of the GLP-1 hormone. This may be because with a rise in the GLP-1 hormone, the risk of diabetes decreases, and since diabetes is linked to chronic inflammation, a low N/L indicates the lack of immune stimulation against any disease inflammation [37]. The study showed that there is a positive correlation between the GIP hormone and white blood cells, hemoglobin, while a study [38] showed that GLP-1 hormone has an effect in reducing the level of cumulative blood sugar in patients with type 2 diabetes because the hemoglobin is inversely affected by the level of the hormone. A study [39] showed that GLP-1 hormone has an effect on the number of platelets and their status within the normal range.

5. The relationship between hormonal GIP, GLP-1 with lipid biomarkers:

The findings of the study showed a positive correlation between total cholesterol and low-density lipoprotein with the hormone GLP-1 but it did not reach the level of significance. It was also observed that there was a significant positive relationship between triglycerides, high-density lipoprotein, and very low-density lipoprotein with the GLP-1 hormone. The findings of the study also showed the correlation between fats and hormones GIP, A positive correlation was found between triglycerides and very low-density lipoprotein with GIP, while a negative correlation appeared between total cholesterol and low-density lipoprotein with GIP. As for high-density lipoprotein, it was associated with a marked adverse association as in Table 5.

A study [40] showed the GIP/GLP-1 hormones work to help in weight loss and control fat metabolism. And agree with a study that showed that the hormone GLP-1 does not affect both low-density fat and high-density fat. This is consistent with, the effect of GIP is weakened in obesity and type 2 diabetes, and the effect is harmful because it affects lipocytes to promote lipid buildup and reduce insulin responsiveness [41]. A study the incretin hormones regulate obesity and fat deposition by sending signals to the central nervous system, thus regulating appetite, satiety, and controlling weight in the long term [42].

6. GLP-1 and GIP as predictive tools for T2DM:

GLP-1 concentration indicates that the sensitivity and specificity rate for diabetes is 66.7% at a cut-off level of less than (46.065 pg/ml) and at a probability level of $p < 0.001$. We also note that the sensitivity rate is 61.9 % and a specific rate of 62.9 % of patients suspected of having type 2 diabetes have type 2 diabetes if the GIP concentration was less than the cut-off level (451.334 pg/ml) and at a probability level of ($p < 0.004$) Table 6 and Figure 1.

As found in a study [43] there is an inverse relationship between low concentrations of the hormones GIP and GLP-1 and the risk of developing type 2 diabetes, and defects in the secretion of [44] GLP-1 and GIP cause early evaluation of incretin hormone deficiency is very useful in avoiding type 2 diabetes [45].

Conclusions

We conclude from the current study that a decrease in the hormones GIP and GLP-1 are considered predictive indicators of diabetes, and they also play an important role in achieving homeostasis in the level of fat concentration and some blood parameters.

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Conflicts of interest: The authors declare that there are no conflicts related to this work**.**

Ethical considerations: The research received the green light from the Medical College's Ethics Committee at Kufa University, and all subjects gave their informed consent agreements.

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TABLE 1. Hematological data of healthy controls (HC) and T2DM patients

Parameters	Control N=21	Patients N=62	p-value
WBC	6.446±0.44	7.191±0.299	0.169
RBC	4.878±0.112	4.033±0.067	0.053
HGB	13.943±0.256	12.685±0.189	0.051
PCV	42.757±0.766	42.024±0.563	0.445
PLT	227.286±13.901	251.032±7.56	0.143
MPV	9.500±0.488	8.494±0.403	0.118
N/L	2.595±0.197	3.119±0.301	0.149

Results expressed as mean ± standard error for normally distributed data

TABLE 2. Lipid profile of healthy controls (HC) and T2DM patients

Parameter	Control N=21	Patients N=62	p-value
TG	1.298±0.075	1.952±0.098	**<0.001**
VLDLc	0.593±0.045	0.891±0.034	**<0.001**
T. Chol	4.695±0.111	5.272±0.149	**0.033**
LDLc	3.033±0.118	3.390±0.144	0.053
HDLc	1.069±0.052	0.991±0.034	0.236

