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STUDY THE ROLE OF OBESITY AND OXIDATIVE STRESS AS FACTORS OF TYPE 2 DIABETES

Edrees M.Ameen[1*], Hassan Y. Muhammad[2] and Sabah N. Alwachi[3]

[1]Biology Department, Science College, Salahaddin University, Erbil-IRAQ.
[2]Biology Department, Science College, Baghdad University, Baghdad-IRAQ.
[3]Biology Department, Faculty of ART and Science, American University of Beirut, Lebanon.

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***Correspondence for Author**
Edrees M. Ameen
Biology Department,
Science College,
Salahaddin University,
Erbil-IRAQ.

ABSTRACT

Background: Diabetes mellitus (DM), commonly referred to as diabetes, is a group of metabolic diseases in which there are high blood sugar levels over a prolonged period It is known that type 2 diabetes mellitus and obesity are bound common metabolic problems. The adipokines or adipocytokines are cytokines (cell signaling proteins) secreted by adipose tissue, their action is thought to modify many obesity-related diseases. Oxidative stress (malondialdehyde the final result of lipid peroxidation is considered a biomarker of oxidative stress) is another underlying cause of inflammation in type-2 diabetes (T2DM) playing a central role in causing late diabetic complications such as insulin resistance and impaired insulin secretion in T2DM. **Materials and Methods:** In the present study, 140 males with the ages between (30-60) years, 90 males of them diagnosed with diabetes type 2 and 50 of them were used as control groups. The subjects were divided into four groups, group (I) control, none-obese (25 samples): group (II) control, obese (25 samples); group (III) none-obese diabetic patients (45 samples), group (IV) obese diabetic patients (45 samples). The parameters in the present study were included Adipokines (Leptin, Resistin, and Apelin) and Malondialdehyde. **Results:** The study showed a highly significant ($P<0.05$) increased in Leptin, Resistin, Apelin in diabetic groups (none-obese and obese) when compared with control groups, with the higher concentration of them were observed in obese diabetic group. The results demonstrated a highly prevalence of abnormal concentration of leptin, Resistin in diabetic groups compared with control groups. Furthermore the results found significant positive correlation between these Adipokines together and with Body mass index (BMI). Regarding

oxidative stress, the results showed a significant increase (P<0.05) in serum Malondialdehyde (MDA) concentration in obese control, none-obese diabetic and obese diabetic groups when compared with none-obese control group, and higher concentration of MDA were recorded in diabetic obese group. Positive correlation were found between MDA with body mass index, leptin, Apelin and resistin in type 2 diabetes.

KEYWORDS: Type-2 diabetes, Obesity, Adipokines, Leptin, Resistin, Apelin, Oxidative stress, Malondialdehyde.

INTRODUCTION

During the last twenty years the prevalence of diabetes has increased dramatically in many parts of the world. As of 2014, an estimated 387 million people have diabetes worldwide with type 2 diabetes making up about 90% of the cases.[1] The number of people with diabetes is expected to rise to 592 million by 2035, this is equal to 8.3% of the adult population,[2] The disease is now a worldwide public health problem.[3]

Diabetes mellitus (DM), commonly referred to as diabetes, is a group of metabolic diseases in which there are high blood sugar levels over a prolonged period,[4] Symptoms of high blood sugar include frequent urination, increased thirst, and increased hunger, if left untreated, diabetes can cause many complications.[5] Serious long-term complications include cardiovascular disease, stroke, kidney failure, foot ulcers and damage to the eyes.[2]

It is known that type 2 diabetes mellitus and obesity are bound common metabolic problems. In 2013 the American Medical Association classified obesity as a disease [6]. It is defined by body mass index (BMI) and further evaluated in terms of fat distribution via the waist–hip ratio and total cardiovascular risk factors. BMI a measurement obtained by dividing a person's weight by the square of the person's height, exceeds 30 kg/m2, with the range(25-30) kg/m2 defined as overweight.[7]

The adipokines or adipocytokines are cytokines (cell signaling proteins) secreted by adipose tissue, their action is thought to modify many obesity-related diseases.[8] Adipose tissue is an endocrine organ that secretes numerous protein hormones, including leptin, Apelin, Resistin and Tumor necrosis factor-α are produced primarily in the adipocytes of white adipose tissue.[9]

Oxidative stress (malondialdehyde the final result of lipid peroxidation is considered a biomarker of oxidative stress) is another underlying cause of inflammation in type-2 diabetes (T2DM) playing a central role in causing late diabetic complications such as insulin resistance and impaired insulin secretion in T2DM.[10] Furthermore, oxidative stress has been shown to contribute to the fibrillization and aggregation of tissue specific as well as non-specific proteins in T2DM,[11] Hence, oxidative stress has been linked to inflammatory pathways in muscle cells and adipocytes and to impaired insulin secretion in pancreatic β-cells.[12] For this purpose, the main aim of the study is to through some light on the effect obesity and oxidative stress in type 2 diabetes.

