

Lipid Profile and Insulin Resistance in Patients with Type-II Diabetes Mellitus

Hadil Walid*

Layla K. Ali**

Majeed A. Atiyah, M.Sc.***

Awaz S. Abdullah****

الخلاصة:

الهدف: إيجاد العلاقة بين الخلل في البروتينات الدهنية ومقاومة الانسولين باعتبارها أحد أسباب الخلل الايضي في مرضى السكري ومقارنة النتائج مع المجموعة الضابطة.

المنهجية: تم جمع نماذج الدم من 35 مريض مصابين بالسكري من النوع الثاني فضلاً عن 35 شخص من الأصحاء كمجموعة ضابطة. تم استعمال المصل في قياس سكر الدم والبروتينات الدهنية والكوليسترول والجليسيريد الثلاثي في مجاميع المرضى والضابطة. تم حساب مقاومة الانسولين وفقاً لطريقة هوما (HOMA-IR).

النتائج: أشارت نتائج الدراسة إلى وجود ارتفاع ذي دلالة معنوية في مستويات كل من سكر الدم، الانسولين، مقاومة الانسولين، الكوليسترول، الجليسيريد الثلاثي، البروتين الدهني القليل الكثافة والبروتين الدهني الأقل كثافة، في حين أظهر الدهن العالي الكثافة انخفاضاً ذا دلالة معنوية واضحة.

التوصيات: توصي الدراسة بإجراء دراسات أخرى تتضمن تفصيلاً في علاقة العوامل الالتهابية وكذلك هرمون اللبتين مع مقاومة الانسولين لدى المرضى المصابين بداء السكري من النوع الثاني.

Abstract:

Objective: To investigate the relation between dyslipidemia and insulin resistance where it is one of the metabolic disorders in patients with type-II diabetes mellitus and compare the results with the control group.

Methodology: Blood samples were collected from (35) patients with type-II diabetes mellitus, besides (35) healthy individuals as a control group were enrolled in this study. The age of all subjects range from (20-50). Serum was used in determination of glucose, insulin, lipid profile (cholesterol (Ch), triglyceride (TG), high-density lipoprotein (HDL-Ch), low-density lipoprotein (LDL-Ch) and very low-density lipoprotein (VLDL), for patients and control groups. Insulin resistance (IR) was calculated according to homeostatic model (HOMA-IR).

Results: The results revealed a statistically significant elevation levels ($P < 0.001$) in each of glucose, insulin, insulin resistance, Ch, TG, LDL-Ch, and VLDL, except HDL-Ch which was decreased in all patients group comparing with control group.

Recommendations: To study the Inflammatory factors and leptin and their relation to insulin resistance in type-II DM.

Keywords: Glycoprotein; Insulin Resistance; Type-II Diabetes Mellitus

Introduction:

Insulin is the primary hormone involved in glucose homeostasis, which is released from pancreas and oscillates within a period of 3-6 minutes^(1,2). Insulin resistance is a common pathological state in which target tissues fail to respond properly to normal levels of circulating insulin. Pancreatic B-cells first compensate for peripheral insulin resistance by increasing insulin secretion to maintain euglycemia⁽³⁾.

*Assistance Instructor, College of Education, University of Baghdad

**Assistance Instructor, Nursing Department, Koya Technical Institute

***Assistance Instructor, Basic Medical Science Department, College of Nursing, University of Baghdad

**** College of Education, University of Baghdad

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Hyperinsulinemia and insulin resistance are not only increased in type-II diabetes, but were also found to be correlated with the occurrence of hypertension, hypertriglyceridaemia and decreased HDL-Ch⁽⁴⁾.

The following values indicate risk factor for insulin resistance^(5,6):

- Fasting insulin ≥ 15 mIU/ml
- Triglycerides ≥ 130 mg/dl
- Triglycerides : HDL ratio > 3.5
- C-reactive protein > 3.00 mg/l

Fifty years later, the researches described a condition of insulin resistance, high insulin levels, high triglycerides, and low HDL-Cholesterol that rise in both type-II diabetes and heart disease risk^(7,8,9).

