# Association of Proinflammatory Cytokines (Interleukin 6 and Interleuken 8) with Diabetes Mellitus Type 2 In Baghdad Population

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**Keywords**: Cytokines, ELISA, and TNF α.

**Received** (June), **Accepted** (December)

**ABSTRACT**

Patients with type 2 diabetes suffer from hyperglycemia do not have the ability to use the insulin they produce, and increased incidence of infections, and the aim of the current study is to investigate correlation proinflammatory cytokines (interleukin 6 and interleukin-8) with type 2 diabetes mellitus. in this study, were collected (100) sample of serum and a representative of the 80 samples of type 2 diabetes and 40 samples from healthy controls of Endocrinology, Diabetes Center at Kindi Hospital, and then measure the concentration of IL6, IL8 using enzymatic and colorimetric methods. This study has shown that there was no significant difference in the production of IL8 for diabetes and amounted to about (0.543 ± 0.16) compared with healthy (0.106 ± 0.01), while production showed significant difference for IL6 production reached (1.056 ± 0.18) compared with healthy (1.969 ± 0.19) and The conclusion Search found high values of IL 6 in type 2 diabetes compared with healthy.

## علاقه السايتوكينات الالتهابيه الاولية IL6 وIL 8 مع مرض السكري من النوع 2 في سكان بغداد

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**الكلمات المفتاحية**: المعلمات المناعية ، انزيم مرتبط المناعي، عامل القاتل الورمي

**الخلاصة**

المرضى الذين يعانون من السكري من النوع 2 يعانون من ارتفاع السكر في الدم ليس لهم القدرة على استخدام الانسولين التي ينتجونها، و زيادة حدوث الالتهابات، والهدف من الدراسة الحالية هودراسة علاقة السايتوكينات الالتهابيه الاولية (انترلوكين 6 وانترلوكين 8) مع داء السكري من النوع 2. وفي هذه الدراسة، تم جمع (100) عينة من مصل الدم وممثل عن 80 عينات من مرض السكري من النوع 2 و 20 عينة من الاصحاء من مركز الغدد الصماء والسكري في مستشفى الكندي، ثم قياس تركيز IL6 , IL8 باستخدام الطرق الأنزيمية واللونية (R and D / الولايات المتحدة الأمريكية). وقد أظهرت هذه الدراسة أنه لا يوجد فارق معنوي في انتاج IL8 لمرض السكري وبلغت حوالي (0.543 ± 0.16) مقارنة مع الأصحاء (0.106 ± 0.01)، بينما ظهرت الإنتاج ل IL6 فرق معنوي وبلغ الانتاج (0.18 ± 1.056) مقارنة مع الأصحاء (1.969 ± 0.19) وخلاصه البحث عثر على قيم مرتفعة ل IL 6 في النوع 2 من داء السكري مقارنة مع الأصحاء

## 1. INTRODUCTION

Diabetes Mellitus is a heterogeneous collection of metabolic disorders, characterzatied by hyperglycemia (1), DM affects approximately 150 million people around the would (2), the two major types of diabetes, called type 1 diabetes (T1DM) and type 2 diabetes (T2DM) display markedly different pathophysiologyical disease process (3), T2DM is multifactorial disorder caused by interactions between genetic and environmental factors (4). T2DM also has been postulated as a disease of the innate immune system (5). There is

increasing evidence that an ongoing T- lymphocyte response are closely related to the pathogenesis of T2DM (6). Obesity is known to represent one of the single most important risk factor for the increased risk of the type 2 DM, In addition an increase in central (Visceral) adiposity confers higher metabolic risk. This is increased metabolic risk associated with subclinical inflammation with several studies demonstrating increased levels of inflammatory patient's adipocytokiese such as IL6 and TNF- **α** in with obesity and T2DM. Activation of pro inflammatory adipocytokinase in adipose tissue (AT), is coordinated through NFKB, ( nuclear factor Kappa light chain enhancer of activator B cell) a key transcription factor inflammotrnatel cascade (7), implication of cytokines for adiponection levels of TNF **α**, IL8, and IL6 as well as expression of these cytokines in AT (8). Patients with diabetes mellitus type 2 also known as non insulin dependent diabetes mellitus (NIDDM) (9), suffer from hyperglycenmia because they are not able to use the insulin produced at their body often due to inadequate function of insulin receptors (10), the incidence of infections is increased in patients with diabetes mellitus (DM) (11). The aim of this study correlation between expression of IL6 and IL8 and Type 2 Diabetes Mellitus in the Iraqi Population

