

## **Study of Some Biochemical Parameters, Insulin resistance and Clinical markers for Male Type2 Diabetes Mellitus Patients in Al-Najaf Al-Ashraf Governorate**

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**Abstract :** Type 2 diabetes mellitus or non-insulin dependent diabetes mellitus (NIDDM) is the most common type of diabetes, accounting for 90-95% of all diabetes. Obesity is found in approximately 55% of patients diagnosed with type 2 diabetes. The result is an excessive release of free fatty acids into the blood stream (due to increased lipolysis), and an increase in hepatic glucose production, both of which have the effect of exacerbating peripheral insulin resistance and increasing the likelihood of NIDDM. This study was conducted in endocrinology and diabetic center in Alsader medical city /Najaf, Samples were fasting selected from the patients attending the diabetic consultation unit at the hospital during the period from February 2016 to November 2016. The patients and control groups were with age ranged between (35-70) years. Study was carried out on 60 patient with type 2 diabetes mellitus (60 male) and 30 apparently healthy subjects (30 male) how dealt with as control group.

Insulin resistance was evaluated by four methods. They include homeostatic model assessment (HOMA), quantitative insulin check index (QUIKI), McAulye (McA), and fasting insulin (FI) methods. Insulin resistance was found in 40 (66.7%), 38 (63.3%), 27 (45%) and 17 (28.3%) out of the 60 type 2 diabetes mellitus patients by HOMA, QUICKi, McA and FI methods respectively. Type 2 insulin resistant (40) diabetic patients (IRP) that obtained through the HOMA method were assessed for the other biochemical Parameters. Results of the present study show was a significant gender difference (female more than male) and significant positive correlation with lipid profile. Serum cholesterol, LDL-cholesterol and TG were significantly high in type2 diabetic patients group compared with normal non-obese control group ( $P < 0.05$ ), while serum HDL-cholesterol level was low in NIDDM patients group compared with normal non-obese control group ( $P > 0.05$ ). The aim of the present study was to investigate the possible relationships between insulin, interleukin-8 and lipid profile levels and NIDDM with or without obesity. Results also show that mean value of serum insulin was significantly high ( $P < 0.05$ ) in type 2 diabetic patients group and in obese group compared with normal non-obese control group. Although Serum IL-8 mean value was high in type 2 diabetic patients group and in obese group than normal non-obese control group, but it shows no significant difference between them ( $P > 0.05$ ).

**Key word :** Male Type2 Diabetes Mellitus, Insulin hormone, IL-8, Obesity and dyslipidemia.

### **Introduction**

**Diabetes mellitus** is a disease of abnormal glucose metabolism resulting in hyperglycemia (high blood sugar) due to either a deficiency of insulin secretion or insulin resistance or both. The World Health

Organization (WHO) recognizes three main forms of diabetes: type I or insulin dependent diabetes mellitus (IDDM), type II or non-insulin dependent diabetes mellitus (NIDDM), and gestational diabetes mellitus (GDM).<sup>[1]</sup>

**Type II diabetes mellitus** or non-insulin dependent diabetes mellitus (NIDDM) is characterized by insulin resistance which may be combined with relatively reduced insulin secretion<sup>[2]</sup>. The defective responsiveness of body tissues to insulin is believed to involve the insulin receptor. However, the specific defects are not known. In the early stage of type 2 diabetes, the predominant abnormality is reduced insulin sensitivity. At this stage hyperglycemia can be reversed by a variety of measures and medications that improve insulin sensitivity or reduce glucose production by the liver. Type 2 diabetes differs from type 1 diabetes in that the body makes some insulin, but not enough; also, the body can't use the insulin efficiently. NIDDM is the most common type of diabetes, accounting for 90-95% of all diabetes. It usually develops after the age of 40. However, in the late 1990's, its incidence increased among young people<sup>[1, 3]</sup>. Experts are trying to determine why that is happening; they think it may be related to the increased incidence of obesity and sedentary lifestyles among young people.

About 80% of those with NIDDM are overweight<sup>[2]</sup>. It is more common among people who are older, sedentary or obese, or have a family history of the disease. Even though there is no cure for diabetes, proper treatment and glucose control enable people with NIDDM to live normal, productive lives. A major advance for people at risk of developing NIDDM - such as family members of those with the condition - occurred recently when it was shown that diet and exercise can prevent or delay NIDDM diabetes. Taking the diabetes medication metformin also reduced the risk<sup>[4]</sup>.

