



Association Of Leptin To Insulin Resistance In Iraqi Male Patients With Cardiovascular Disease Undergoing Angiography

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ABSTRACT

Cardiovascular diseases (CVD) remain the biggest cause of deaths worldwide. Leptin is a peptide hormone playing an important role in the regulation of food intake and body weight . It has been noted that insulin resistance and hyperleptinemia are associated with metabolic syndrome and cardiovascular disease (CVD).

Keywords: Cardiovascular disease, leptin, insulin resistance.

INTRODUCTION

Coronary artery disease (CAD), stroke, and ischemia are the leading cause of mortality worldwide. Cardiovascular mortality rates have declined in many high-income countries. At the same time cardiovascular deaths and disease have increased at a fast rate in low- and middle-income countries [1]. The causes of cardiovascular disease are diverse but atherosclerosis and/or hypertension are the most common [2]. Atherosclerosis is a complex, chronic disease characterized by lipid accumulation and inflammation within the intima layer of vessel wall [3]. Leptin ,a peptide hormone, is mainly secreted by adipose tissue plays an important role in regulating food intake, energy expenditure and adiposity [4]. Interestingly, leptin has a structure similar to that of the family of helical cytokines namely interleukins. Thus, leptin shares an extreme functional pleiotropy with other cytokines and is involved in quite diverse physiological functions [5]. Plasma leptin displays a strong association with cardiovascular risk factors, including insulin resistance and metabolic syndrome, even after controlling for measures of body fat mass [6].

Several clinical studies [7,8] demonstrate that hyperleptinemia predicts acute cardiovascular events, restenosis after coronary injury such as angioplasty, and cerebral stroke independent of traditional risk factors. In addition to its pleomorphic activities leptin may also exert actions related to cardiovascular homeostasis that are potentially atherogenic, thrombotic, and angiogenic [9]. Therefore, the aim of the study to assess the relationship of leptin to insulin resistance in patients with coronary artery disease undergoing angiography.

MATERIALS AND METHODS

In this study, serum fasting concentration of leptin was measured using an ELISA kit in 60 male patients with angiographically proved CVD (mean age \pm SD was 57.6 ± 10.2 y) and 30 subjects as a healthy control group (mean age \pm SD was 55.7 ± 9.95 y). Fasting blood glucose and lipid profile were measured by spectrophotometrically. The mean BMI of CVD patients and control group was 29.07 ± 2.96 and 28.7 ± 2.58 kg/m², respectively.

Methodology

Patients: Sixty male patients with angiographically proved by CVD attending Open Heart Center in Al - Sader Medical City in Najaf province from January 2013 to April 2013. The patients ages ranged between (30-80) years. Any acute disease and chronic diseases other than coronary artery diseases such as diabetes mellitus (DM), Rheumatic disease, etc were excluded from the study.

Controls: A healthy subject group of 30 male was included in the study as a control group. Their age and weight ranges were comparable to that of the enrolled patients. None of these subjects had an obvious systemic diseases.

Blood samples: Venous fasting blood samples (5ml) were collected from both patients before coronary angiography and healthy control group in plain tubes containing no anticoagulant. Disposable syringes and needles were used for blood collection. After allowing the blood to clot at room temperature for about 15 min, blood samples were centrifuged at 2555.7 xg for 15 min. Resulted serum was separated in two aliquots into plain tubes, where stored in -20°C until to be assayed.

Methods

Determination of body mass index (BMI): The body mass index was calculated using the formula [10]
$$\text{BMI} = \text{weight (Kg)} / \text{height (m}^2\text{)}$$

Determination of fasting glucose and lipid profile: Serum glucose and lipid profile (total cholesterol, triglyceride, high density lipoprotein cholesterol (HDL-C) were measured spectrophotometrically by enzymatic reactions using ready for use kits, supplied by Biolabo, France. Serum HDL-C was determined after precipitation of other lipoproteins by the reagent containing sodium phosphor-tungstate along with magnesium chloride and the cholesterol contents in the supernatant were measured by the cholesterol kit [11]. Very low density lipoprotein cholesterol (VLDL-C) was calculated from TG/5 and low density lipoprotein (LDL-C) from Friedewald's formula: LDL-cholesterol = total cholesterol – HDL-C – VLDL-C [12].