## 2. MATERIALS AND METHODS

### Sampling

Serum was collected from (100) a representative sample of 80 sample of non insulin dependent diabetes mellitus (40 males and 40 females, age ranged from 25 to 65 years) and 40 healthy samples from Endocrinology and diabetes center in Al-kindi Hospital during the period from March/2013 till January/2014

### The IL6 and IL8 detection assay

The IL6 and IL8 concentration was detected using IL6 and IL8 enzyme linked immuno sorbent assay (ELISA) for *in vitro* quantitative determination of cytokine in supernatant, buffered solutions, serum samples. The cytokine kit is a solid phase sandwich ELISA, a monoclonal antibody specific for IL6 or IL8 has been coated onto the wells of the microtiter plate ( Linbro$^{R}$, USA).

### A: The IL6 Cytokine Kit

**Calibrator Diluent RD6-11 (1X)** Dilute 10 mL of Calibrator Diluent RD6-11 Concentrate into 10 mL of deionized water to prepare 20 mL of Calibrator Diluent RD6-11 (1X). (12)

**IL-6 Standard**, Reconstitute the IL-6 Standard with 5.0 mL of the appropriate Calibrator Diluent (Calibrator Diluent RD6-11 Concentrate for serum samples). This reconstitution produces a stock solution of 10 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 500 µL of Calibrator Diluent RD6-11 Concentrate into each tube. Use the stock solution of standard IL6 to produce a dilution series. Mix each tube thoroughly before the next transfer. The reconstituted standard stock serves as the high standard (10 pg/mL). **Wash Buffer of IL6 and IL 8**, If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 100 mL of Wash Buffer Concentrate into deionized water to prepare 1000 mL of Wash Buffer.

### Protocol

Add 100 µL of Assay Diluent RD1-75 to each well, Add 100 µL of Standard and sample per well. The Covered plate was incubated for 2h at room temperature 25ºC and shaker at 500 rpm. The plate was washed as follow: The washing buffer was aspirated from

each well, 0.4ml of washing solution was dispensed into well then the content of well was aspirated (Washing was repeated twice). Add 200 µL of IL-6 Conjugate to each well. The Covered plate was incubated for 2h at temperature 25ºC and shaker at 500 rpm then washed as in up step, add 50 µL of substrate solution to each well, the covered plate was incubated for 1h at temperature 25ºC and shaker at 500 ± 50 rpm. Add 50 µL of Amplifier Solution to each well, incubate for 30 minutes at room temperature. Than add 50 µL of Stop Solution to each well, the absorbance of each well were read on a ELISA microplate reader (Asys/ Austrlia) at 490nm.

**B: The IL8 Cytokine Kit**

**Substrate Solution of IL8,** Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light.

**IL-8 Standard** Reconstitute the IL-8 Standard with 5.0 mL of Calibrator Diluent RD6Z. This reconstitution produces a stock solution of 2000 pg/mL. Pipette 500 mL of Calibrator Diluent RD6Z into each tube. Use the stock solution to produce a dilution series. Mix each tube thoroughly before the next transfer. The undiluted standard serves as the high standard (2000 pg/mL).

**Protocol**

Add 100 mL of Assay Diluent RD1-85 to each well, add 50 µL of Standard and sample per well. The Covered plate was incubated for 2h at room temperature 25ºC and shaker at 500 ± 50 rpm. The plate was washed as follow: The washing buffer was aspirated from each well, 0.4ml of washing solution was dispensed into well then the content of well was aspirated (Washing was repeated twice). Add 200 µL of IL-8 Conjugate to each well. The Covered plate was incubated for 1h at room temperature 25ºC and shaker at 500 ± 50 rpm then washed as in up step, add 200 µL of substrate solution to each well, the covered plate was incubated for 30 minutes at room temperature 25ºC and shaker at 500 ± 50 rpm (Protect from light). Than add 50 µL of Stop Solution to each well, the absorbance of each well were read on a ELISA microplate reader (Asys/ Austrlia) at 450nm.

