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Preptin Hormone in Patients with Type 2 Diabetes Induced Post Coronavirus Infection (Covid-19)

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A B S T R A C T

The research included a study of the levels of the hormone preptin and some biochemical variables in the blood serum of people with type 2 diabetes (T2D) induced post coronavirus infection (Covid-19). Those variables included: glucose, cholesterol, triglycerides, high-density lipoprotein (HDL), low-density lipoprotein (LDL) and measurement of levels of antioxidants (Albumin, uric acid, fucose and glutathione), as well as levels of oxidants compounds: for exaple malondialdehyde and peroxynitrite on (16) a sample for patients and (40) samples for the control group, whose ages ranged between (35-70) years in Mosul city.

The results showed that there was a significant increase in the levels of the preptin hormone, glucose, cholesterol and LDL in addition to the decrease in HDL, among T2D patients induced after corona compared with the control group. No significant difference was observed in the TG, HDL and uric acid between the patients' group and control group.

The results also indicated that there was a state of high oxidative stress reflected in the low levels of antioxidants for glutathione, albumin, and fucose, and a significant increase in the levels of malondialdehyde and peroxynitrite in people with diabetes mellitus developed after corona compared with the control group.

The study concluded that the hormone preptin is a good indicator that reflects the status of T2D patients induced Post coronavirus infection (Covid-19), by comparing them with the levels of antioxidants and oxidants, as well as the levels of fucose and glucose.

1 Introduction

The emerging coronavirus disease in 2019 (Covid-19) led to a serious pandemic in 2020, as classified by the World Health Organization, which led to a great loss in the global economy, loss of lives and lifestyle changes (Goyal *et al*., 2020). The clinical symptoms of coronavirus differ from one person to another, and it may be asymptomatic or accompanied by serious complications that lead to death (Sheraton *et al*., 2020). Coronavirus (Covid-19) mainly targets lung systems, but it has unexpected effects on many organs. Previous studies have shown a number of long-term complications of the Corona virus (Nalbandian *et al*., 2021). Including fibrosis of the lungs, heart attack, stroke, mood disorder and diabetes developed after infection with the virus (SeyedAlinaghi *et al*., 2021), which is the most important focus of our research.

As coronavirus can cause the emergence of diabetes in people with it (Chee *et al*., 2020). As a result of multidirectional changes in glucose metabolism resulting from a defect in the organs and tissues responsible for the main metabolism, including beta cells in the pancreas, fatty tissue, small intestine and kidney (Bornstein *et al*., 2020). Coronavirus binds to the angiotensin-converting enzyme 2 receptor. It works by inhibiting the angiotensin II-to-angiotensin (7-1) pathway that plays a protective role in diabetes by

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improving pancreatic cell survival, stimulating insulin secretion, and reducing insulin resistance (Ni *et al*., 2020).

Preptin hormone is a peptide hormone with a molecular weight of 3,948 Daltons that was first isolated in 2001 from beta cells of the pancreatic gland of mice (beta-TC6-F7) by Buchanan *et al*. (2001). Preptin hormone consists of 34 amino acids as it is secreted with insulin and amylin from secretory granules of beta cells. Preptin is the newest member of the insulin family discovered. Preptin hormone has many functions in the human body, but the most important function of preptin is energy balance and carbohydrate metabolism by stimulating it to secrete insulin from beta cells when the blood glucose level is high, where it participates along with insulin, amylin and pancristatin in glucose metabolism. It is a physiological enhancer of insulin secretion caused by high glucose concentration (Wang *et al*., 2020).

Oxidative stress is a factor that causes metabolic and physiological changes and various diseases of the organism, as it is associated with some chronic diseases such as diabetes, heart diseases and cancer, and is also associated with some types of viral infections that are carried out through RNA, such as coronavirus (Baqi *et al*., 2020). A study has proven the association of oxidative stress with complications that affect corona patients, such as severe inflammation, blood clotting and cellular oxygen deficiency (Sudre *et al*., 2021). It has been observed that coronavirus can generate oxidative stress due to the excessive levels of reactive oxygen and nitrogen species in Corona patients, which are randomly generated by immune cells to infected cells and surrounding tissues as a result of acute inflammation. As a result of the oxidative stress caused by infection with coronavirus (Mohiuddin and Kasahara, 2021). Peroxynitrite is the largest of the potent oxidants produced by immune cells and the main mediator associated with inflammation (Ahmed *et al*., 2020).