## • **Materials and Methods**

### **1-Patients and control groups**

The study comprised 60 Male Type2 Diabetes Mellitus Patients, with age ranged from 35-70 years, duration of Male Type2 Diabetes Mellitus Patients for at least four years and had obese. The samples were obtained from the Diabetes Mellitus and endocrinology center in Alsader medical city /Najaf from February 2016 to November 2016. Also 30 non-Diabetes Mellitus were enrolled to serve as control group, with age range 30-70 years.

The laboratory work was carried out in the hormones laboratory, internal laboratory, Clinical chemistry unit and immunity unit in Alsader medical city/Najaf. Type2 Diabetes Mellitus Patients was diagnosed by consultant doctors. The information of patients were obtained through a questionnaire consisted of the name, sex, age, weight, height, duration of the Type2 Diabetes Mellitus Patients, smoking habits, complications and other diseases.

### **2- Collection of blood samples**

After an overnight fasting (8-12 hours), about five milliliters of venous blood was aspirated using disposable syringes. Samples were collected between 09.00-10.30 am. The blood was allowed to clot in plain tubes for 30-45 minutes at room temperature and serum was recovered by centrifugation at 2000 x g for 10 minutes and transferred into plain plastic tubes and kept frozen at -20°C until analysis in disposable serum tubes.

## • **Biochemical measurement:**

### **1-Measurement of body mass index:**

Body mass index values were calculated from the following equation:

$$\text{BMI} = \text{Weight (kg)} / \text{Height (m}^2\text{)}$$

## **2- Determination of fasting blood glucose concentration:**

Glucose kit for quantitative determination of glucose in human serum was supplied by Bio Merieux, France.

## **3- Determination of Glycated Hemoglobin (HbA1c):**

Glycated Hemoglobin kit for quantitative determination of Glycated Hemoglobin in human serum was supplied by Bio Merieux, France.

## **4- Measurement of Total Cholesterol (TC):**

Total Cholesterol kit for quantitative determination of Total Cholesterol in human serum was supplied by Bio Merieux, France.

## **5-Measurement of High Density Lipoprotein-Cholesterol (HDL-C):**

HDL-C kit for quantitative determination of HDL-C in human serum was supplied by Bio Merieux, France.

## **6-Measurement of Low Density Lipoprotein-Cholesterol (LDL-C):**

LDL-C kit for quantitative determination of LDL-C in human serum was supplied by Bio Merieux, France.

## **7- Determination of triglyceride(TG)concentration:**

Triglyceride kit for quantitative determination of TG in human serum was supplied by Bio Merieux, France.

## **8- Determination of fasting insulin (FIN) level:**

Insulin ELISA kit for the quantitative determination of human insulin concentration in serum was supplied by DRG, Germany.

## **9-Determination of Serum Interleukin-8 concentration:**

Interleukin-8 ELISA kit for the quantitative determination of human Interleukin-8 concentration in serum was supplied by DRG, Germany.

- **Biostatistical analysis**

Analysis of data was performed by using minitab, megastatistical and SPSS for excel in the home computer. The results were expressed as (mean  $\pm$  standard deviation) . p-value  $<0.05$  was used as a level of statistically significant.

- **Results and Discussion:**

### **Characteristics of the patient and control groups**

The clinical and characteristics of the participants are shown in Table (1). The data include both, Type 2 Diabetes Mellitus and the control group were the number of patients and the control group, age, weight, height, body mass index (BMI) and duration.

**Table (1) . Information of the enrolled Patients and the control groups**

Parameters	Control Mean± SD	range	patients Mean± SD	range
No.	30	-----	60	-----
Age (years)	37.60±4.49	35-70	34.43 ±6.5	35-70
Weight (Kg)	69.43±5.45	63-61	76.45±17.40	50-97
Height (m)	176.30±4.90	166-187	174.65±5.90	158-189
Duration (years)	-----	-----	8.11 ±4.90	4- 23

### **Effect of Type 2 Diabetes Mellitus on Serum Lipid Profile, Fasting blood glucose and Glycated hemoglobin.**

Serum cholesterol, LDL-Cholesterol and TG were significantly high in NIDDM patients group than normal non-obese control group ( $P < 0.05$ ), while serum HDL-Cholesterol level was lower in NIDDM than normal non-obese control group but it shows no significant difference as shown in Table (2).

Firstly, serum cholesterol shows a positive correlation with LDL-Cholesterol at 0.01 level of significant ( $r = 0.919$ ) with FBG% at 0.05 level of significant ( $r = 0.417$ ) respectively. Secondly, TG shows a non-significant negative correlation with HDL-Cholesterol ( $r = -0.207$ ) and a non-significant positive correlation with LDL-Cholesterol and FBG%.

