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Study of insulin resistance, cortisol hormone and some biochemical parameters in Iraqi type 2 diabetic patients

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***Abstract*— The most prevalent type of diabetes disease is type 2. It resulted through cells' failure for recognizing and responding to insulin, which can increase the risk of this disease if improperly managed. This work seeks to assess few biochemical variables connected to glycemic index in people with type 2 diabetes (T2D). In this study, certain biochemical markers were taken from 37 patients with diabetes mellitus (DM). In the current study, lipid profile, kidney function, insulin, glycated hemoglobin (HbA1c), fasting blood sugar (FBS), uric acid, insulin resistance (HOMA-IR) and cortisol hormone were measured. The blood serums of the 37 patients were taken from diabetic center in Yarmouk hospital and 27 control samples were taken from healthy people in the University of Baghdad. The ages for healthy and unhealthy patients were (19 to 47) years old. The results showed that, diabetic participants had higher HbA1c, FBS, HOMA-IR, cortisol, urea, uric acid, lipid profile, and creatinine levels comparing to the control samples at P≤0.05. We concluded from this study, abnormally elevated levels of serum cortisol, FBS, HbA1c, and insulin. Increasing HOMA-IR indicated that IR is a risk factor for T2DM development. This work revealed a relationship between T2DM and kidney function parameters. It was found that patients with T2D having higher levels of creatinine and urea.**

Keywords— Type 2 diabetes mellitus, Insulin resistance, Kidney function, Cortisol and Lipid profile.

I. INTRODUCTION

Diabetes mellitus Type 2 (T2DM) considers the highest percentage (90%) of all diabetes cases. In T2DM, the response to insulin is diminished, and this is defined as insulin resistance. During this state, insulin is ineffective and initially countered by an increase in insulin production to maintain glucose homeostasis, however, the insulin production decreases, resulting in T2DM. T2DM is mostlthappen for people older than 45 years. It is also happen in children and adolescentsdue to rising levels of obesity, physical inactivity, and energy-dense diets [1].

The prevalence of T2D and its comorbidities has reached epidemic levels. Almost more than 90% of all cases of diabetes are T2D, and its incidence and prevalence are quickly rising globally. According to International Diabetes Federation, there will be 629 million adults with diabetes all over the world by 2045, up from the current 425 million cases. The percentage of T2D cases also increased globally. An early therapy may prevent or delay full-blown disease, thus it's crucial to identify those who are most at risk to develop T2D.

Impaired insulin resistance (IR) and insulin secretion in the liver and muscle are the primary pathophysiological characteristics of T2D. Also, T2D is frequently preceded through protracted period of pre-diabetes, which is defined by IR and an increase in fasting or 2-hour glucose (impaired fasting glucose or glucose tolerance) levels in the OGTT [2]. Diabetes recognizes when the pancreas no longer has the ability to secrete enough insulin for compensating IR in peripheral insulin-sensitive tissues. Insufficiency incretion hormone or resistance in the hyper-glucagonemia and gastrointestinal tract are other issues that T2D patients experience during the insulin secretion [3]. The emergence of glucose intolerance and T2D are significantly influenced by IR because cells do not respond to insulin normally. In spite of increasing of glycogen synthesis insulin-stimulated state and, skeletal muscle is a key tissue for the uptake of glucose. Through controlling glycogenolysis and gluconeogenesis, the liver is crucial in preserving appropriate glucose levels. Insulin typically inhibits the genes responsible for gluconeogenesis and reduces the glucose amount produced by liver. Insulin inhibits rapid lipolysis inside fat cells [4]. The aim of the study is to evaluate some biochemical parameters and relationship of the cortisol hormone in patients with type2 diabetes.