Determination of serum insulin levels: Serum insulin was determined by enzyme linked immune sorbent assay (ELISA) based on the sandwich principle (DRG kit, Germany) [13].

Determination of insulin resistance: Insulin resistance was measured by Homeostasis model assessment
$$\text{HOMA-IR} = [\text{Glucose (mmol/l)} \times \text{Insulin (}\mu\text{IU/ml)}] / 22.5$$
 [14].

Determination of serum leptin levels: Leptin ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle (DRG, Germany) [15].

RESULTS AND DISCUSSION

Selection of patients and parameters: Table 1: enroll parameters of 60 CVD patients, and 30 healthy individuals. It includes two groups: patients and control individuals respectively. Mean, Standard

Deviation (SD), and Range values of Age, weight, height and BMI were tabulated. No statistically significant difference in age and BMI were observed between the two groups (age and weight matched).

Table 1: Clinical characteristics of CVD Patients and control group

Parameters	CVD Patients (n= 60)	Control (n= 30)	P-Value
	Mean \pm SD (Range)	Mean \pm SD (Range)	
Age (y)	57.6 \pm 10.2 (35–79)	55.7 \pm 9.95 (38–74)	NS
Weight (kg)	83.2 \pm 10.1 (66–129)	85.1 \pm 9.34 (68–110)	NS
Height (m)	1.69 \pm 0.04 (1.57–1.82)	1.72 \pm 0.05 (1.6–1.9)	0.013
BMI (kg/m ²)	29.07 \pm 2.96 (26.2–39.9)	28.7 \pm 2.58 (24.6–36.3)	NS
SBP (mmHg)	129 \pm 12 (100–170)	124.5 \pm 6.2 (110–135)	0.022
DBP (mmHg)	77.3 \pm 6.5 (70–95)	74.8 \pm 4.8 (70–80)	0.044

SD (Standard Deviation), BMI (Body Mass Index), CVD (cardiovascular disease)
SBP(systolic blood pressure), DBP (diastolic blood pressure), NS (No significant)

Biochemical parameters determined in CVD patient & control groups: The measured parameters of study were tabulated for CVD patient and control group, each one of these characteristics is presented by (Mean \pm SD) , range values and p -values as shown in Table 2. The results of the present study showed significantly high serum levels of leptin ($p < 0.05$) , insulin ($p < 0.001$), HOMA-IR ($p < 0.001$), TC ($p < 0.05$), TG ($p < 0.001$),VLDL-C ($p < 0.001$) and TG/HDL-C ($p < 0.001$) in CVD patients when compared with those of the control group. While, no significant ($p > 0.05$) of HDL-C, LDL-C levels, TC/HDL-C and LDL-C/HDL-C ratio in CVD patients were observed when compared with those of the control group. in CVD patients when compared with those of the control group.

Table 2: Biochemical parameters in CVD patients & control group.

Parameters	CVD Patients (n= 60)	Control Group (n= 30)	P- Value
	Mean \pm SD (Range)	Mean \pm SD (Range)	
Leptin (ng/ml)	14.9 \pm 14.7 (1.8–82.3)	8.7 \pm 6.3 (1.5–25.9)	0.006
Insulin (μ IU/mL)	14.14 \pm 6.44 (6.5 – 30.8)	9.82 \pm 4.57 (4.6 –21.6)	< 0.001
HOMA-IR	2.81 \pm 1.27 (1.27 – 6.87)	1.94 \pm 0.88 (0.87 – 4.03)	< 0.001
TC (mg/dl)	179.6 \pm 50.2 (80–310)	160.1 \pm 28.2 (85 – 195)	0.01
TG (mg/dl)	174.4 \pm 61.9 (75 – 286)	130.1 \pm 34 (70 – 185)	< 0.001

HDL-C (mg/dl)	37.3 ± 9.4 (22 – 71)	37.07 ± 7.5 (22 – 54)	NS
LDL-C (mg/dl)	107.5 ± 51.5 (12 – 243.8)	96.9 ± 30.8 (24.4 – 147)	NS
VLDL-C (mg/dl)	34.87 ± 12.4 (15 – 57.2)	26 ± 6.8 (14 – 37)	< 0.001
TC/HDL-C	5.1 ± 2 (2 – 11.5)	4.5 ± 1.4 (2.07 – 8.18)	NS
TG / HDL-C	4.9 ± 2.1 (1.87 – 10.2)	3.7 ± 1.3 (1.52 – 7.1)	0.001
LDL-C/HDL-C	3.2 ± 1.8 (0.28 – 9)	2.8 ± 1.3 (0.6 – 6)	NS