Thirdly, LDL-Cholesterol has a non-significant positive correlation with FBG% and a non-significant negative correlation with HDL-Cholesterol.

Finally, HDL-Cholesterol shows a non-significant negative correlation with FBG% ( $r = -0.094$ ). These results are in a good agreement with the previous reports<sup>[5]</sup>. The lipid profile abnormalities associated with insulin resistance affect all lipid fractions. They are characterized by elevated fasting triglyceride levels, elevated postprandial triglyceride rich remnant lipoproteins, low HDL-Cholesterol, and small dense LDL-Cholesterol particles. The results show high significant of HbA1c in Type 2 Diabetes Mellitus when compared with control groups. This pattern correlates strongly with cardiovascular risk, and treatment decreases this risk. The result is consistent with other authors<sup>[6,7]</sup>.

### **Effect of Type 2 Diabetes Mellitus on Serum Insulin.**

Results of the present study show that serum insulin mean value was significantly high in NIDDM patients compared with normal non-obese control group ( $P < 0.05$ ) as shown Table (2). Serum insulin has a positive correlation with BF% at 0.05 level of significant ( $r = 0.258$ ). It also has a positive non-significant correlation with IL-8, cholesterol, LDL-Cholesterol and BMI ( $r = 0.164$ ). The results obtained in this study are in consistent with previous reports.<sup>[8]</sup> in which insulin resistance increases with increasing body mass index, and waist circumference. These reflect increased adiposity especially increased levels of visceral adipose tissue. NIDDM and the metabolic syndrome would be the most common clinical syndromes associated with insulin resistance. Insulin resistance typically predates the development of diabetes and is commonly found in unaffected first-degree relatives of diabetic patients<sup>[9]</sup>.

### **Effect of Type 2 Diabetes Mellitus on Serum IL-8.**

Although serum IL-8 mean value was high in NIDDM patients than normal non-obese control group, but it shows no significant difference between them ( $P > 0.05$ ) as shown in Table (2).

IL-8 shows non-significant positive correlation with cholesterol, LDL-cholesterol, HDL-Cholesterol, BMI and BF% ( $r = 0.089$ ). While it shows a negative correlation with TG. IL-8 besides its implications for its association with different inflammatory processes and because the adipose tissue is able to produce and release various cytokines and IL-8 has been implicated in the atherosclerotic process<sup>[10,11]</sup>, founding it is of interest to investigate the ability of human adipose tissue and isolated adipocytes to express and release IL-8 and this will be discussed by discussing the combined effect of diabetes and obesity.

**Table (2): Mean  $\pm$  SD values of Triglycerides, Cholesterol, HDL-C, LDL-C, HbA1c, Insulin, IL-8 and BMI in Type 2 Diabetes Mellitus patients compared to non-diabetic non-obese control group.**

Parameters	Type 2 Diabetes Mellitus (No=60)	Non-Diabetic Non-Obese (N0=30)	P value
FBG (mmol/l)	9.99 $\pm$ 0.39	4.87 $\pm$ 0.68	< 0.03
HbA1c (%)	7.16 $\pm$ 0.21	4.87 $\pm$ 0.37	0.29
Cholesterol (mg/dl)	214.5 $\pm$ 47	153.7 $\pm$ 27.7	< 0.05
TG (mg/dl)	148.5 $\pm$ 21.9	82.7 $\pm$ 22.1	< 0.05
LDL-C (mg/dl)	140.1 $\pm$ 25.1	100 $\pm$ 24.8	< 0.05
HDL-C (mg/dl)	42.5 $\pm$ 10.7	44.9 $\pm$ 6.4	NS
Insulin ( $\mu$ IU/ml)	20.1 $\pm$ 4.3	11.5 $\pm$ 3.1	< 0.05
IL-8 (pg/ml)	31.9 $\pm$ 4.6	27.9 $\pm$ 2.6	NS
BMI (kg/m <sup>2</sup> )	32.9 $\pm$ 10.2	20.3 $\pm$ 3.9	< 0.001

### Effect of Type 2 Diabetes Mellitus and Obesity on Serum Insulin

Serum insulin was significantly high in NIDDM obese patients group as compared with normal non-obese control group (P<0.001) as shown Table(3).

Serum insulin has a non-significant positive correlation with IL-8 and BMI .

### Effect of Type 2 Diabetes Mellitus and Obesity on Serum IL-8

Although serum IL-8 mean value was high in NIDDM obese patients group compared with non-diabetic non-obese control group , it shows no significant difference between them (P > 0.05) as shown in Table (3).