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II. MATERIAL AND METHODS

A. A.Study design

This work was conducted in Department of Biotechnology, Faculty of Sciences, Univ. of Baghdad. A total of 37 people with DM who visited the diabetic center in Yarmouk hospital and 27 healthy control subjects. The ages of the participants of patients and control were from 19 to 47 years old. The time consumed of this study was from August 2022 to February 2023. All participants from patients and inpatients (control) were male. A number of specific tests, such as the glycated hemoglobin (HbA1c), urea, serum cortisol, fasting blood sugar (FBS), uric acid, creatinine, insulin, lipid profile, homeostatic model assessment for BMI and insulin resistance (HOMA-IR) were performed in this work. Samples were gathered, and then analyzed in the University of Baghdad's Chemical Laboratory by the Biotechnology Department of the College of Science.The participants fasted for16hours , and then the blood samples were collected. A 5ml blood sample was taken from every subject by using a needle and syringe. After collection the whole blood samples, the samples left at room temperature for 15 minutes to allow them to clot. The clot was removed by centrifuging at 3000 xg for 10 minutes, the resulting supernatant is desired serum.

B. Determining serum total cholesterol (TC):

Commercially available kit (bio-Merieux) was used to assessed the total concentration (TC) concentration following the enzymatic method [5]. TC value was specified using Spectro-photometrically to be 500 nm,.

C. Determination of the serum high density lipoprotein (HDL-c):

The (bio-Merieux) kit was used for measuring the HDL-c level with the use of an enzymatic method [6]. The main idea behind this approach is to precipitate lipoproteins and chylomicrons of LDL and VLDL by adding of phosphotungestic acid with the presence of magnesium ions . The HDL, which contains cholesterol and phospholipids, was included in the supernatant that was created following centrifuging. At 500 nm, HDL has been spectro-photometrically defined.

D. Determining the serum triglycerides (TG):

With the use of Bio-Merieux kit and the enzymatic method proposed by Prencipel and Fossati [7], the total concentration of the serum (TG) was determined. The 500nm value was designated as the TG total serum concentration.

E. Determination of serum VLDL-C:

VLDL had been determined by using the conventional equation of Fridewald *et al*., [8]. VLDL-c (mg/dl) = 0.20 x TG (mg/dl).

F. Determination of serum LDL-c:

Serum LDL was determined depending on Friedewald's eq. LDL-c =T.Chol. – (HDL-c + VLDL-c)**.**

G. Determining fasting blood sugar (FBS)

FBS has been enzymatically estimated using glucose oxidase GOD PAP(Kit)(Liquid)GL-2624.

H. Determining serum insulin

The German company AESKULISA ELISA kit measures serum insulin levels.. The specific antigen-coated microplates are incubated with diluted serum samples (1:101). The patient's antibodies bind to antigen. The next step was washing the unbound fraction away. Afterwards, anti-human immunoglobulins that are conjugated to the horseradish peroxidase (i.e. conjugate) were incubated and reacted with the antigen-antibody complex of samples in micro-plates. The next step was washing out any unbound conjugate. A colorimetric (blue) enzymatic reaction is produced by adding TMB-substrate and is stopped by dilute acid (color has been changed into yellow). The amount of the conjugate that was coupled to the antigen-antibody complex determines intensity of the color production from the chromogen, and this amount was proportional to initial concentration regarding relevant antibodies in patient sample.

I. Determination of HOMA-IR

The two equations that were used to calculate HOMA-IR are:

fasting glucose (mg/dl) × fasting insulin) / 405

(fasting glucose (mmol/L) x fasting insulin) /22.50 .

J. Determining serum Creatinine:

Creatinine levels in the serum were tested colorimetrically using commercially available kits (BIOLABO). The kidneys excrete the creatinine that is released during the metabolism of creatine phosphate. The colourful complex of creatinine picrate, which carries the ionic bounds, is created when the creatinine is combined (in a 1:1 ratio) with the alkaline picrate. The rate of colored complex production is inversely correlated with creatinine content.

K. Determination of serum urea level:

Serum Urea was measured colormetrically following Scott and Fawcett, [8], using Randox kit.

L. Determination of serum cortisol

Serum Cortisol was specified through competitive immune detection method by Ichroma kit.

M. Determination of glycated hemoglobin (HbA1c)

HbA1c was measured using (Stanbio Glycohemoglobin –pre-fil-procedure No.P350) quantitative colorimetric Glycohemoglobin determination in whole blood.

N. Determining uric acid

Serum uric acid was measured colormetrically depending on Prencipel and Fossati, [8], using BIOLABO kit.

