

## **Interleukin-2 and Interleukin-12 levels of Type 1 Diabetes Mellitus Patients in Thi-qar Province**

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### **Abstract:**

The current study was conducted at the center for Diabetes and Endocrinology of the Health Directorate in Thi- Qar province, during the period from October 2013 to May 2014. The study aimed to evaluate immune status of diabetes type I patients by measuring the levels of interleukins (IL-2, IL-12) in the serum using a technique enzyme-linked immune sorbent adsorptive (ELISA), the study included a total of 72 patients with type I diabetes they were (37 males and 35 females) and they were aged between 1-40 years. When compared with 12 apparently healthy people as control. The results showed a significant increase ( $P \leq 0.01$ ) in the levels of interleukins in the serum (IL-2, IL-12) of all patients with type I diabetes compared to the control group.

**Key words:** IL-2 , IL-12 , T1DM , Thi-Qar

مستويات انترلوكين ٢ وانترلوكين ١٢ لمرضى داء السكري النوع الاول  
في محافظة ذي قار

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الخلاصة :

أجريت الدراسة الحالية في مركز السكري والغدد الصماء في مديرية الصحة في محافظة ذي قار، خلال الفترة من تشرين الاول ٢٠١٣ إلى ايار ٢٠١٤. هدفت الدراسة الى تقييم الحالة المناعية لمرضى السكري من النوع الأول عن طريق قياس مستويات الحركيات الخلوية ، التي شملت (IL-2 ، IL-12) في مصل الدم باستخدام تقنية الإمتزاز المناعي المرتبط بالإنزيم (ELISA) ، شملت الدراسة دراسة ٧٢ فردا من المرضى الذين يعانون من داء السكري من النوع الأول (٣٧ ذكور و ٣٥ إناث) تراوحت أعمارهم بين ١-٤٠ سنة. بالمقارنة مع ١٢ شخصا من الأصحاء ظاهريا. وأظهرت النتائج زيادة معنوية ( $P \leq 0.01$ ) في مستويات الحركيات الخلوية في المصل (IL-2 ، IL-12) في جميع المرضى الذين يعانون من داء السكري من النوع الأول مقارنة مع مجموعة السيطرة.

## **Introduction :**

Diabetes mellitus type 1 (also known T1DM ,formerly insulin dependent diabetes mellitus (IDDM) , or juvenile diabetes [1,2]. T1DM is a chronic systemic metabolic disorders characterized by increased levels of glucose in the blood (hyperglycemia) and abnormalities in the metabolism of protein [3] , that results from the autoimmune destruction of insulin-producing pancreatic beta cells[4,5],by autoreactive T-lymphocyte[6] , and that leading deficiency of insulin [7] , and this disease was once thought to be mediated exclusively by CD4\_ T cells and is now recognized as one in which autoreactive CD8\_ T cells play a fundamental pathogenic role [8] . The etiology of T1DM is complex and involves both genetic and environmental factors which play important roles [9,10,11] . A permissive genetic background is required for the development of the islet autoimmune process generating antibodies (Ab) against insulininsulin auto antibodies (IAA), glutamic acid decarboxylase isoform 65 (GADA65-Ab), and protein tyrosine phosphatase (IA2) [12,13] .

The incidence of T1DM is reported to be increasing by 3-5% per year, and the number of people with diabetes is estimated to reach 380 million by 2025 [14]. The incidence rate of childhood T1DM is increasing dramatically in many countries over the past 20 years[15] .The classical symptoms of type 1 diabetes include polyuria (frequent urination),polydipsia (increased thirst),Xerostomia (dry mouth),polyphagia (increased hunger), fatigue, and weight loss[16,17] .

The diabetic complications are divided into microvascular (nephropathy, retinopathy, and neuropathy) and macrovascular (cardiovascular) disease. Once considered a part of type 1 diabetes itself, the observation that similar complications also arise in patients with secondary diabetes led to the view that complications are caused by the hyperglycaemic milieu. The blood glucose values that define diabetes itself are set by the risk of diabetic microvascular complications [18,19] .