The study aims to know the role of the preptin hormone and its relationship with some other measurements in patients with type 2 diabetes developed after infection with coronavirus (Covid-19), and to know the state of oxidative stress.

2. Materials and Methods

This study included (18) patients diagnosed with diabetes developed after infection with coronavirus (Covid-19), where the clinical diagnosis of each case was made by a specialized doctor, and samples were collected during the time period from the beginning of September 2021 to the end of November 2021 from patients treated at the Al-Wafa Specialized Center for Diabetes and Endocrinology in Mosul city, their ages range from (35-70) years.

The control group consists of 40 normal healthy individuals who have no history of disease and who match the age and body mass index with the patients. Data were collected for patients and the control group, including age, sex, height, weight, body mass index, family medical history, and smoking.

Ten milliliters of venous blood were taken from the patients and control groups and left for 15 minutes at room temperature for coagulation. Then the serum was separated by centrifugation at (3000 x g) for 15 minutes and divided into two parts and kept frozen at (-20°C) until it worked the analysis.

2.1: Methods used in measuring preptin hormone and the variables specified in the research:

The serum preptin was estimated based on linked enzyme immunosorbent assay (ELISA) technique using ready-made assay kits from BT LAB Company /China, which is an immunological method for quantification based on the sandwich principle.

The levels of glucose, total cholesterol, triglycerides and high-density lipoprotein cholesterol (HDL-C) were estimated using ready-made assay (kits) from the company (BIOLABO) and using enzymatic methods (Ambade *et al*., 1998; Fossati and Prencipe, 1982; Lopez-Virella *et al*., 1977; Allain *et al*., 1974), the concentration of low-density lipoprotein cholesterol (LDL-C) in serum was calculated using the following Friedewald equation (Farukhi and Mora, 2018):

LDL-C (mmol/L) =
Total cholesterol - HDL-C - (TG/2.2)

The albumin concentration was estimated using the Bromocresol green method, in which a ready-made (Kits) from the French company (BIOLABO) was used, which depends on the amount of albumin bound with the reagent (3,3̅,5,5̅-Tetrabromocresol green, BCG) to form the albumin-BCG complex, and the absorbance intensity is measured at the wavelength 630 nm (Doumas *et al*., 1971). Also, uric acid was estimated using Kits, which depends on the enzyme uricase in the oxidation of uric acid to allantoin and hydrogen peroxide (Walker *et al*., 1990).

The measurement of fucose was based on the principle of direct interaction between sulfuric acid with components of the blood serum, as the thiol group (-SH) of sulfuric acid interacts with carbohydrates in the serum, and these reactants bind with the amino acid cysteine and form a colored product, which is measured by the intensity of absorption at wavelengths 396 and 430nm (Dische *et al*., 1948).

The concentration of GSH in serum was determined using the modified method used by researchers (Sedlak and Lindsay, 1968), The method is based on the use of Ellman's reagent, which is [5-5¯ dithio bis (2-nitrobenzoicacid)], DTNB, which reacts quickly with GSH and reduces it by means of the thiol group (SH group) of GSH forming a colored product, the absorbance is read for it at 412 nm.

MDA was measured in the serum using the method of researchers (Guidet and Shah, 1989) and the method depends on the reaction of MDA with thiobarbituric acid (TBA) and this reaction takes place in an acidic medium and it produces a colored product whose absorbance intensity is measured at the wavelength 532 nm. Peroxynitrite (ONOO-) levels were measured using the method of researcher Vanuffelen *et al*., 1998), which depends on ONOO- in the nitration of phenol to nitrophenol, which reflects the concentration of ONOO- and its absorbance intensity can be read at 412 nm.

2.2: Statistical analysis

The statistical program SPSS-17 was used to determine the mean ($\bar{X}$) and standard error (SE) and the t-test was chosen to compare two variables and find the difference between the values that appeared through the P-value that occurs at ($P \leq 0.05$) significant difference, and at ($P \geq 0.05$) non-significant difference (Hinton, 2004).

3. Results and Discussion

3.1: Comparison of levels of preptin hormone, glucose and lipids in diabetic patients after infection with Covid-19 compared to the control group.

