

Study a level of TNF- α and INF-Y in patients with Type I and II Diabetes Mellitus
in diayla governorate

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Mellitus in diayla governorate

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Abstract

The aim of this study was to determine a level of TNF- α and INF-Y in patients with Type I and Type II Diabetes Mellitus, this cytokines measured by enzyme-linked immunosorbent assay (ELISA). A total of 70 patients and 20 healthy volunteers were included in this study, collected during the period from the beginning of August 2015 to the end of May 2016. The results showed a significant increase in the concentration of INF-Y in the serum of both types diabetes mellitus with the control group there were, the level of INF-Y in patients with type I and type II diabetes mellitus was 740.1, 738.5 pg / mL respectively while the level in control group was 431.9 pg / mL .As well as the results showed a significant increase in the concentration of TNF- α in the serum of both types diabetes mellitus than control group there were, the level of TNF- α in patients with type I and type II diabetes mellitus was 339.2 , 228.4 pg / mL respectively while the level in control group was 107.8 pg / mL.

Key word: Diabetes Mellitus , TNF- α , INF-Y .

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دراسة مستوى عامل التنخر الورمي الفا وانترفيرون كاما للمرضى المصابين بداء السكري النوع الاول والثاني في محافظة ديالى

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الخلاصة

تهدف هذه الدراسة الى تحديد مستوى عامل التنخر الورمي الفا و انترفيرون كاما في المرضى المصابين بمرض السكري النوع الأول والنوع الثاني باستخدام طريقة الامتزاز المناعي المرتبط بالأنزيم (ELISA). تم جمع (70) عينة دم من مرضى السكري لكلا النوعين وتم جمع (20) عينة كمجموعة سيطرة ، تم إجراء هذه الفترة من بداية اب 2015 إلى نهاية ايار 2016. أظهرت النتائج ارتفاع مستوى تركيز عامل التنخر الورمي الفا و انترفيرون كاما لدى المرضى المصابين بمرض السكري من النوع الاول والنوع الثاني مقارنة مع مجموعة السيطرة حيث بلغ تركيز انترفيرون كاما للأشخاص المصابين بالنوع الأول والنوع الثاني لمرض السكري (506.9 ± 740.1) ، (576.6 ± 738.5) على التوالي مقارنة مع الأشخاص الاصحاء (147.4 ± 431.9) ، كذلك اظهرت نتائج الدراسة ارتفاع تركيز عامل التنخر الورم ألفا TNF- α في مصل المصابين لكلا النوعين مقارنة مع العينة القياسية ، حيث بلغ تركيزه كاما للأشخاص المصابين بالنوع الأول والنوع الثاني لمرض السكري (205.6 ± 339.2) ، (224.6 ± 228.4) على التوالي مقارنة مع الأشخاص الاصحاء (107.8 ± 29.6) .

الكلمات المفتاحية: مرضى السكري ، عامل التنخر الورمي الفا ، انترفيرون كاما

Introduction

Diabetes mellitus is a group of metabolic disorders that end with Hyperglycemia , and produces either a defect in the secretion of the hormone insulin or insulin reaction or both(3). It is a major worldwide health problem predisposing to markedly increased cardiovascular mortality and serious morbidity and mortality related to the development of nephropathy , neuropathy and retinopathy (39). Diabetes is a common disease that occurs due to lack of control over the level of glucose in blood, which over time leads to a significant damage to be influential in the structure and functions of the body tissue disease (38). There are three main