## **Cytokines:**

Cytokines are central mediators of inflammation by controlling innate and adaptive immune responses as well as tissue damage, defense, repair, and remodeling. Type 1 diabetes is an inflammatory disease of the pancreatic islet, in which insulin-producing  $\beta$ -cells are preferentially destroyed to varying degrees by the concerted action of autoreactive T-cells and monocytic cells [20] .The physiological response to physical exercise includes secretion of pro- and anti-inflammatory cytokines, counterregulatory hormones, and growth factors [21,22]. All elements of this complex response pattern are relevant to children with type 1 diabetes (T1DM) [23].

### **Interleukin-2(IL-2):**

IL-2 is a 15 kDa 4-bundled  $\alpha$ -helical protein mainly produced by activated CD4<sup>+</sup> T lymphocytes. However, the expression of IL-2 by naive CD8<sup>+</sup> T cells, dendritic cells, and thymic cells has also been reported [24,25]. The magnitude and duration of the T cell immune response is dependent on the interaction of IL-2 with its high-affinity IL-2 receptor (IL-2R) which is composed of  $\alpha$ ,  $\beta$  and  $\gamma$  subunits. The intermediate affinity IL-2R is composed of IL-2R $\beta$  (CD122) and IL-2R $\gamma$  (CD132) and is constitutively expressed on resting T lymphocytes. However, IL-2R $\alpha$  (CD25) is only induced after T-cell activation, which allows the formation of the high-affinity IL-2R[26]. IL-2 have been identified as contributors to the breakdown of central and/or peripheral tolerance, although the precise mechanisms associated with pathogenesis remain to be defined [27,28,29,30,31,32].

Circumstantial evidence suggests that regulatory T cells (T-reg cells) control the progression of diabetes. Disruption of T-reg cell development and homeostasis by blocking of the CD28-B7 pathway or IL-2 activity in NOD mice leads to acceleration of diabetes [33,34].

### **Interleukin -12 (IL-12) :**

Interleukin-12 is a cytokine produced by antigen presenting cells like Dendritic Cells (DC), macrophages also by NK cells. It plays a critical role in cell mediated immunity. It affects a variety of stages in the immune response; it prompts NK cells and T cells to produce pro-inflammatory cytokines, such as IFN- $\gamma$ , IL-2, IL-3 and TNF- $\alpha$ ; it contributes to NK cell maturation [35]; and, along with other pro-inflammatory factors, it stimulates CD4<sup>+</sup>CD25<sup>-</sup> T cell activation in the presence of regulatory T cells [36]. Interleukin-12 also regulates naive T cell differentiation into T helper type 1 lymphocytes (Th1), and inhibits differentiation into T helper type 2 lymphocytes (Th2) [37]. It has been documented that increased systemic inflammatory activity in patients with coronary artery disease is associated with a prominent Th1 response [38]. Current data suggest that IL-12 plays a critical role in the pathogenesis of T1DM [39].

The significance of IL-12 in human autoimmunity is not clear, and serum levels of IL-12 in diabetes mellitus have not been well established. Elevated levels of this cytokine have been observed in most autoimmune diseases [40,41].

IL-12 is a disulfide linked heterodimer composed of a heavy chain of 40 kDa (p40) and a light chain of 35 kDa (p35). The two subunits of IL-12 are in contrast to most cytokines, which possess only one polypeptide chain. Many cell types express the p35 chain, while the p40 subunit is expressed mainly by activated macrophages and B cells [42].

## **Materials & Methods :**

### **study design :**

This study was performed on (72) Iraqi patients with T1DM patients, who attended the consultant clinic for Type 1 diabetes mellitus in endocrine and diabetic center in Al-Nasiriya city in the period from beginning of October 2013 to the end of May 2014. Also, this study included (12) person apparently healthy individuals as a control group, who have no history or clinical evidence of T1DM or any other chronic disease, and no obvious abnormalities.

