University of Thi-Qar Journal of Science (UTJsci)
E-ISSN:2709-0256, P-ISSN: 1991-8690, Vol. (10), No.1(Special Issue: ISCAMET), April. 2023

Evaluation of leptin hormone and Tumor necrosis factor levels in patients with type two diabetes mellites

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Abstract **Background: Type 2 diabetes mellitus (T2DM) is a leading cause of mortality and morbidity worldwide. Leptin, an adipokine with the primary function of regulating energy balance, is found to mediate insulin secretion and sensitivity in peripheral tissues. Aims: Hence, we aimed to determine the role of leptin in the development of insulin resistance (IR) in newly diagnosed T2DM patients and determine tumor necrosis factoralpha (TNF-α). Materials and methods: A case control study a total of 80 patients and 50 control groups, used Cobas E311 device to measure glucose, whereas HbA1c used D-10 hemoglobin Hba1c device whereas other parameters used ELISA methods were used to analyses 130 samples Results: Highly significant increase level of Fasting blood sugar, hemoglobin A1c, Insulin, Leptin and HOMA- insulin resistance whereas significant decrease level of TNF alpha and , Plasminogen activator inhibitors -1 in patient type two diabetes mellitus compared to the control group. Conclusion: increased the level of leptin levels while TNF-α levels were decreased in type 2 diabetes patients.**

Keywords—: Leptin, TNF-α, insulin resistance, type 2 diabetes mellitus, pro-inflammatory cytokines.

I. Introduction

Diabetes Meletus (DM) is a set of metabolic conditions defined and distinguished by hyperglycemia in the absence of medication. Insulin secretion deficiencies, insulin action defects, or both, as well as carbohydrate, lipid, and protein metabolic disturbances, are all examples of heterogeneous etiopathology of DM. DM problems such as retinopathy, nephropathy, and neuropathy, among others, have long-term serious impacts (Artasensi et al., 2021). Heart disease, peripheral arterial and cerebrovascular disease, obesity, cataracts, erectile dysfunction, and non-alcoholic fatty liver disease are all more common in people with diabetes. They are also more susceptible to infectious infections, such as Tuberculosis (TB) (Kazi & Blonde, 2019).

Leptin is an adipocyte-derived hormone, which is also known as an adipocytokine as it plays a role in the inflammatory process involving adipose tissues(Conde et al., 2011). The role of leptin in altering glucose metabolism and insulin sensitivity in T2DM is not fully explored. Since IR is the basis behind the development of T2DM, we designed the present study to estimate the levels of leptin and IR (using HOMA-IR) in newly diagnosed T2DM patients, in a view to determine the role of leptin in the development of IR in T2DM (Moonishaa et al., 2017).

Development of insulin resistance and pathogenesis of type 2 diabetes mellitus (T2DM) are directly interlinked with each other. Among various causative factors that are decisively involved for the pathogenesis of T2DM and development of insulin resistance, pro-inflammatory and/or oxidative stress mediators are most important(Conde et al., 2011). Among the various pro-inflammatory cytokines, tumor necrosis factoralpha (TNF-α) is one the most important pro-inflammatory mediator that is critically involved in the development of insulin resistance and pathogenesis of T2DM. TNF-α is mainly produced in adipocytes and/or peripheral tissues, and induces tissue-specific inflammation through the involvement of generation of ROS and activation of various transcriptional mediated pathways. The raised level of TNFα induces insulin resistance in adipocytes and peripheral tissues by impairing the insulin signaling through serine phosphorylation that leads to the development of T2DM(Akash et al., 2018; Wieser et al., 2013).

Aims of this study determine the role of leptin in the development of insulin resistance (IR) in newly diagnosed T2DM patients and determine tumor necrosis factor-alpha (TNF-α), and show the correlation of leptin and TNF-α with insulin, Homo insulin resistance , and Plasminogen activator inhibitors 1 biomarkers in patients with diabetes Mellitus compared to the control group.

II. Materials and Methods

A. Study population and sample collection

A case control study of a total of 130 patients and control groups [patient type 2 diabetes mellitus group as a total N= 80 (male=38, female =42), and control group as a total N=50 (male=28, female=22)], with an age range from (33 to 73) years, who attended Al-Shafaa teaching hospital in Basra. All patients in this study were diagnosed by specialist physicians in Al-Shafaa teaching hospital in Basrah throughout the period from November 2021 to February 2022.

B. Blood Samples collection and processing

From each participant, five milliliters of blood were drawn, three milliliters were transferred to sterilized test tubes, the sample was coagulated at room temperature for 30 minutes, the sample was separated by centrifugation at 3000 rpm for 15 minutes, the serum was isolated and stored (minus twenty ^{0}C) until analyzed, and 1.8 milliliters of blood were put in an EDTA tube, used Cobas E311 device for measuring glucose (Roche / Germany), HbA1c used D-10 Hemoglobin Testing System - Bio-Rad device (D-10 /USA) device whereas other parameters used a ELISA methods(Sun long / Korea) were used to analyses 130 samples.