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types of diabetes Type I and Type II diabetes and gestational diabetes, diabetes type I, which is known diabetic insulin-dependent diabetes is characterized by pancreatic β -cell destruction mediated by immune mechanisms which leads to a lack of insulin secretion, whereas type II non insulin dependent diabetes mellitus (NIDDM), on differently from the first type which leads to insulin resistance or reduced insulin sensitivity, in addition to relatively low secretion of insulin (4). Add to another type of diabetes is almost latent called gestational diabetes and there are other types of secondary can get because of other diseases (5). Some cytokines associated with the levels of risk of diabetes (33). This cytokines include tumor necrosis factor alpha TNF- α , which is secreted preliminarily by phagocytic cells (Macrophage) and also by a wide range of other cells, including adipocytokine (7). Protein interferon-gamma (IFN- γ), also known Type II interferon or Macrophage-activating factor (MAF) of cytokines derived from T cells type 1 helper cells (Th1) which supports the immune system to complete the cell decomposition of the target cells, as well as a documented increase in patients with diabetes (34). Cytokines are produced by a wide range of Leukocytes and Non leukocytes and help to facilitate communication between cells as well as working on differentiation and reproduction of cells that affect the immune system and that works either self (Autocrine) or may work on other neighboring cells target (Paracrine action) lit work hormonally (Indocrine action) cells and long distances(12). Note has been an increase of the level of dynamics of cellular pro-inflammatory in patients with type II diabetes (27). It is already clear on a large scale that cytokines play a key role in the development of Type I diabetes. Research has indicated a link interferon alpha and interferon-gamma with Type I diabetes strongly (29; 26), therefor ,the aimed of this study was to determine a level of TNF- α and INF- γ in patients with Type I and Type II Diabetes Mellitus in diayla governorate .

Materials and Methods

Collecting 70 blood samples from patients with diabetes as (35) serum sample for each type, all patients diagnosis by a physician specialist in advisory clinic in Baquba Teaching Hospital in Diyala province where the number of males for type I (14) and the number of females (21) and the number of males for type II (17) and the number of females(18). Twenty apparently healthy volunteers from both sexes were included in this study as a control group. They were

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12 males and 8 females. Samples were collected from those individuals only if they were not receiving any medication and did not had a history of a chronic or acute illness. From each individual included in this study, 5 ml of blood was drawn by vein puncture , then the sera was separated by centrifugation for 5 minutes, and divided into aliquots (250 μ l) and stored at -20°C till examination. Each aliquot of the serum was used once to avoid thawing and freezing. All sera and reagents were allowed to stand at room temperature before use in the test.

Principle of TNF- α and INF- γ measurement

The tumor necrosis factor levels alpha and interferon-gamma was quantified using the Sandwich ELISA test ,according to the instructions obtained in the kit made by shanghai company. This kit uses enzyme-linked immune sorbent assay (ELISA) based on biotin double antibody sandwich technology to assay Human Tumor necrosis factor α (TNF- α) and Interferon gamma (INF- γ). Add Tumor necrosis factor α (TNF- α) and Interferon gamma (INF- γ) to wells that are pre-coated with Tumor necrosis factor α (TNF- α) monoclonal antibody and Interferon gamma (INF- γ) monoclonal antibody then incubate. After incubation, add anti TNF- α antibodies and anti INF- γ labeled with biotin to unite with streptavidin-HRP, which forms the immune complex. Remove unbound enzymes after incubation and washing, then add substrate A and B. The solution will turn blue and change to yellow with the effect of acid. The shades of solution and the concentration of TNF- α and INF- γ are positively correlated (6).

Statistical analysis

Data have been analyzed statistically using Statistical Package for Social Sciences program version 22 with descriptive formula has been described by formula number and percentage and were compared using the chi square test As for variables with numerical formula has been described using the average and standard deviation of the rate and were compared between groups using test(t-test) between the two groups or test (ANOVA) during the comparison of more than two groups, use the correlation coefficient test to find the possible relationship between two variables measure (25).

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Results and Discussion

A-Distribution of the study groups according to sex and age

This study included 70 samples were divided into two groups, first group type I diabetes includes 35 sample, 14(15.5 %) male and 21 (23.3%)female and second group Type II diabetes includes 35 sample,17(18.8%)male and 18(20%)female , while the control group includes (20) sample, 12 (13.3%) male and 8 (8.8 %)female .As shown in (Figure1) .