3.1.1: Preptin hormone

The results shown in Table (1) that there is a significant increase in the level of preptin hormone in the serum of people with induced diabetes after infection with coronavirus, compared with the healthy group at (P=0.036). The reason for the rise of the preptin hormone is due to the rise in blood glucose in the serum of patients with diabetes, and that the function of the preptin hormone is to stimulate the secretion of insulin in the event of high blood glucose, the higher the level of blood glucose, increased the concentration of preptin hormone (Alubaidi *et al*., 2018).

Table 1: Comparison of levels of preptin hormone, glucose and lipids in diabetic patients after infection with COVID-19 compared to the control group.

Biochemical variables	Control		Patients		p-value
	$\bar{X}$	SE	$\bar{X}$	SE	
age (year)	48.5 **a**	1.90	48.14 **a**	4.64	**0.574**
BMI (body mass index, kg/m^2)	30.3 **a**	1.22	31.08 **b**	2.02	**0.066**
Proptin (ng/mL)	1231.9 **a**	50.61	1407.65 **b**	194.4	**0.036***
Glucose (mg/100mL)	93.9 **a**	3.03	134.42 **b**	5.98	**0.017***
Cholesterol (mg/100mL)	162.77 **a**	8.79	174.71 **b**	9.04	**0.045***
Triglyceride (mg/100mL)	121.19 **a**	15.06	142.57**b**	16.38	**0.036***
(HDL) (mg/100mL)	46.02 **b**	2.10	35.24 **a**	5.69	**0.042***
(LDL) (mg/100mL)	93.6 **a**	7.36	144.52 **b**	6.34	**0.010***

*The difference in the letters (a, b) horizontally indicates that there is a significant difference at a probability level of less than or equal to 0.05 between the studied groups**.**

3.1.2: Glucose:

The results shown in Table (1) indicated that there is a significant increase at the probability level of 0.017 in the concentration of glucose for patients with diabetes developed after infection with coronavirus compared to healthy group, and these results are consistent with the results of previous studies that showed that there is an increase in blood glucose in some Coronavirus patients (Ren *et al*., 2020).

The cause of high blood glucose after infection with coronavirus may be the result of the entry of virus through the receptor of the angiotensin-converting enzyme 2 (ACE2) into sites and organs in the body that express these receptors, such as the cells of the pancreas gland, and their infection with infections, which leads to infection of beta cells and a decrease in insulin secretion (Lutz *et al*., 2020; Murray *et al*., 2020), or as a result of a defect in ACE2 itself that prevents the conversion of angiotensin II to angiotensin (1-7) and leads to hyperactivity of angiotensin II, which is a critical inducer of insulin resistance(Ren *et al*., 2020) as well as disrupts the activities of insulin-stimulating pathways, impairing the transmission of Glut-4 (Glucose receptor) is attached to the membrane of insulin-sensitive tissues such as muscle, liver, and adipose tissue (Khunti *et al*., 2021). In addition, Ang11 also contributes to inflammation by both immune cells and cells in tissues, which leads to an increase in proinflammatory cytokines that block insulin receptors in beta cells leading to a state of insulin resistance similar to that seen in type 2 diabetics (Mahmudpour *et al*., 2022).

3.1.3: Lipid levels:

The results in Table (1) indicated that there was a significant increase in cholesterol at a probability level of 0.045. These results are consistent with previous studies that showed that cholesterol in the lipids of the cell membrane is a key element in the entry of the virus and the promotion of viral infection, as it plays an important role in the interaction and linkage between the S protein in coronavirus and the angiotensin-converting enzyme receptor 2 and facilitating the process of viral phagocytosis (Meher *et al*., 2020). Studies have found that using effective lipid-lowering and cholesterol-lowering treatment significantly suppresses coronavirus infection (Katsiki *et al*., 2020).

The results also indicated a significant increase in triglycerides (TG) and LDL-C in patients compared with healthy people at a probability level of 0.036 and 0.01, respectively, and that there was a significant decrease at a probability level of 0.042 in HDL-C in the group of patients compared to the healthy group, and these results are consistent with a previous study that showed that inflammation and the increase of cytokines for immune cells as a result of infection change the shape and metabolism of lipids in corona patients and work on an increase in TGs and a decrease in HDL-C (Sorokin *et al*., 2020).

3.2. Comparison of antioxidants and oxidants levels in patients with diabetes developed after infection with coronavirus with the healthy group.