O. Statistical analyses:

SPSS for Windows, version 22 program was utilized in order to conduct the data analyses. Data was represented as mean ± standard deviation (SD). The

designed parameters was checked the Shapiro-Wilk normality test to see if they can fit to the gaussian distribution. After doing the analysis of variance (ANOVA), Bonfferoni Post Hoc test was utilized for many comparisons. Using Pearson's analysis of correlation, the levels of the association were examined. Significant was defined as a p value < 0.05 [9].

III. RESULTS AND DISCUSSION

A. Effect of some biochemical parameters on T2DM

A total of 37 DM patients and 37 control subjects were participated in this study. T2D' and control participants' results for various biochemical parameters are displayed in (Table1). The current work evaluated to some biochemical parameters that were involved in DM-related problems. FBS, HbA1c, HOMA-IR, insulin, cortisol and uric acid levels were tested. In contrast to controls, T2D' patients showed significantly greater amounts of HbA1c, FBS, HOMA-IR, insulin, cortisol, and uric acid ($p < 0.05$). (Table 1). The values mentioned in this study may be a factor in diabetes conditions.

Table 1-Biochemical parameters in T2D and control subjects

Parameter	Control group	Patients group	*P* value
FBS (mg/dl)	91 ± 9	188 ± 29.3	< 0.05
HbA1c %	5.3 ± 0.4	7.9 ± 1.2	< 0.05
Insulin (µIU/ml)	12.4 ± 1.1	13.1 ± 0.9	< 0.05
HOMA-IR	2.7 ± 0.3	6 ± 0.7	< 0.05
Cortisol (ng/ml)	7.7 ± 0.6	8.8 ± 1.1	< 0.05
Uric acid (mg/dl)	4.3 ± 0.5	7.2 ± 1.2	< 0.05

FBS: fasting blood sugar, HbA1c: glycated hemoglobin, insulin, HOMA-IR: homeostatic mofel assessment for isulin resistance, cortisol and uric acid.

The primary energy source for cells is glucose. Nevertheless, without insulin, glucose can't enter the cell. An adequate quantity of insulin creates by pancreas that responsible for transporting the glucose into the cells. Thus, glucose that accumulates in blood, its concentration rises, and diabetes mellitus (DM) results. The research's findings revealed a considerable rise in glucose levels in T2DM patients comparing to the controls. This condition typically develops around the age of 40, and might be caused by weak β-cells, little insulin production and/or function, and rising IR [10].

Final product of purine metabolism in humans is uric acid (UA). It produces when the enzyme xanthine oxido-reductase converts the hypoxanthine into xanthine and then to the uric acid. Uric acid produces the superoxide anion and other ROS by using molecular oxygen as electron acceptor. Moreover, UA is a physiological free radical scavenger and one of the main factors in plasma antioxidant capacity [11]. As a result, UA functions as pro-oxidant as well as antioxidant. T2DM increases the production of the free radicals and weakens body's endogenous antioxidant defense system, both of which contribute to oxidative stress [12]. According to earlier research, hypouricemia and T2DM are related. According to Mehrdad *et al.*, [13] uricosuria and glucosuria have a positive relation. Also, it was found that a higher degree of hyperglycemia was linked to a faster rate of uric acid excretion and a decrease in levels of the plasma uric acid . A thorough evaluation of hypouricaemia and tubular transport regarding uric acid has been done [13]. According to reports, increased glomerular hyper-filtration due to the improper tubular urate handling causes higher urate clearance in T2DM [14].

B. Effect of lipid profile in patients who have type 2 diabetes

Table 2 displays significant lipid profile differences between 37 patients with T2DM and 37 control participants. When T2DM sufferers are compared to control subjects, there is a significant increase in T.ch, VLDL-C, T.G, and LDL-C.