MATERIALS AND METHODS

Patients Selection

This study was carried out on (120) Iraqi diabetic patients. They were ranged between (30-60) years of ages. Informed consent was obtained from both patients and controls group to fill the study protocol sheet before venipuncture. Those patients were diagnosed according to the level glucose. The clinical examination was performed under supervision of physician specialist in diabetes and endocrinology, and their classification into groups was according to criteria of ADA.[13]

The experimental groups

This study included 120 individuals (male), they are divided into four groups:

1. Group 1 (45) whom have diabetes-2 and Obese (BMI≥30) .
2. Group 2 (45) whom have diabetes-2 and non-Obese (BMI≤25).
3. Group 3 (15) Control (Non-diabetes) with BMI≥30 (Obese).
4. Group 4 (15) Control (Non-diabetes) with BMI≤25 (Non-obese).

The study was conducted for a period from April 1st 2013 to August 1th 2014.

Collection and handling of samples

From each subject, 10ml of blood were obtained by venepuncture, using a 10 ml disposable syringe. The blood sample was centrifuged at 3000 rpm for ten minutes to collect serum. The serum was stored in Deep freezer at -20 degree celcieus (c^{o}) until it was assayed.

Measurement of Body Mass Index (BMI)

BMI is calculated for all groups, as weight (kg) divided by height squared (m^2).

$$\text{BMI}=\frac{Weight\,(Kg)}{\{Height\,(m)\}2}$$

Laboratory investigations

Plasma glucose was measured by enzymatic colorimetric assay, leptin, Resistin , Apelin and MDA measured by enzyme-linked immunosorbent assay (ELISA).

RESULTS

The role of obesity in Diabetes type-2

Table (1): showed significant differences (P<0.001) in serum leptin level between all groups and the higher level of them were recorded with diabetes obese group (22.868 ng/ml) when compare with other three groups (4.691, 10.676 and 9.701ng/ml respectively). Significant differences (P<0.001) were appeared in serum resistin between different studied groups. Control none-obese group showed lower value of resistin when compare with control obese, patients none- obese and patients obese groups and higher level of resistin were recorded with patients obese group. Significant variation in the level of serum apelin were recorded between obese groups when compare with none-obese groups. Highly significant elevation (P<0.001) in both obese control and obese diabetes type-2 groups were observed compared with none-obese diabetes-2 and none-obese control groups.

Table (1): The value of Leptin, Resistin and Apelin between control and diabetes patients (means ± standard errors)

Groups	Leptin (ng /ml)	Resistin (ng/ml)	Apelin (ng /ml)
Group1: Control (none-diabetes, none-obese)	4.691 ± 0.984[a]	9.460 ± 1.067[a]	1.45± 0.13[a]
Group 2: Control (none-diabetes, obese)	10.676 ± 0.961[b]	22.170 ± 1.743[c]	2.71± 0.14[b]
Group3: Diabetes type 2, none-obese	9.701 ± 0.528[b]	15.319 ± 0.906[b]	1.91 ± 0.12[a]
Group4:Diabetes type 2, obese	22.868 ± 1.162[c]	28.793 ± 1.339[d]	2.95 ± 0.22[b]
p-value	0.001	0.001	0.001

p- value ≤ 0.05 considered significant.

Post Hoc Duncan- test: no differences between groups with the same letter

The pathophysiologic link between obesity and type-2 diabetes is not entirely understood, but adipokines seem to play an important role. Leptin may also directly regulate glucose homeostasis independently of its effects on adiposity; leptin regulates glycemia at least in part via the CNS, but it may also directly regulate the physiology of pancreatic β-cells and

peripheral insulin-sensitive tissues.[14] Resistin down-regulated insulin receptor expression levels (necessary for maintenance of β-cell mass) in clonal β-cell and hence decreased cell viability. Resistin actually induced insulin resistance in pancreatic islets causing a subsequent reduction in glucose-stimulated insulin secretion (GSIS).[15] Recent evidence suggests that apelin is itself expressed in pancreatic islets, particularly in β- and α-cell, raising the possibility of autocrine/paracrine effects.[16]

Table (2): The value of MDA between control and diabetes patients(means ± standard errors).