IL-8 shows non-significant positive correlation with insulin . It is proposed that IL-8 is one of the adipocyte-derived cytokines <sup>[12,13]</sup>

IL-8 is produced mainly by macrophages and monocytes and plays a role in modulating an inflammatory response <sup>[14]</sup>. Inflammation might contribute to the pathogenesis of atherosclerosis <sup>[15]</sup>, and IL-8 is probably also an atherogenic factor. Oxidized low-density lipoprotein (LDL) particles are able to stimulate production and secretion of IL-8 by macrophages from human atherosclerotic plaques and high levels of IL-8 in macrophage-derived human foam cells were found <sup>[16]</sup>. IL-8 is a potent chemo attractant and may be responsible for the recruitment of neutrophils and T lymphocytes into the sub endothelial space. It also induces adhesion of monocytes to endothelium <sup>[17]</sup> and migration of vascular smooth muscle cells <sup>[18]</sup>. All those processes lead to intimal thickening and atherosclerosis <sup>[17]</sup>. Another function of IL-8 is inhibition of tissue inhibitor of metalloproteinase expression, which results in the increased release of matrix-degrading metallo-proteinases and in consequence the instability of atherosclerotic plaque <sup>[19]</sup>. Elevated serum IL-8 levels were found in type 1 and type 2diabetic subjects, and it is suggested that this cytokine might also contribute to the development of diabetic macroangiopathy <sup>[20]</sup>. Recently, two elegant studies of Bruun *et al.* <sup>[12, 13]</sup> .

Revealed, through the in vitro experiments, that IL-8 is produced and secreted by human adipocytes. IL-8 was released from adipose tissue fragments into the medium, and IL-8 mRNA was found in isolated adipocytes. Therefore, the authors suggested that the correlation found between the severity of obesity and the development of atherosclerosis and cardiovascular disease might be related to the ability of human adipose tissue to produce and release IL-8 . However, there are no in vivo data about plasma IL-8 levels in obesity. Additionally, it was found that adipocyte IL-8 mRNAexpression and protein synthesis and release are stimulated by IL-1 and TNF <sup>[13]</sup> and inhibited by the insulin-sensitizing agents' thiazolidinedione ciglitazone and biguanide metformin. Both insulin resistance and TNF overexpression in adipose tissue and skeletal muscle are important features of human obesity, related to each other, as TNF induces insulin resistance by acting via autocrine-paracrine pathway <sup>[21]</sup>. It is well known that insulin resistance plays a role in the pathogenesis of

obesity-related complications<sup>[22]</sup>.TNF may also increase cardiovascular risk in obesity via other mechanisms<sup>[23]</sup>.

**Table (3): Mean ± SD values of Insulin, IL-8, Body mass index in diabetic patients compared to non-diabetic non-obese control group.**

Parameters	Diabetic Obese N=30	Non-Diabetic non-Obese N=30	P value
Insulin(μ IU/ml)	21.9±3.9	11.5±3.1	< 0.001
IL-8 (pg/ml)	34.8±4.2	27.9±2.6	NS
BMI (kg/m <sup>2</sup> )	36±3.2	20.3±3.9	< 0.001

### Evaluation of insulin resistance in type 2 diabetic patient and the control groups

Insulin resistance (IR) was evaluated by using four methods; Homeostasis Model Assessments (HOMA), Quantitative Insulin Sensitivity Check Index (QUICKI), McAuley's index (McA) and Fasting Insulin level (FI). As shown in Table (4), 40 out of 60 patients (66.7%) were found to be insulin resistant by HOMA, 38 out of 60 patients (63.3%) were found to be insulin resistant by QUICKI, 27 out of 60 patients (45%) were found to be insulin resistant by McA and 17 out of 60 patients (28.3%) were found to be insulin resistant by FI. However, one healthy person exhibited insulin resistance through the evaluation by HOMA, QUICKI and FI methods, but not by McA method Table(5).

The evaluation of insulin resistance is a promising approach in the management of diabetes mellitus. Several methods are used for such evaluation. The euglycaemic insulin clamp method is a direct method. Intravenous glucose tolerance test is an indirect method (IVGTT), can only be used in small research trials and minimal model approximation of the metabolism of glucose (MMAMG). These are standard methods for the measurement of insulin resistance in research<sup>[24]</sup>. However, they are impractical in clinical practice and are difficult to be carried out in population based research studies<sup>[25]</sup>.

