



## Estimation the levels of interleukin 6 and tumor necrosis factor-alpha patients with diabetic type 2 in Tikrit city

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### ABSTRACT

Type 2 is the most common metabolic disorder characterized by the increased concentration of glucose in blood due to insulin resistance or relative insulin deficiency. Changes in human behavior and lifestyle over the last century have resulted in a dramatic increase in the incidence of diabetes worldwide. Interleukin 6 is pro inflammatory cytokines secreted by immune cells, adipose tissue , Muscles is able to accelerate or inhibit the inflammatory processes .High circulating IL-6 levels have been associated with insulin resistance and greater risk of T2DM .

80 patients with type 2 diabetes mellitus (40 male and 40 female )are diagnosed by specialized in medicine in general Salah- Aldin Hospital in Tikrit city and 40 apparently healthy Controls (20 male and 20 female ) were included in this study This study carried out in Tikrit city from 1<sup>st</sup> October 2017 to 1<sup>st</sup> of April 2018 Anthropometric measures include, Age , BMI , were done for all participants Fasting serum samples were obtained and used for the measurement of serum glucose Interleukin- 6 (IL-6) and Tumor necrosis factor alpha (TNF- $\alpha$ ) .

The results of current study showed that Fasting serum glucose (FBS), Interleukin-6 (IL-6), Tumor necrosis factor alpha (TNF- $\alpha$ ) are High significant increase ( $p < 0.01$ ) in diabetic patients when compared to control group.

In Conclusion, serum interleukin -6 (IL-6) and Tumor necrosis factor – alpha (TNF- $\alpha$ ) has high significant increases level in diabetic patients compared to control.

### Introduction

Diabetes mellitus (DM) is a combination of heterogeneous disorder commonly presenting with episodes of hyperglycemia and glucose intolerance , as a result of lack of insulin defective insulin action or both [1].

Type 2 diabetes mellitus is the most common form of diabetes mellitus accounts for 90-95% of diabetes mellitus cases, caused by a combination of insulin resistance and pancreatic cells insulin secretory dysfunction leading to a disorder of metabolism[2]. Type 2 diabetes mellitus is usually preceded by prediabetes, in which levels of blood glucose are above normal but not high enough yet for a diagnosis of diabetes [3].Type 2 diabetes mellitus also called adult onset diabetes mellitus or non-insulin dependent diabetes mellitus – NIDDM [4].

Insulin resistance and impaired insulin secretion are the two main metabolic disturbances in the pathogenesis of type 2 diabetes [5]. Type 2 diabetes develops when the pancreas is unable to secrete more insulin to compensate for existing insulin resistance . Both impaired insulin secretion and insulin resistance are influenced by genetic and environmental factors such as hyperglycemia, free fatty acids, inflammatory mechanism [6].

Chronic inflammation is a crucial factor contributing to the development and progression of type 2 diabetes which is associated with elevated circulating, biomarkers of innate immunity activation, including Interleukin-6 (IL-6) and Tumor necrosis factor- alpha (TNF- $\alpha$ ), activation these alteration are also present in pre diabetes and metabolic syndrome in human,

Both insulin resistance and diabetes can result from diverse genetic defects affecting the function of individual organs, including liver, fat, muscles, langerhans islets in pancreas and neuronal tissue, thus insulin resistance result can from defects and various inflammatory immune genes, autoimmunity and adaptive immune system have a rate in the pathogenesis of type 2 diabetes mellitus by which high pro inflammatory cytokines production has been associated with insulin resistance and type diabetes mellitus development[7].

Immune inflammatory mediators are responsible for the effects of environmental factors on insulin resistance and beta cell function [8].

Tumor necrosis Factor- alpha (TNF- $\alpha$ ) and Interleukin-6 (IL-6) pro inflammatory cytokines can alter insulin sensitivity by triggering different key steps in the insulin signaling pathways, stimulating phosphorylation of serine residues instead of tyrosin in insulin receptor substrate-1 (IRS-1).

Obesity modifies cytokines (adipokines) secretion from adipose tissue, by which alters gene expression in the adipose cells and activates inflammatory processes within the adipose tissue; Furthermore obese adipose tissue secretes lower levels of anti-inflammatory dipokines such as adiponectine and higher levels of pro-inflammatory cytokines, including IL-6 and TNF- $\alpha$ , the release of free fatty acids (FFAs), which activate inflammatory signaling also increased in obesity as a results of activated lipolysis [9].

#### the aim of the study

To investigate the physiological relationship between interleukin 6 and tumor necrosis factor – alpha in type II diabetic patients' in Tikrit city.