Results expressed as mean ± standard error for normally distributed data

TABLE 3. Comparison of GLP-1 and GIP between healthy controls (HC) and T2DM patients

Parameter	Patients N=62	Control N=21	p-value
GLP-1 pg /ml	88.847± 1.598	21.234± 2.375	0.001
GIP pg /ml	540.973± 5.634	331.117± 6.245	0.004

Results expressed as mean ± standard error for normally distributed data

TABLE 4. Correlation between GIP and GLP-1 with hematological parameters

Variables	GLP-1	GIP
WBC	0.151	0.176
RBC	0.070	-0.124
HGB	-0.010	0.067
PCV	-0.004	0.064
PLT	0.131	-0.150
MPV	0.249	-0.069
N/L	**-0.346****	0.028

***: p<0.01*

TABLE 5. Correlation between GIP and GLP-1 with lipid profile and atherogenic indices parameters

Variables	GLP-1	GIP
T.Chol	0.119	-0.078
TG	**0.311***	0.094
HDLc	**-0.381****	**-0.264***
VLDLc	**0.311***	0.094
LDLc	0.117	-0.049
CRI-I	**0.453****	0.141
CRI-II	**0.365****	0.069
AIP	**0.397****	0.208
AC	**0.453*****	0.141

**: p<0.05, **: p<0.01, ***: p<0.001*

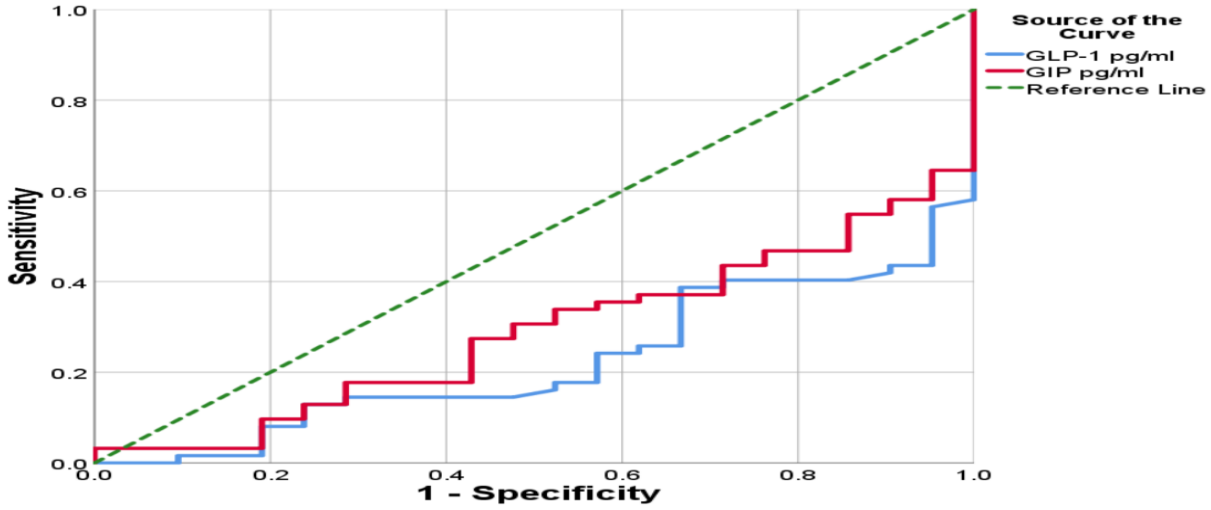

Figure 1. Receiver operating characteristic curves of GIP and GLP-1 for diagnosis of T2DM patients

TABLE 6. Receiver operating characteristic-area under curve (AUC) analysis of GIP and GLP-1 for diagnosis of T2DM patients

Variable	Cut-off	Sensitivity %	Specificity %	Youdin's J statistic	AUC (95% CI)	p
GLP-1* pg/ml	46.065	66.7	66.7	0.334	0.788(0.677-0.879)	<**0.001**
GIP* pg/ml	451.334	61.9	62.9	0.248	0.712(0.599-0.825)	**0.004**

**: parameters that decreased in T2DM patients. CI: Confidence interval, AUC, area-under curve*

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