The American Association of Clinical Endocrinologists (AACE) cites the risk factor for insulin resistance syndrome is being overweight or obese, being over forty years age and elevated triglycerides/low HDL-Cholesterol⁽³⁾.

Recent studies indicate that the acute elevation of non-esterified fatty acids (NEFA) causes an increase in endogenous glucose production and a decrease in clearance of insulin (without increase in insulin secretion) resulting in hyperinsulinemia^(10,11).

Methodology:

Thirty five patients with type-II DM of (20-50) years-old age who attend the National Diabetes Center (NDC) and thirty five healthy individuals as a control group were enrolled in this study. 10 ml of blood samples were collected into disposable plain plastic tube from the patients and the healthy control after an overnight fasting, and centrifuged at 3000 rpm within 30 minutes of collection. The resulting serum was used for determination of glucose, lipid profile (Ch, TG, HDL-Ch, LDL-Ch) which were all measured by enzymatic methods. Insulin was measured by enzymatically immunoassay. Serum glucose was determined by using a kit from LINEAR Chemicals, S.L. Spain. According to Trider reaction, the glucose is oxidized to D. gluconate by the glucose oxidase (GOD) with the formation of hydrogen peroxide. In the presence of peroxidase (POD), a mixture of phenol and 4-amino antipyrine is oxidized by hydrogen peroxide, to form a red quinoneimine dye⁽¹²⁾. Lipid profile includes cholesterol (CH), triglyceride (TG), high-density lipoprotein (HDL-Ch), low-density lipoprotein (LDL-Ch) and very low-density lipoprotein (VLDL-Ch). The cholesterol was determined in serum by using a kit from LINEAR Chemicals, S.L.-Spain. In this method, three enzymes were used; cholesterol esterase (CE), cholesterol oxidase (CO) and peroxidase (POD). In the presence of the former the mixture of N-ethyl - N-propyl -m- anisidine (ADPS) and 4- amino antipyrine are condensed by hydrogen peroxide to form a quinoneimine dye⁽¹²⁾. Triglycerides were measured by using a kit from LINEAR Chemicals, S.L. - Spain. The method is based on the enzymatic hydrolysis of serum or plasma triglyceride to glycerol and free fatty acids (FFA) by lipoprotein lipase (LPL)^(12,13). The glycerol is phosphorylated by adenosine triphosphate (ATP) in the presence of glycerolkinase (GK) to form glycerol-3-phosphate (G-3-P) and adenosine diphosphate (ADP). Glycerol-3-phosphate is oxidized by glycerophosphate oxidase (GPO) to form dihydroxy acetone phosphate (DHAP) and hydrogen peroxide. A red chromogen is produced by the peroxidase (POD) catalyzed coupling of 4-aminoantipyrine and phenol with hydrogen peroxide (H₂O₂). High density lipoproteins-Cholesterol was determined by using a kit from LINEAR Chemicals, S.L. - Spain. This technique uses a separation method based on the selective precipitation of apolipoprotein B-containing lipoproteins (VLDL and LDL) by phosphotungstic acid/MgCl₂, sedimentation of the precipitant by centrifugation, and subsequent enzymatic analysis of HDL-Ch as residual cholesterol remaining in the clear supernatant⁽¹²⁾.

Low-density lipoprotein cholesterol concentration is most commonly calculated by using the empirical Friedwald formula which was based on the assumption that VLDL-Ch is present in serum at a concentration equal to one fifth of the TG concentration⁽¹⁴⁾.

$$\text{LDL-Ch (mg/dl)} = \text{Total Cholesterol} - \left(\text{HDL-Ch} + \frac{\text{TG}}{5} \right)$$

$$\text{VLDL} = \frac{\text{TG}}{5}$$

Insulin concentration was determined by using the DSL-10-1600 ACTIVE insulin ELISA which is an enzymatically amplified (one-step) sandwich-type immunoassay from Diagnostic systems laboratories, Inc., USA. In this assay, standards, controls and patients serum samples are incubated with HRP-labeled anti-insulin antibody in microtitration wells which have been coated with another anti-insulin antibody. After incubation and washing, the wells are incubated with the substrate tetramethylbenzidine (TMB). An acidic stopping solution is then added and the degree of enzymatic turnover of the substrate is determined by dual wavelength absorbance measurement at 450 and 620nm. A set of insulin standards is used to plot a standard curve of absorbance versus insulin concentration⁽¹²⁾. The degree of insulin resistance was estimated at the baseline by HOMA. Scoring was performed by using fasting insulin and glucose for calculation (insulin measured in (μ IU/ml), glucose was measured in (mmol/L) according to the following reaction⁽¹³⁾.