### **Blood Samples Collection:**

Blood samples were collected by venipuncture from 72 patients and 12 controls (five milliliters of venous blood) were drawn by disposable syringe under aseptic technique. The blood samples were placed in a sterile plane tube and allowed to clot, then serum was separated by centrifugation at 4000 rpm for 15 minutes. The serum was stored at  $-10\text{ C}^{\circ}$ . These sera (72 T1DM patients and 12 controls) were used for estimating the concentration of interleukin (IL-2, and IL-12).

### **Methods :**

Kit of (IL-2&IL-12) provided by (CUSABIO company, Germany). The sera of patients and controls were assessed for the level of two cytokines, which were IL-2, IL-12, by means of ELISA that were based on similar principles.

### **A - Principles of Assay :**

This assay employs the quantitative sandwich enzyme immunoassay technique. Antibody specific for IL-2, IL-12 has been pre-coated onto a micro plate. Standards and samples were pipetted into the wells and any IL-2, IL-12 present was bound by the immobilized antibody. After removing any unbound substances, a biotin-conjugated antibody specific for IL-2, IL-12 was added to the wells. After washing, avidin conjugated Horseradish Peroxidase (HRP) was added to the wells. Following a wash to remove any unbound avidin-enzyme reagent, a substrate solution was added to the wells and color develops in proportion to the amount of IL-2, IL-12 bound in the initial step. The color development was stopped and the intensity of the color is measured.

### **B – Assay procedure :**

All reagents were brought and samples to room temperature before use. The samples were centrifuged again. It is recommended that all samples and standards be assayed in duplicate.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.

2. Refer to the Assay Layout Sheet to determine the number of wells to be used and put any remaining wells and the desiccant back into the pouch and seal the ziploc, store unused wells at 4°C.
3. Add 100µl of standard and sample per well. Cover with the adhesive strip provided. Incubate for 2 hours at 37°C. A plate layout is provided to record standards and samples assayed.
4. Remove the liquid of each well, don't wash.
5. Add 100µl of Biotin-antibody (1x) to each well. Cover with a new adhesive strip. Incubate for 1 hour at 37°C. (Biotin-antibody (1x) may appear cloudy. Warm up to room temperature and mix gently until solution appears uniform.)
6. Aspirate each well and wash, repeating the process two times for a total of three washes. Wash by filling each well with Wash Buffer (200µl) using a squirt bottle, multi-channel pipette, manifold dispenser, or autowasher, and let it stand for 2 minutes, complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
7. Add 100µl of HRP-avidin (1x) to each well. Cover the microtiter plate with a new adhesive strip. Incubate for 1 hour at 37°C.
8. Repeat the aspiration/wash process for five times as in step 6.
9. Add 90µl of TMB Substrate to each well. Incubate for 15-30 minutes at 37°C. Protect from light.
10. Add 50µl of Stop Solution to each well, gently tap the plate to ensure thorough mixing.
11. Determine the optical density of each well within 5 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. Subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

**Statistical analysis :** The analysis of data was expressed as mean  $\pm$  SD. The comparisons between each T1DM patient group with matched healthy control were performed with T-test by using computerized Minitab 14 program.  $P < 0.01$  was considered to be the least limit of significance, the statistical analysis was done by using Pentium-4 computer through the ( SPSS program) Statistical Package For Social Sciences (version-20).

### **Results :**

The present study showed the presence of a significant increase ( $P \leq 0.05$ ) in the rate of concentrations of IL-12, IL-2 in sera of patients with type I diabetes, compared with the average concentration in the sera of healthy control group, as was the rate of concentration of IL-2 in patients (39.099pg/dl) compared to the control group (19.623pg/dl) with significant difference

(0.01), while IL-12 concentration (60.154 pg/dl) for patients compared to the healthy control (29.417pg/dl ) with a significant difference (0.01) .