There are many indirect methods that are used for the assessment of insulin resistance. Biochemical studies have indicated to the suitability of HOMA, QUICKI<sup>[26,27]</sup> and McA methods as indirect criteria for insulin resistance<sup>[28,29]</sup>. Among the various indirect methods, HOMA was suggested to be the most appropriate method with respect to results of the MMAMG direct method which is called, the gold standard method<sup>[30,31]</sup>. In some reports, McA was demonstrated to be the most precise rational for prediction of insulin sensitivity<sup>[32]</sup>. To select insulin resistant patients as accurate as possible in the present investigation, the four indirect methods of evaluation of insulin resistance were examined. The data exhibited high rate of insulin resistance through the HOMA and QUICKI methods (66.7% and 63.3% of diabetics were insulin resistant respectively) and low rate with McA and FI methods (45% and 28.3% of diabetics were insulin resistant respectively).

In the current investigation, the HOMA method was implicated for the selection of insulin resistant diabetics. Two factors have strongly led us for the HOMA implication. The first is the wide use of HOMA in the previous works mentioned in literatures<sup>[33]</sup>. The second is that 40% of the enrolled patients were overweight or obese. Insulin resistance is a serious mechanism involved in the pathogenesis of type 2 diabetes mellitus in particular those of abnormal weight. Thus the data of the HOMA method was highly suggestive to be used for selection of insulin resistant type 2 diabetics, therefore 40 out of 60 patients were categorized as insulin resistant diabetics. The results of the HOMA method of the insulin resistance assessment demonstrated one out of 30 healthy individuals of the control group was insulin resistant subject. This individual was excluded from the control group and only 29 subjects were considered for the comparison in the next sections.

The results of the current study are in agreement with Erus et al. (2007)<sup>[34]</sup>. In regards to the data of HOMA methods. However, inconsistency was also observed in comparison with Lukshmy, et al. (2006)<sup>[25]</sup>. The most satisfactory reasons for the difference may be the patient's status and the number of the enrolled diabetics. Some authors have mentioned that FI method is also an alternative rational for the evaluation of insulin resistance and could demonstrate comparable results for those of other indirect methods. The present

study is inconsistent with such findings and determination of fasting insulin level seemed to be inappropriate for the prediction of insulin resistance accurately<sup>[25]</sup>.

**Table 4: The Incidence of insulin resistance and sensitivity in type 2 diabetes mellitus**

Method	No.of IR Patients (%)	No.of IS Patients (%)
HOMA	40 (66.7%)	10 (16.7%)
QUIKI	38 (63.3%)	15 (25%)
McA	27 (45%)	22 (36.7%)
FI	17 (28.3%)	31 (51.7%)

**Table 5: The Incidence of insulin resistance and sensitivity in type 2 diabetes mellitus and the control groups**

Method	Insulin resistant subjects		Insulin sensitive subjects	
	Patients	Control	Patient	Control
HOMA	40 (66.7%)	1(3.3%)	10 (16.7%)	29 (96.7%)
QUICKI	38 (63.3%)	1(3.3%)	15 (25%)	29 (96.7%)
MCA	27 (45%)	0 (0%)	22 (36.7%)	30 (100%)
FI	17 (28.3%)	1 (3.3%)	31 (51.7%)	29 (96.7%)

### Conclusions:

1. About 40 out of 60 patients of type 2 diabetic patients are presented with insulin resistance.
2. There was a non-significant positive correlation between IL-8 and obesity in somewhat it might due to the fact that insulin.
3. There were significant elevations in serum insulin, cholesterol, and LDL-cholesterol with a non-significant decline in HDL-cholesterol in NIDDM patients in comparison with normal subjects due to metabolic effect of diabetes itself and to the obesity accompany diabetes.
4. There were significant elevations in serum insulin, and TG with a non-significant decline in HDL-cholesterol in obese subjects in comparison with normal subjects this can be explained by fact that BMI is a measure of adiposity.
5. The combination of obesity and diabetes makes the change in the above parameters more dramatic.
6. There was a non-significant positive correlation between IL-8 and obesity in somewhat it might due to the fact that insulin.

### Recommendations:

1. Studies of the correlation between sex hormones and development of diabetes mellitus in obese subjects (prospective study).
2. Studies of the correlation between elevated cortisone level, insulin resistant and dyslipidemia with the development of vascular complications in type 2 diabetic patients.
3. Studies of the correlation between Thyroxine hormone and development of diabetes mellitus in obese subjects (prospective study).
4. Studies of the effect of daily exercise on ACTH and/or insulin resistant.

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