$$\text{HOMA} = [(I_o \times G_o) / 22.5]$$

I_o = Fasting serum insulin

G_o = Fasting blood glucose

Data were presented as mean and standard deviation SD. Study of T-test (p) was used to compare the significance of the difference in the mean values of any groups ($p < 0.05$) were considered statistically significant. The overall predictive values for the results in all studied groups were performed according to biostatistics⁽¹⁵⁾.

Results:

The levels of fasting glucose, insulin and insulin resistance (IR) in sera of 35 patients with diabetes mellitus and 35 healthy individuals as control group were measured which expressed as mean \pm SD, (Table 1).

Table 1. Levels of glucose, insulin and insulin resistance in sera of patients with diabetes mellitus and control group

Subjects	Glucose concentration mg/dl	Insulin μ IU/ml	IR	T-test
Control group	95.8 \pm 9.5	7.1 \pm 1.23	0.23 \pm 0.02	P<0.001
Patients group	180.2 \pm 38.1	18.12 \pm 10.4	7.9 \pm 0.96	P<0.001

IR= Insulin Resistance; mg/dl= Milligram per deciliter; μ IU/ml= Micro International Unit per milliliter

The concentrations of cholesterol (Ch) triglyceride (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) in sera of 35 patients with diabetes mellitus and 35 healthy individuals as control group were determined and shown in table (2).

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Table 2. The level of Ch, TG, HDL, LDP and VLDL in sera of patients with diabetes millets and control group

Subject	Ch mg/dl	TG mg/dl	HDL mg/dl	LDL mg/dl	VLDL mg/dl	T-Test
Control group	170.5±15.2	71±25	53±5	101.3±5	16.2±5	P<0.001
Patients group	231±22.6	165.3±50.8	46.6±9.1	151.3±3.34	33.06±10.16	P<0.001

Ch=cholesterol; HDL=High-density Lipoprotein; LDL=Low-density Lipoprotein; mg/dl= Milligram per deciliter; P= Probability Level; VLDL=Very Low-density Lipoprotein

Discussion:

Unfavorable changes in lipoproteins may help explain the increased risk for D.M. observed with IR states^(16,17). The study reported that IR has been proposed to be an important cause of dyslipidemia⁽¹⁸⁾.

Lipoproteins mediate feeding cycle after a meal eating by transporting lipids from the intestine as chylomicrons and from the liver as VLDL to most tissues for oxidation and to adipose tissue for storage. Lipid is mobilized from adipose tissue as free fatty acids (FFA) attached to serum albumin. Abnormalities of lipoprotein metabolism cause various hypo or hyperlipoproteinemias. The most common of these is diabetes mellitus, where insulin deficiency causes excessive mobilization of FFA and underutilization of chylomicrons and VLDL, leading to hypertriacylglycerolemia⁽¹⁹⁾.

The results revealed that there is a statistically significant elevation (P<0.001) in each of glucose, insulin and insulin resistance in all patients group compared with that found in control group.

The results revealed that there is statistically significant elevation (P<0.001) in each of Ch, TG, LDL, VLDL except HDL which showed significant reduction in all patients group compared to control group. The results are in agreement with the results of previous study which had been reported that the low HDL and elevation of TG, Ch were associated with IR⁽²⁰⁾.

Conclusion:

The study concluded that there is a statistically significant elevation in the levels of glucose, insulin, insulin resistance, Ch, TG, LDL-Ch, and VLDL except HDL-Ch which was decreased in all patients comparing with control group.

Recommendations:

1. We recommend to study the inflammatory factors and cytokinaes (e.g. c-reactive protein, tN α , Interleukins ... etc) in type-II diabetic patients and their relation to insulin resistance.
2. To have further studies on leptin hormone levels in type-II DM and its relation to insulin resistance.

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