C. Statistical Analysis

The data are expressed as means ± standard deviations (SD). The t-test and the chi-square test were used to see whether there were any differences in the means of the groups. Variable correlations were also investigated. SPSS for Windows was used to conduct all statistical analyses (version 26, USA). The Mann Whitney test was used in addition to the non-parametric and T-test used for the normal distribution. P < 0.05 was regarded as statistically significant.

III. Results and discussion

In table 1 showed the Sex and Residence parameters were non significantly p-value > 0.05 when compared control with patient groups, whereas Smoking and Blood pressure parameters were significantly p-value < 0.05, We note that the numbers of diabetics are more susceptible to blood pressure than healthy people because patients with type 2 diabetes at high risk for cardiovascular events, targeting a systolic blood pressure (Garrick, 2011)**,** Raised blood pressure is more common in people with type 2 diabetes than in the general population. In patients with type 2 diabetes the risk of diabetic complications was strongly associated with raised blood pressure. Any reduction in blood pressure is likely to reduce the risk of complications, with the lowest risk being in those with systolic blood pressure less than 120 mm Hg(Stratton et al., 2000), also blood pressure is one of the risk factors affecting type 2 diabetes patients according to the study (DeFronzo et al., 2015)

Table 1 Statistical distribution (frequency of percentages) between control and patients' groups by their (sex, address, smoking and blood pressure).

Items		Groups				Sig.*
		Control (N=50)		Patient (N=80)		
		Freq.	%	Freq.	%	
Sex	Male	28	56.0%	38	47.5%	0.346
	Female	22	44.0%	42	52.5%	
Residence	Central	22	44.0%	41	51.3%	0.421
	Peripheral	28	56.0%	39	48.8%	
Smoking	Yes	4	8.0%	28	35.0%	0.001
	No	46	92.0%	52	65.0%	
	Yes	8	16.0%	62	77.5%	0.0001
Blood Pressure	No	42	84.0%	18	22.5%	

* Chi-Square Test

For each FBS, BMI, and HbA1C, in the table 2 displays statistically significant difference p- values 0.05, whereas age was non-significant difference p- values > 0.05, was agreed with previous research(Chakrabarti et al., 2022; Gupta, 2016; Ouchi et al., 2022)**.** In order to track a diabetic's glycemic management, glycated hemoglobin (HbA1c) levels are routinely monitored. With the aim of lowering, it in diabetics who are receiving treatment, the level of circulating HbA1c is considered the gold standard for monitoring glycemic control. Hemoglobin A1c (HbA1c) is the gold standard for monitoring glycemic control and serves as a surrogate for diabetes-related complications. Although HbA1c measures mean glycemic exposure during the preceding 2 to 3 months, it does not provide information about day-to-day changes in glucose levels. Self-monitoring of blood glucose represents an important adjunct to HbA1c because it can distinguish between fasting, preprandial, and postprandial hyperglycemia (Dailey, 2007). As a result, the HbA1c level may be used as a potential biomarker to identify individuals who are at high risk of developing dyslipidemia and cardiovascular disease (Baranwal et al., 2017). Improved glycemic control, as demonstrated by a reduced HbA1c level, has been shown to have a positive impact on patients' lipid profiles in certain prior research, whereas the aforementioned parameters were found to have either no significant link or a negative relationship in other studies(Deshmukh et al., 2015; Naeem et al., 2016; Ozder, 2014; Samdani et al., 2017; Sarkar & Meshram, 2017)**.**

Table 2 Differences of the age, BMI, FBS and Hb A1c between the study groups

Variables	**Control (N=50)**	**Patients (N=80)**	**P. Value**
	Mean±SD	**Mean±SD**	
Age (years)	56.1±8.75	53.1±9.61	0.079
BMI (Kg/m^2)	26.080±7.964	30.160±5.053	0.001
FBS (mg/dl)	89.36 ±8.861	189.74±94.623	0.001
HbA1C (%)	5.580 ±1.004	8.9275 ±2.335	0.001

In this table 3 shown increase significant level of insulin and HOMA -IR (P value 0.001), this study was agree with previous studies(Tangvarasittichai, 2015). In the general population, insulin resistance develops several years before the onset of type 2 diabetes and has multiple factors, including genetics(Reaven, 2008; Unger, 2008). The two most significant features of the pathophysiology of T2DM are insulin resistance and reduced insulin production(Dedoussis et al., 2007; Tangvarasittichai, 2015).