Groups	MDA (µmol /L)
Group1: Control (none-diabetes, none-obese)	3.754 ± 0.585^{a}
Group 2: Control (none-diabetes, obese)	7.125 ± 1.016^{b}
Group3: Diabetes type 2, none-obese	8.786 ± 0.665^{b}
Group4: Diabetes type 2, obese	12.803 ± 0.670^{c}
p-value	0.001

p- value ≤ 0.05 considered significant.

Post Hoc Duncan- test: no differences between groups with the same letter

Table (2) showed The highest value of oxidative stress marker (serum MDA) were observed in patients obese group (12.803 µmol/L) but the lower value of them were appeared in control none-obese group (3.754 µmol/L).

The role of Oxidative stress in Diabetes type-2

These findings strongly confirmed the evidence that diabetic patients were susceptible to oxidative stress and higher blood glucose level had an association with free radical-mediated lipid peroxidation. During the course of diabetes, every cell in the patient is exposed to abnormally high glucose concentrations. However, high glucose-related damage only targets specific tissues, including retina, kidney, and nerve tissues. Cells in these tissues are deficient in the ability to change glucose transport rates when faced with elevated extracellular glucose concentrations, thereby leading to high intracellular glucose. In cells with high intracellular glucose concentrations, higher amounts of glucose are metabolized and oxidized through the tricarboxylic acid (TCA) cycle.[17]

Correlation between Body mass index with Apelin, Leptin and MDA in diabetes type-2

Table(3) shown the results of current data showed significant positive correlation between BMI with Apelin , Leptin and MDA, when body mass index increase that lead to increase in Apelin, Leptin and MDA .

Table (3): Pearson correlation (r) between BMI with Apelin, Leptin and MDA

	Apelin	Leptin	MDA
Body mass index	0.466**	0.582**	0.447**

****correlation is significant at 0.01**

Obesity-induced insulin resistance is physiologically regulated through the release of adipokines by adipose tissue. Adipokines control amongst others insulin sensitivity, inflammation, angiogenesis, and lipid metabolism. Adipocytes, together with macrophages infiltrating the adipose tissue, secrete pro-inflammatory cytokines such as IL-6 and TNF-α, and adipokines such as leptin, adiponectin, resistin, visfatin and apelin [18, 19]. It has been shown that hyperleptinemia plays a key role in the formation of lipid peroxides thus mediating oxidative stress, corroborated by the previous observation that leptin increased the oxidative stress in tissues where there is high rate of fatty acid oxidation, including muscle tissues. Leptin is strongly correlated with MDA, this is association is even greater for obese diabetic individuals indicating that these people are under severe oxidative stress. However, confirmed that BMI alone may be a strong predictor of clinical manifestations of obesity as a strong and significant correlation exists between leptin and BMI in obese diabetic population [20]. Chronic high exposure to glucose and lipids lead to a decrease of β-cell function. Several glucose-related pathways like the autoxidation, oxidative phosphorylation, glycosylation and the glycosamine pathways induce the formation of reactive oxygen species (ROS) in β-cells.[21]

Correlation between Apelin, Leptin , Resistin and MDA in diabetes type-2

As shown in table (4) significant positive correlation were found between resistin, leptin, Apelin and MDA, when increased any parameter another parameter was increased , these results are show highly correlation between this parameter as factors in diabetes type-2.

Table (4): Pearson correlation (r) between Apelin, Resistin, Leptin and MDA

	Resisten	Leptin	MDA
Apelin	0.425**	0.516**	0.320**
Resistin		0.598**	0.439**
Leptin			0.557**

**** correlation is significant at 0.01**

Adipocytes of patients with obesity have a lower insulin receptor density and a higher density of beta-3 adrenergic receptors, thus increasing the lipolysis rate with release of Free Fatty acid (FFA), a situation that has several metabolic consequences in which the following are present: increase in the production of oxygen-derived free radicals; induction of insulin resistance; synergism in the action of Adiopkines and induction of apoptosis in pancreatic beta cells; taken together, these effects are categorized as lipotoxicity. Lipotoxicity causes both anatomical and functional injury in different cell lines. Adipose tissue dysfunction as well as lipotoxicity comprises two mechanisms that explain the proinflammatory state and insulin resistance (IR).[22,23]

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