Table 2- Levels of the lipid profile in T2D and control subjects

Parameter	Control group	Patients group	*P* value
T.ch (mg/dl)	147 ± 9	177 ± 8.6	< 0.05
T.G (mg/dl)	99 ± 4.7	122 ± 5.7	< 0.05
HDL-C (mg/dl)	39 ± 3.6	38 ± 4.4	< 0.05
VLDL-C (mg/dl)	19.8 ± 1.4	24.4 ± 2.9	< 0.05
LDL-C (mg/dl)	88.2 ± 3.3	114.6 ± 4.1	< 0.05

T.ch : total cholesterol, T.G: triglycerides, HDL-C: high density lipoprotein, VLDL-C: very low density lipoprotein, LDL-C: low density lipoprotein.

From the table above, the results showed a statistically significant increase in the total cholesterol in diabetes patients comparing to the controls. Such observation might be explained by a muscle activity reduction or by suppression of cholesterol catabolism [15]. Also, it was discovered that diabetes patients' triglyceride levels were substantially higher than those of controls. This can be explained due to the low insulin levels, which lead to hyperglycemia and the release of the fatty acids from adipose tissue. Adipose tissue's fatty acids have been mobilized for the energy purposes, and any excess that is accumulated in the liver where they're transformed into triglycerides [16]. By comparing with controls, diabetes patients' VLDL cholesterol levels were noticeably higher. This rise might be a result of hyperinsulinemia that causes LDL, triglycerides and VLDL cholesterol to rise. It understood that the insulin and growth hormone increase production of Apo-E and Apo-B 48, as well as adipose tissue lipolysis and hepatic triglyceride synthesis, all of which contribute to the creation of VLDL cholesterol . Those with T2DM showed considerably higher plasma LDL cholesterol levels when compared to controls. As insulin increases the number of the LDL receptors, chronic insulin deficiency, like the ones that are found in the T2DM, may be linked to a decreased amount of the LDL receptor and an increase in the plasma LDL-c levels [17].

Patients with T2DM had considerably lower levels of plasma HDL-c in comparison with the healthy controls. This result might be explained by urinary loss of the Lecithin: Cholesterol Acytransferase (LCAT) that causes severe deficit and restricts HDL's ability to absorb excess

cholesterol from extra hepatic tissues [18]. Also, this has been compounded through a notable decrease in hepatic HDL-C receptors. Low levels of serum HDL-C play a role in the functional and structural alterations that had resulted in arterial rigidity.

C. Effect of kidney function on type 2 diabetes mellitus

Table (3) shows increase significance $p \leq 0.05$ in creatinine and urea in T2D patients in comparison with control group.

Table3- level of kidney function in control and T2DM subjects.

Parameter	Control group	Patients group	*P* value
Urea (mg/dl)	16.9 ± 3.4	23 ± 3.9	< 0.05
Creatinine (mg/dl)	0.90 ± 0.20	1.9 ± 0.40	< 0.05

Through measuring the levels of urea and creatinine in the serum of non-diabetic and diabetics controls, it was possible to evaluate the extent to which T2DM had affected kidney function. Serum creatinine as well as urea are well-known indicators of GFR, despite the fact that serum creatinine is the more sensitive indicator of kidney dysfunction. Every time there was impaired kidney function or kidney damage, the urea value increased. Renal damage caused by increased blood sugar levels when the concentration of urea increases along with the level of sugar. Feng *et al* .,[19] study discovered that increasing levels of urea and serum creatinine in the diabetic rats is an indication for progressive kidney damages . According to the findings, T2DM participants' serum creatinine, blood glucose, and urea concentrations have all been shown to be higher [19]. Research demonstrated that higher serum levels of creatinine and urea in diabetes patients might suggest pre-renal disease. According to Saudi research conducted in 2017 through Abdulrahman Aldukhayl, Iraq had medium prevalence of diabetic nephropathy, UAE had the highest and Bahrain had lowest prevalence, in a case when compared to other Arabian nations [20].

IV. CONCLUSION

Among T2D individuals, this investigation discovered abnormally elevated levels of serum cortisol, FBS, HbA1c, and insulin. Increased HOMA-IR, which indicates IR, is a risk factor for T2DM development. This work revealed a link between T2DM and kidney function parameters. Patients with T2D have higher levels of creatinine and urea.

CONFLICT OF INTEREST

Authors declare that they have no conflict of interest.

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