#### Material and Methods

This study was achieved by collecting (120) blood samples of (80) diabetic patients (40 male and 40 female) diagnosed with type 2 Diabetes mellitus and (40) subjects as Control (20 male and 20 female) patient study carried out in Salah- Aldin Hospital in Salah-Aldin governorate from October 2017 to April 2018 on study population age ranged from (32-63 years) old.

#### Serum samples treatment

Approximately 5 ml of fasting human blood was collected from each subject (patients and control) and transferred into sterilized test tubes and allowed for 30 minute to clot at room temperature, the sample was centrifuged for 15 minutes at 3000 rotations per minute and the serum was immediately separated and stored at (- 20 ° C) till used for interleukin 6 (IL-6) and Tumor necrosis factor-alpha (TNF- $\alpha$ ) .

#### Determination of fasting blood glucose

Fasting blood glucose was determined by enzymatic colorimetric method using Randox diagnostic kits (UK) [10].

#### Determination of Interleukin 6 (IL-6)

IL-6 Concentration was estimated in serum human using ELISA kit from Koma BIOTICH , Company

(Korea) [11].

#### Determination of Human Tumor necrosis factor alpha (TNF- $\alpha$ )

TNF- $\alpha$  Concentration was estimated in serum human using ELISA kit from Koma BIOTICH , Company (Korea) [12].

#### Statistical analysis

All data were presented as a mean and standard deviation, (SD). P value less than 0.05 was accepted as a significant value.

Un paired Student T test was used to compare between the mean of measured variables.

#### Results and Discussion

A total number of 120 subjects were participated in the present study, distributed as follow:-

**Group 1:** Forty normal (40), apparently healthy subjects as controls.

**Group 2 :**(80) Patients with Diabetes Mellitus type 2. **Age and Anthropometric parameters of all Diabetes Mellitus type 2 Vs control subjects.**

As in Table 1, the control subjects are matched with DM patients in age and gender. So there is no significant difference regarding age of patients (52.38  $\pm$  9.6 years) as compare with control subjects, (49.7  $\pm$  6.1 years).

Also, in the present study, the range age of DM patients is from (45 to 63) years. So, aging process is associated with reduced function of beta cells of the pancreas and increase resistance to insulin that contributes to insulin resistance DM, (Type 2). This finding agrees with previous result[13].

**Table (1) shows that the age, body weight, height and body mass index (BMI), of DM patients and control subjects.**

Parameters	Controls	Patients (62)	P $\leq$ value
Age (years)	49.7 $\pm$ 6.1	52.38 $\pm$ 9.6	NS
Body weight (Kg)	74.9 $\pm$ 4.5	96.4 $\pm$ 20.8	0.01 **
Height (Cm)	173.9 $\pm$ 5.9	169.2 $\pm$ 5.8	NS
BMI (Kg/m <sup>2</sup> )	24.1 $\pm$ 1.4	29.2 $\pm$ 4.2	0.01 **

(\*\*High significant )

By using questionnaire, the result of present study shows that most of patients are lacking daily physical activity. Decreased physical activity/less exercise in the elderly has also contributed to the occurrence of obesity[14].

In the present study, Table (1) shows that there were high significant elevation (P  $\leq$  0.01). in body weight of DM patients, (96.4  $\pm$ 20.8 Kg), as compared with control subjects, (74.9  $\pm$ 4.5Kg). Moreover, there were high significant increase (P $\leq$  0.01) in BMI of DM patients, (29.2  $\pm$ 4.2Kg/ m<sup>2</sup>), as compared with control subjects, (24.1  $\pm$  1.4 Kg/ m<sup>2</sup>) .

Higher body weight and elevated body mass index, (BMI) are indicators of obesity and associated with increased insulin resistance as in type 2 DM [15]. It means that obesity enhances insulin resistance [16]. The association between increases in fat mass are mostly due to adipocyte hypertrophy. Because large adipocytes are less insulin sensitive than small adipocytes, the expanded fat depot loses most of its

ability to take up glucose in response to insulin because the enlarged lipid droplet pushes cell organelles, such as mitochondria, against the cell surface, the role of mitochondria may be important especially in visceral fat [17].

### Results of IL-6 and FBS and TNF- $\alpha$ in DM type2 patients and Control

Table (2) showed that the values of IL-6 and FBS in DM patients and controls. There was a high significant increase ( $P \leq 0.01$ ) in the concentration of (IL-6 pg/ml) in DM –type 2 patients ( $288.1 \pm 73.8$  pg/ml) as compare with control subjects, ( $171.7 \pm 3.7$  pg/ml).