**Table (1) :** Comparison of serum (IL-2,IL-12) concentrations (pg/dL)

of the patient groups with healthy controls group

Parameter	Subject	No of cases	Mean± SD	T-value	Df	P-value
IL – 2	Pateints	70	39.099 ± 25.900	2.530	80	0.01
	Control	12	19.623 ± 14.322			
IL – 12	Pateints	70	60.154 ± 39.477	2.622	80	0.01
	Control	12	29.417 ± 21.445			

### **Discussion:**

The results of the current study showed a high level of concentration of IL-12 in the patient group compared to the healthy control group and a significant difference ( $p \leq 0.01$ ) for patients ( $60.154 \pm 39.477$ ) and the healthy control group ( $29.417 \pm 21.445$ ) .These results agree with the results studies of all [35,39.43], whose indicated to a increase in the concentration of IL-12 in the sera of patients with type I diabetes.The increase of IL-12 in most autoimmune diseases such as multiple sclerosis [44], as well as events in arthritis and psoriasis[45]..

The elevated serum IL-12 levels in T1DM were related to the excessive pro-insulin secretion. Therefore, this cytokine may play a critical role in the pathogenesis of T1DM, since IL-12 is important in immune response to infections. It has been shown that in the absence of infection, IL-12 induced auto-reactive T cell responses might predispose to self-destructive immunity but the significance of IL-12 changes in the blood of patients with T1DM remains unclear. The IL-12 accelerates the development of macrovascular complications in the disease [46]. Additionally, it has been noted that elevated glucose levels in diabetic animals stimulates inflammatory reactions related to IL-12 cytokine gene expression [47].However, it is not known whether factors related to the course of T1DM , such as metabolic compensation, beta cell secretory dysfunction and insulin resistance affect IL-12 concentrations, but in a recent study, a multiple regression analysis revealed that the IL-12 serum level in T1DM primarily was dependent upon fasting pro-insulin concentration [43].It has been suggested that cytokines released by monocytes/macrophages, including IL- 1beta, IL-12 could have an initial role in islet  $\beta$ -cell damage [35] . Pro-inflammatory cytokines may be increased by hyperglycemia in subjects with impaired glucose tolerance, this result was confirmed, because all diabetics had elevated serum level of IL12. This cytokine may be useful biomarkerfor early detection of diabetes[48] .

The IL-2 Tests showed the results of the current study, the increase in the level concentration of IL-2 in the patient group ( $39.099 \pm 25.900$ ) compared to the healthy control group ( $14.322 \pm 19.623$ ) and a significant difference ( $P \leq 0.01$ ). Type I diabetes is a chronic inflammatory disease, some's researches indicated the cytokines increase when the injury chronic inflammatory diseases and malignant diseases [49,50,51,52,53,54]. The results of this study are agree with the results of a study [55], whose have indicated the high level of concentration of IL-2 in the sera of patients with type I diabetes.

The reason for the increase in the concentration of IL-2 is associated with elevated concentration of IL-12 as the IL-12 released from APCs activates TH1-type CD4+ T cells, causing the immune balance between effector and regulatory cells to breakdown. TH1 cells produce IL-2, which activates b cell-specific pre-cytotoxic T cells (Pre CTL) to become cytotoxic (CTL), and IFN-g, which may cause macrophages (MØ) to become cytotoxic. These cytotoxic macrophages release b cell-cytotoxic cytokines including IL-1b, TNF-a, and IFN-g, and free radicals. TH1 cells also secrete cytokines that are directly cytotoxic to b cells.  $\beta$ cell antigen specific CD8+ cytotoxic T cells (CTL) recognize antigens expressed on b cells in association with MHC class I molecules. These CTLs release granzyme and perforin(cytolysin), which are toxic to b cells. In addition, Fas- and TNFR-mediated apoptosis are involved in b cell destruction. In this way ,macrophages , T cells, and cytokines synergistically act to destroy b cells, resulting in the development of autoimmune type 1 diabetes[56].

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