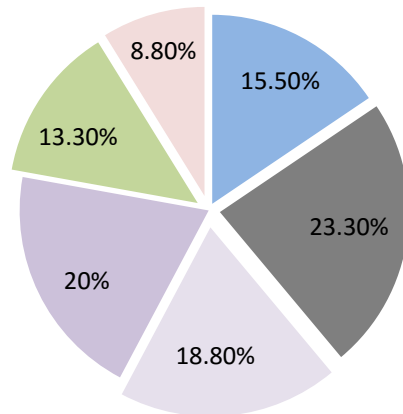


Figure 1. The distribution of the study According to gender

The result of this study show the female patients type I and type II diabetes mellitus was higher than male patients there were , the percentage of female was (23.3 ,20) % respectably , while the percentage of male (15.5, 18.8)% respectively compared with the control group(13.3 ,8.8)% respectively with no significant difference. This could be due to the hormonal influence , the hormonal influence in the case of diabetes for women is more than men. The female sexual hormonal differences are related to Endocrinopathy ,Menstruation, Pregnancy , Menopause and Hormonal Contraceptive. It is also believed that the sexual hormone receptors have an impact on the functions of inflammatory and immune cells (35). The results of this study are compatible with other studies who did not find any difference in the distribution of the groups studied by gender(13,17,32). The results of this study showed that the mean age of patients with diabetes type I was (17.9 \pm 18.5) years while the mean age of patients with type II diabetes (56.3 \pm 10.2) years, while in the control group was (37.7 \pm 10.9)

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years, with a significant difference was noticed between then , with the p-value ($p < 0.0001$). As shown in the table (1).

Table 1. Distribution of the studied groups (type I ,type II and apparently healthy control) according to the age

Age (years)	Study group		
	Control	Type 1	Type2
Number	20	35	35
Mean \pm S.D	37.75 \pm 10.99	17.94 \pm 18.54	56.34 \pm 10.22
P value	0.0001<		

The studies have shown that aging in Iraqi society is associated with increasing insulin resistance , refusing to endure the glucose , increasing fat tissues, heart diseases ,increasing blood pressure , and atherosclerosis (8, 9,31) . These complications usually appear in patient with diabetes typeII because the probability of infecting with this disease increases with aging as a result of a weakened immune system of the diabetic people which is due to physiological changes for patients with diabetes type II , which may alter the immune cell activity of the immune system (40, 28) . Previous studies have shown that the high infecting average of type I diabetes category (10-19 years) may be due to physiological changes that occur in puberty and the increase of sexual hormones. It may be due also to the influence of environmental factors, malnutrition, and fatigue (16, 37).

B - TNF- α and INF- γ level

The study showed a remarkable increase in the concentration of interferon- gamma in the serum of patient with type I and type II compared with the sample (healthy), the concentration in the blood of patient with diabetes type I(740.1 \pm 506.9) pg/ml, while the concentration in the blood of patient with type II diabetes (738.5 \pm 576.6) pg / mL, also the level in control group was(431.9 \pm 147.3) pg / mL, as shown in table(3). Where the results of the current study show no significant differences in the concentration of interferon- gamma among patient with diabetes type I and type II with p .value of probability (0.0489). But when measuring tumor necrosis factor level alpha (TNF- α) study results showed high concentration for type I and type II compared with the standard sample (healthy), concentration in the blood

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of patient with diabetes type I (339.2 ± 205.6) ng / L, while the concentration in the blood with diabetes type II (228.4 ± 224.6) ng / L, and also the level in control group was (107.8 ± 29.65) ng / L, as shown in the table (2).

Table 2. Serum level mean of TNF α and IFN- γ DM (type I, type II) and apparently healthy control

Pg / ml		Study group		
		control	Type 1	Type2
TNF-alpha	N	20	35	35
	mean \pm SD	107.8 \pm 29.65	339.2 \pm 105.6	228.4 \pm 124.6
	p.value	0.0002		
INF -Y	N	20	35	35
	mean \pm SD	431.9 \pm 147.3	740.1 \pm 506.9	738.5 \pm 576.6
	p.value	0.0489		