Also, there was a high significant increase ( $P \leq 0.01$ ) in fasting blood sugar, ( $250.1 \pm 70.5$  mg/dl) in type 2 DM patients as compared with control subjects, ( $89.9 \pm 12.4$  mg/dl).

Also, there was a high significant increase ( $P \leq 0.01$ ) in the concentration of (TNF- $\alpha$  ng/ml) in DM –type 2 patients ( $188.3 \pm 13.8$  ng/ml). as compared with control subjects, ( $140.4 \pm 4.9$  ng/ml).

**Table 2 The mean values and SD of IL-6 and FBS in DM – type 2 patients and control subjects.**

Parameters	Control	Patients	P value
IL-6 pg/ml	$171.7 \pm 3.7$	$288.1 \pm 73.8$	0.01 **
FBS mg/dl	$89.9 \pm 4.9$	$250.3 \pm 70.5$	0.01 **
TNF- $\alpha$ ng/ml	$140.4 \pm 4.9$	$188.3 \pm 13.8$	0.01 **

(High significant \*\*)

Interleukin-6 (IL-6) is a multifunction cytokine that regulates immune response, acute phase reactions and

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hematopoiesis and may play a central role in host defense mechanisms, and it's one of several pro inflammation cytokines that have been associated with insulin resistance[18].

Circulating IL-6 levels have been reported to be elevated in subjects with type 2 diabetes. Moreover, IL-6 independently predicts the risk of type 2 diabetes [19].

TNF- $\alpha$  has been shown to be secreted in the adipocytes of both mice and human. In the adipose tissue o f rodents with genetic obesity and insulin resistance, increased levels of TNF- $\alpha$  was described, supporting a link between obesity, diabetes and TNF- $\alpha$  [20].

A high level of TNF- $\alpha$  is believed to induce insulin resistance and is considered to contribute to the development of diabetes [21].

TNF- $\alpha$  was believed to induce insulin resistance by inhibiting phosphorylation of kreb cycle substrate [22].

The present study showed that blood sugar increased in diabetic patients rather than control. In type (2) diabetes patients can still produce insulin , but do so relatively inadequately for their body's needs. in many cases this actually means the pancreas produces larger than normal quantities of insulin.

A major feature of type 2 diabetes is a lack of sensitivity to insulin (insulin resistance) by the cells of the body (particularly fat and muscle cells).

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## تقدير مستوى انترليوكين 6 وعامل تنخر الاورام الفا لدى المرضى المصابين بداء السكر النوع الثاني في مدينة تكريت

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### الملخص

يعد داء السكر من النوع الثاني الاضطراب الاستقلابي الاكثر شيوعا اذ يتميز بارتفاع معدل سكرالكلوكوز في الدم وقد يكون ذلك بسبب مقاومة الانسولين او نقص الانسولين النسبي. ان التغيير في سلوكيات وانماط الحياة خلال القرن الاخير ادى الى زيادة مثيرة في نسبة المصابين في هذا المرض عالميا . انترليوكين 6 وهو من الساييتوكينات المرافقة للالتهابات تفرزها الخلايا المناعية والانسجة الدهنية والعضلات ولها القدرة على تسريع او تثبيط العمليات المناعية وقد ارتبط ارتفاع مستوى انترليوكين 6 مع مقاومة الانسولين وزيادة خطر الاصابة بمرض السكر النوع الثاني. تضمنت الدراسة الحالية (80) ثمانون عينة دم لاشخاص مصابين بداء السكر النوع الثاني (40 ذكور 40 اناث) وقد تم تشخيص العينات من قبل الكادر المتخصص في مستشفى صلاح الدين العام في مدينة تكريت وقد تم اختيار (40) اربعون عينة دم لاشخاص (20 ذكور 20 اناث) كمجموعة سيطرة. اجريت في مدينة تكريت خلال الفترة من بداية شهر تشرين الاول 2017 الى بداية شهر نيسان 2018 . وقد تم الحصول على نماذج دم لجميع المشاركين من اجل تقدير تراكيز نسبة السكر في الدم وانترليوكين 6 وعامل تنخر الاورام الفا . اظهرت نتائج الدراسة الحالية ارتفاع معنوي ( $P \leq 0.01$ ) في تركيز سكر الكلوكوز وانترليوكين 6 وعامل تنخر الاورام الفا في مصل الدم لدى المرضى مقارنة بمجموعة السيطرة.

نستنتج من هذه الدراسة ارتفاع معنوي في مستوى انترليوكين 6 وعامل تنخر الاورام الفا لدى مرضى داء السكر النوع الثاني مقارنة بمجموعة السيطرة .