SD (Standard Deviation), TC (Total cholesterol), TG (Triglyceride), HDL-C (High density lipoproteins cholesterol), LDL-C (Low density lipoproteins cholesterol), CVD (cardiovascular disease), NS (No significant)

Correlation of leptin levels with biochemical parameters in CVD patients: Table 3: showed the linear regression analysis used to analyze the correlation of the studied biochemical parameters with leptin in sera of CVD patients. The results indicated a significant negative correlation observed between leptin and HDL-C (P=0.015), and positively correlated (p<0.01) with BMI, FI (P=0.006), HOMA-IR (P=0.019), TG (P=0.04), VLDL-C (P=0.04), TC/HDL-C (P=0.002), TG/HDL-C (p<0.01) , LDL-C/HDL-C (P=0.01). While, no significant positive correlations of TC and LDL-C with leptin level were observed.

Table 3: Correlation of leptin with the studied biochemical parameters in CVD patients

Parameters	Leptin (ng/ml)	
	r	P
BMI (kg/m²)	0.507	< 0.001
Insulin (μIU/ml)	0.351	0.006
HOMA-IR	0.303	0.019
TC (mg/dl)	0.237	NS
TG (mg/dl)	0.266	0.04
HDL-C (mg/dl)	-0.314	0.015
LDL-C (mg/dl)	0.223	NS
VLDL-C (mg/dl)	0.266	0.04
TC/HDL-C	0.393	0.002
TG/HDL-C	0.451	<0.001
LDL-C/HDL-C	0.331	0.01

r (regression analysis) TC (Total cholesterol), TG (Triglyceride), HDL-C (High density lipoproteins cholesterol), LDL-C (Low density lipoproteins cholesterol) , NS (No significant)

Leptin and cardiovascular disease: In the present study, serum leptin levels significantly higher in patients with CVD as compared to the control subjects. These results are consistent with previous studies [16,17,18] and in disagreement with Couillard C, et al.1998 and Piemonti L, et al. 2003 [19,20] who did not find an association between leptin and CVD. Several clinical studies demonstrate that hyperleptinemia predicts acute cardiovascular events, restenosis after coronary injury such as angioplasty, and cerebral stroke independent of traditional risk factors [7,8]. In addition to its pleomorphic activities, leptin may also exert actions related to cardiovascular homeostasis that are potentially atherogenic, thrombotic, and angiogenic [9, 21].

In one previous study leptin was showed to be an independent predictor of myocardial infarction in patients with arterial hypertension [22]. Furthermore, leptin has been related to coronary artery calcification in type 2 diabetic patients [23] and with several CV risk factors and vascular dysfunction in humans [24]. By contrast, no correlation between leptin and intima-media thickness is noted in 403 elderly men without ischemic heart disease [25] or in children with obesity or type 1 diabetes mellitus [26]. It is not clear why leptin levels are correlated with preclinical atherosclerosis in some studies but not others. This may depend on the pathophysiological context of the patients studied, medications taken, or other factors.

In the present study, the high leptin levels of angiographically confirmed CVD patients may increase the possibility of its role in development and progression of CVD. This may occur by its direct effects through the stimulation of vascular smooth cell proliferation, accelerates vascular calcification, induces oxidative stress in endothelial cells that may contribute to atherogenesis, and promotes coagulation by increasing platelet adhesiveness.⁽¹⁰⁾ or its indirect effect through augmentation of IR [10].

Leptin and insulin resistance in CVD patients: The present study showed a significantly the correlation between leptin levels and insulin resistance in obese CVD patients. Insulin potentiates leptin induced NO release by enhancing leptin-stimulated phosphorylation of endothelial NO synthase. This raises the possibility of cross talk between insulin and leptin signaling [27]. At physiological level, leptin inhibit insulin biosynthesis and secretion in pancreatic β -cells. In turn, insulin stimulates leptin secretion from adipose tissue, establishing a hormonal regulatory feedback loop, the so called adipoinular axis. Multiple signal transduction pathways are involved in leptin signaling in pancreatic β cells. In most overweight individuals, physiological regulation of body weight by leptin seems to be disturbed, representing "leptin resistance." This leptin resistance at the level of the pancreatic β -cell may contribute to dysregulation of the adipoinular axis and contribute to development of hyperinsulinemia and manifest type 2 diabetes mellitus in overweight patients [28].