3.2.1: Antioxidants Levels:

The results in Table (2) indicate that there is a significant decrease in the levels of antioxidants, including fucose and glutathione, in patients with diabetes developed after infection with coronavirus, at a probability level of 0.042 and 0038, respectively, and these results are consistent with the results of a previous study that indicated a decrease in antioxidant levels in corona patients (Polonikov, 2020).

The reason for the low levels of antioxidants, especially glutathione, in corona patients is due to its depletion in balancing the effect of free radicals, whose level is high due to the increase in oxidative stress in corona patients (Mohiuddin and Kasahara, 2021). As it plays a vital role as an antioxidant, in addition to its other vital properties, has the ability to act as an anti-inflammatory, anti-tumor, and immune-boosting agent. It is used in anti-aging cosmetics, nutritional supplements, or as a treatment for some diseases (Iqbal *et al*., 2021; Hameed and Al-Helaly, 2021), so we note low levels in patients. In addition, glutathione is one of the most important antioxidants and the main detoxifier of metallic or otherwise, as it works to remove many toxic compounds xenobiotics and remove reactive oxygen species (ROS). It is important in the functioning of the immune system and it is one of the main antioxidants produced by cells (Million *et al*., 2020; Hameed and Al-Helaly, 2020).

The results also indicate a significant decrease in patients' albumin levels at a probability level of 0.028, and these results are consistent with a previous study (Chen *et al*., 2020). The reason for the low albumin levels is due to a defect in liver function as a result of infection with coronavirus, as it is an important biomarker for assessing liver function (Clark *et al*., 2021). The reason for this decrease may also be due to the inflammation caused by the infection of coronavirus, which increases the permeability of the capillaries, which leads to the escape of albumin outside the blood vessels and a decrease in its concentration in the blood (Spada *et al*., 2021; Qin *et al*., 2020). The results also indicate an insignificant decrease in uric acid, one of the antioxidants, in patients with diabetes developed after

infection with coronavirus, compared to healthy patients.

3.2.2: Oxidants Levels:

On the other hand, the results in Table (2) showed that there was a significant increase in the level of MDA in diabetic patients induced after infection with t coronavirus compared to the healthy group at a probability level of 0.015. This is due to the increase in the production of free radicals in corona patients, which in turn works; it destroys lipid membranes and occur lipid peroxidation, and elevated levels of MDA as by-products of the oxidation process (Aninagyei *et al*., 2019).

Table (2): Comparison of oxidation levels of antioxidants in diabetic patients after infection with corona compared to the control group.

Biochemical variables	Control		Patients		p-value
	X̄	SE	X̄	SE	
Albumin (g/100mL)	4.79 **b**	0.14	2.27 **a**	0.11	0.029*
Uric acid (mg/100mL)	5.89 **a**	0.35	6.21 **a**	0.36	0.748
Fucose (mg/100mL)	12.44 **b**	1.18	8.14 **a**	1.88	0.042*
Glutathione (µmol/L)	7.83 **b**	0.55	4.25 **a**	1.46	0.038*
Malondialdehyde (µmol/L)	5.46 **a**	0.43	11.45 **b**	1.87	0.015*
Peroxynitrite (µmol/L)	54.39 **a**	2.95	73.42 **b**	2.26	0.035*

*The difference in the letters (a, b) horizontally indicates that there is a significant difference at a probability level of less than or equal to 0.05 between the studied groups.

The results also indicated that there was a significant increase in the levels of ONOO- in diabetic patients who developed after infection with the coronavirus compared to the healthy group at the probability level of P<0.05. These results are consistent with previous results that indicated high levels of oxidative compounds, including ONOO-, in corona patients (Ntyonga-Pono, 2020; Derouiche, 2020).

The reason for the high level of ONOO- in corona patients is the inflammation resulting as the body's immune response to protect against different types of infections, and during infections, excessive production of nitric oxide and superoxide anion radical occurs, which results in the formation of ONOO- in the infected body and the resulting oxidation problems different and increase the complications of the disease (Ahmed *et al*., 2020).

4. Conclusions

The study concluded that the preptin hormone is a good indicator that reflects the status of patients with type 2 diabetes that developed after infection with corona, by comparing it with the levels of antioxidants and oxidants, as well as levels of fucose and glucose, and patients should increase the intake of antioxidants of various kinds to reduce their state of oxidative stress.

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Conflict of Interest: The authors declare that there are no conflicts of interest.

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