**Statistical Analysis**

The Statistical Analysis System SAS (2010) was used to effect of different factors in study parameters. Least significant difference LSD test (T-test) was used to significant compare between means in this study (13).

## 3. RESULTS

The strong correlation between elevations in the local and circulating proinflammatory cytokine IL6, IL10, IL8 and TNF α, that have association with diabetes mellitus, the study appeared a significantly lower production of IL6 (1.056± 0.18) compared with healthy (1.969± 0.19), however, IL8 has been shown no significant differences between patients and control shown table (1) and Figure (1) appeared different production of IL6 and IL8 between patient and healthy by using Models show significant difference to productivity cytokine.

Table 1: Compare between Type 2 Diabetes Mellitus and Control in IL-6 & IL-8.

| Group | No. | Mean pm/ml ± SE | |
|---|---|---|---|
| | | IL-6 | IL-8 |
| Patients | 80 | 1.056 ± 0.18 | 0.543 ± 0.16 |
| Control | 40 | 1.969 ± 0.19 | 0.106 ± 0.01 |
| T-test | --- | 0.598 * | 0.465 NS |

* (P<0.05).
NS: No significant differences
SE: Stander error

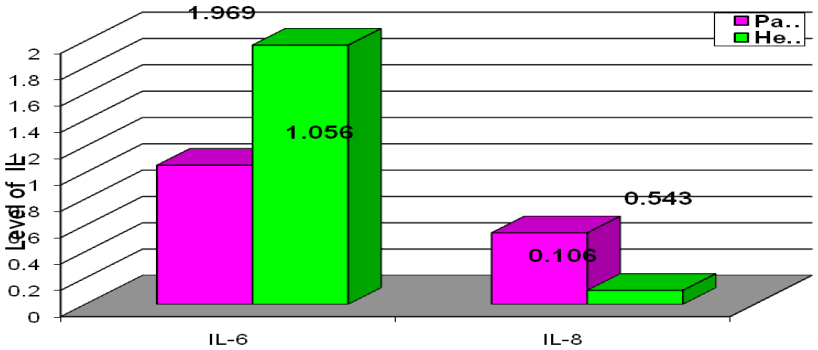


Figure 1: Models show significant difference to productivity cytokine from Diabetes Mellitus type 2 and healthy.

Type 2 diabetes mellitus (T2DM) has been recognized as an immune mediated disease leading to impaired insulin signaling and selective destruction of insulin producing beta cells in which cytokines play an important role (5). This stresses the fact that type 1 and 2 diabetes mellitus may be considered different entities with a probable different pathophysiologic pattern. Zozulinska, (1999), shown elevated resting values of IL6 and IL8 in diabetic patients compared to non diabetic controls, and in another study of type 2 DM patients showed a significantly higher production of IL6 compared with type 1 DM patients and control (14), TNF α and IL6 have been shown to attenuate insulin sensitivity in vivo and in vitro (15), and the plasma concentration of IL6 independently predicts future risk of developing type 2 diabetes mellitus (16).

Serum IL8 levels are increased in diabetic patients (14) and IL8 has been suggested to be involved in the pathogenesis of atherosclerosis (17). Proinflammatory cytokines have also been shown to be elevated in type 2 diabetes. IL6 has been shown to have the strongest correlation with insulin resistance and type2 diabetes (18). The conclusion elevated values of IL 8 and low values of IL6 were found in diabetes mellitus type 2 compared to healthy.

## CONCLUSION

A high value of IL 6 in type 2 diabetes compared with healthy.

## ACKNOWLEDGEMENTS

Authors are thankful to whom sharing their knowledge, and all who participates in this work.

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