Leptin and dyslipidemia in CVD patients: Dyslipidemia plays a major role in the pathogenesis and progression of atherosclerosis and cardiovascular diseases [29, 30]. Evidence has shown that elevated TG and VLDL-C serves as excellent biomarkers for atherosclerosis in part because of their association with atherogenic cholesterol-enriched remnant particles, which may be directly incorporated by macrophages and may participate in pro-atherothrombotic inflammatory signaling pathways [31]. On the other hand, a characteristic dyslipidemia is also associated with insulin resistance [32]. Several studies have reported the possibility that newly addressed lipid profiles might be more useful than the traditional ones used for CVD prediction, and measuring these variables might help identify insulin resistance and CVD [33]. Kimm, et al. 2010 [33] demonstrated that the lipid ratios of TC/HDL-C, LDL-C/HDL-C and TG/HDL-C, as well as TG and HDL-C are independently associated with insulin resistance and risk factors of CVD.

Results in the present study showed an association of atherogenic lipid profile parameters with CVD. Leptin and insulin resistance were positively correlated with high TG, mVLDL-C and TG/HDL-C ratio and negatively correlation with HDL-C. That are consistent with previous studies [7,34,35], and may indicate an adverse effects of high leptin with progression of CVD. Leptin stimulates lipoprotein lipase secretion in cultured human and murine macrophages [36]. Leptin increases accumulation of cholesterol esters in foam cells, especially at high glucose concentrations [37]. It directly stimulates phosphorylation and activation of AMP-activated protein kinase (AMPK) in skeletal muscle, increasing phosphorylation of acetyl-CoA

carboxylase (ACC) and fatty acid oxidation [38], In addition, it is known that leptin in adipocytes inhibits the synthesis of ACC, the rate limiting enzyme in the conversion of carbohydrates to long chain fatty acids and hence in the storage of energy as triacylglycerol [34]. Moreover, long-term treatment of wild-type mice with large leptin doses increases mRNA levels of the key lipolytic enzyme hormone-sensitive lipase but reduces those of the lipogenic enzyme fatty acid synthase [39]. Hormone-sensitive lipase levels are more immediately controlled by cellular levels of cAMP, so it seems that leptin, like glucagon and catecholamines, might stimulate lipolysis primarily by increasing cAMP concentrations. Furthermore, rats lacking functional leptin receptors have high levels of acyl-CoA synthetase and glycerol-3-PO4 acyltransferase (two enzymes required for lipogenesis), but low levels of acyl-CoA oxidase (ACO) and carnitine palmitoyl transferase I (two enzymes involved in fatty acid oxidation). It has been hypothesized that one of the functions of leptin is to keep the triacylglycerol content of non-adipocytes low, thereby protecting them from steatosis and lipotoxicity [39]. In addition to the indirect effect of leptin on lipid metabolism and its reduction of the lipogenic effects of insulin, the addition of insulin to cultured leptin-deficient adipocytes increases the synthesis of ACC, fatty acids and triacylglycerol to a greater extent than in adipocytes that do produce leptin, possibly due in part to leptin inhibition of insulin-adipocyte binding. Like the direct effects of leptin, this action also extends to non-adipocytes: the insulin-induced increase in triacylglycerol synthesis and decrease in fatty acid oxidation in isolated mouse skeletal muscle are reduced by simultaneous administration of leptin [39]. Serum leptin levels were significantly higher ($p < 0.05$) in CVD patients when compared with those of the control group. There was a significant positive correlation between leptin with, BMI, HOMA-IR, triglyceride, VLDL-C, TC/HDL-C, TG/HDL-C and LDL-C/HDL-C, and there was a significant negative correlation between leptin with HDL-C in CVD patients.

APPLICATIONS

These results are applicable to assess the relationship of leptin to insulin resistance in patients with coronary artery disease undergoing angiography

CONCLUSIONS

High leptin level is associated with insulin resistance in male patients with coronary artery disease proved by angiography.

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