Modern lifestyle variables including physical inactivity, abdominal obesity, and an overproduction of adipokines can contribute to insulin resistance. The first step in maintaining normal glucose tolerance is compensatory hyperinsulinemia. About 25% of non-diabetic patients in the same individuals have insulin resistance. The continuing increases and/or reductions in insulin secretory compensatory responses that have been seen in T2DM patients led to a reduction in glucose tolerance. Oxidative stress, ROS production, and the activation of stress transduction factor pathways all rise with elevated glucose, FFA, and insulin levels. By decreasing insulin action and secretion, this may hasten the onset of T2DM(Reaven, 2008; Tangvarasittichai, 2015). In this table 3 shown significant decrease level P value= 0.049 in patient diabetes mellitus compared to control groups, our study match with(Rodrigues et al., 2017; Swaroop et al., 2012). Tumor Necrosis Factor Alpha 1 (TNF-) is an adipocytokine that has a function in systemic inflammation and triggers the acute phase response. The bulk of TNF-producing cells, including adipocytes, are macrophages, although there are several other cell types that function just as well. TNF- influences glucose metabolism and prevents insulin from being transmitted. As obesity and insulin resistance have been connected to metabolic illnesses, including type 2 diabetes mellitus, it has been hypothesized that TNF- metabolism abnormalities may also have an effect on the development and progression of this disease, but this mechanism is unclear(Swaroop et al., 2012; Zou & Shao, 2008).

Plasma leptin levels in T2DM patients were significantly higher in our study (P = 0.001) compared to the control group, which is in agreement with earlier research(Andrade-Oliveira et al., 2015; Katsiki et al., 2018). Elevated leptin levels are linked to the development of insulin resistance and T2DM. It has also been reported that CHD patients have higher leptin levels After myocardial infarction(MI), serum leptin concentrations rise (Katsiki et al., 2018) also the leptin level in T2DM patients were significantly associated with the presence of smoking, obesity, hypertension, dyslipidemia, and metabolic syndrome(Sari et al., 2010). Leptin has an inhibitory effect on the insulin secretion, which is due to the leptin-induced proinflammatory cytokines such as C-reactive protein and Interleukin-6, causing apoptosis of pancreatic β-cells (Moonishaa et al., 2017; Sun & Rutter, 2011). The 50-kDa glycoprotein known as plasminogen activator inhibitor-1 is a member of the protein family known as serine protease inhibitors (serpins) (PAI-1). PAI-1 is the main regulator of the endogenous fibrinolytic system. PAI-1 is made up of three beta sheets and nine alpha helices. PAI-1 can attach to the somatomedin B domain in addition to interfering with proteasome activity and cell adhesion to the extracellular matrix. Only two tissues express and manufacture PAI-1: the liver and spleen. The synthesis of PAI-1 is regulated by insulin, very-low-density lipoprotein
(VLDL), low-density lipoprotein (LDL), and glucose
(Rabieian et al., 2018).

Table 3 Differences of the biomarker levels indicators between the in this study.

Variables	Control (N=50)	Patients (N=80)	P. Value
	Mean±SD	Mean±SD	
Insulin mIu/L	13.0480 ±9.247	67.2513±30.08803	0.001
HOMA IR	2.8596±2.045	34.0201±29.95485	0.001
TNF ng/L	68.3566±59.717	54.8964±67.36606	0.049
Leptin ng/ml	30.5484±30.811	76.4338±35.24778	0.001
PAI-1 Au/ml	18.5468±17.364	5.6123±7.58196	0.001

study groups * Mann Whitney-U Test

In the table 4 show positive correlation between insulin with both Homo-IR & Leptin, this study was agree with previous study (Moonishaa et al., 2017). In compliance to this theory, leptin levels should have an only negative correlation with insulin levels, which is opposite to what we see in the present study. This can be explained by the underlying IR leading to elevated insulin levels, along with a possible leptin resistance in our study population. The possible explanation could be because leptin and insulin share the same pathway for their actions, leading to a leptin-insulin crosstalk. Sun and Rutter described that the central appetite controlling actions of leptin and insulin are mediated through the hypothalamic AMP-Kinase pathway(Moonishaa et al., 2017; Sun & Rutter, 2011). Other mechanisms for the leptin-mediated insulin sensitivity are by inhibiting the Sterol regulatory element binding protein-1 and stearoyl-CoA desaturase-1(Rondinone, 2007). Leptin also stimulates the insulin receptor substrate and PI-3K in the insulin signaling pathway, thus increasing the peripheral insulin sensitivity by way of increased glucose uptake and fatty acid oxidation in the tissues. Leptin resistance, which is more common with obesity, also leads to IR, linking leptin's role in the pathogenesis of T2DM(Welsh et al., 2009).

Table 4 Spearman's correlations among biomarkers including in patient group.

Variable		HOMA IR	TNF alpha	Leptin	PAI-1
Insulin	R	.826	-.03	.236	-.04
	Sig.	.000	.80	.035	.703
HOMA IR	R		.10	.016	-.18
	Sig.		.36	.889	.100
TNF alpha	R			.009	.088
	Sig.			.93	.438
Leptin	R				.003
	Sig.				.98

IV. Conclusions

Hyperleptinemia reflecting leptin resistance plays an important role in the development of IR in T2DM patients, making leptin a possible biomarker for assessing IR levels in T2DM patients, especially in the obese, TNF-α levels were decreased in patients. Our data suggest that serum leptin and TNF-α levels may serve as markers of obesity and type I diabetes mellitus.

Conflict of interest: There is no conflict of interest.

Acknowledgments: We would like to thank all participants

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