The results of this study showed that the presence of high levels of interferon-gamma concentration in patient with diabetes for type I and type II and the effective role that is represented in the crash of beta cells in patient with type 1 diabetes, these findings are consistent with the results of each of (15,23,20, 24) by capturing high concentrations of interferon-gamma in a sample infected compared with the standard sample (healthy), where there are many studies that supported the results of this study show a clear concept to beta crashed cell associated with increased expression dynamics of cellular pro-inflammatory such as IL-12, IL-2, IL-1, TNF- β , TNF- α , IFN- γ and INF- α (19). Interferon-gamma is working to induce and accelerate the crash of beta cells in patient with diabetes type I where the crashing directly mechanism or indirectly, to direct the crash cellular detachment of the cytokines by helper T cells type I mechanism (Th1) and included interferon gamma show their effects either when macrophages, leading to increased filtration of these cells in the pancreatic islets cells sites, leading to increased filtration of these cells in the pancreatic islets cells sites, which speeds up the beta cells of the crash during the liberation of nitric oxide nitric oxide and the roots of oxygen oxygen radicals(21). The rise in the blood glucose in patients with Type II diabetes increases with inflammation markers that help predict diabetes type II (1, 22). There are many studies that have supported the current results of the study showed that high concentration of tumor necrosis factor alpha for patients with diabetes compared with the

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control group, these results coincided with the results of (30, 18, 14). The high level of tumor necrosis factor alpha is an indicator of the increased insulin secretion (10). Tumor necrosis factor alpha production may limit the activity of T cells and the organization promotes the induction of immune interaction and phase influencing the response of lymphoid cells. Therefore, the low production of tumor necrosis factor may lead to enhance the function of regulatory T cells that limit the occurrence of immune reaction. In this way, tumor necrosis factor plays an important role in adaptive immunity (35). Additionally some researcher was noticed increase a level of pro-inflammatory cytokines in patients with diabetes mellitus type I and II (11). But when compared with INF-Y and TNF- α with sex for the patients and healthy controls. He was found INF-Y for patient with diabetes type I in males (797.3 ± 634.8) pg / mL higher than females (701.9 ± 413.6) pg / mL with no significant differences for both sexes, but for patient with diabetes type II in males (811.9 ± 667.9) pg / mL higher than females (669.2 ± 484.4) pg / mL with no significant differences for both sexes, as for the control group were males (431.9 ± 161.0) pg / mL and females (431.9 ± 134.7) Pg / mL with no significant differences for both sexes. As shown in Table (3).

Table3. Relationship between INF-Y and sex in studied groups

INF y with sex	Control		Type1		Type2	
	male	female	male	female	male	female
Number	12	8	14	21	17	18
mean	431.9	431.9	797.3	701.9	811.9	669.2
Std.D	161.0	134.8	634.8	413.6	667.9	484.4
Std.E	46.47	47.61	169.7	90.26	162.0	114.2
Minimum	201.0	167	227	284	164	331
maximum	658	599	2008	1942	1969	1836
p.value	0.9995		0.5308		0.4723	

Also it found the level of TNF- α for type 1 diabetes in males (366.4 ± 221.4) ng / L higher than females (321.1 ± 197.8) ng / L with no significant differences between the sexes and for type II diabetes among males (290.7 ± 246.0) ng / L higher than females (286.3 ± 209.6) ng / L with no significant differences between the sexes. The control group were males ($104.8 \pm$

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29.78) ng / L less than females (112.3 \pm 30.65) ng / l with no significant differences between the sexes. As shown in Table (4).

Table 4. Relationship between TNF- α and sex in studied groups

TNF- α with sex	Control		Type1		Type2	
	Male	female	male	female	male	female
gender						
number	12	8	14	21	17	18
mean	104.8	112.3	366.4	321.1	290.7	286.3
Std.D	29.78	30.65	221.4	197.8	246	209.6
Std.E	8.598	10.84	59.16	43.17	59.66	49.41
Minimum	51.0	91.0	170	128	102	112
Maximum	166	184	754	759	758	744
p.value	0.5962		0.5931		0.9545	

The absence of a statistically significant difference in patients with the two types of diabetes for both sexes may be due to the mechanics of the immune defense of the male and female .The immune response in the patient's body may be similar to some extent regardless of sex because the interaction within the patient's body leads to activation of immune cells which are responsible for the immune response in the serum of patients with diabetes. It can be concluded that the kind of gender has no effect on the level of cellular dynamics in the body (2).

Conclusions

The present study showed a significant increase the levels of TNF- α and INF- γ in patient with diabetes mellitus type I and II compared to the control group.

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