The Effect of High Insulin Resistance and Obesity on serum level of IL-12 in a sample of Iraqi patients with Type 2 Diabetes Mellitus

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الخلاصة

يلعب IL-12 دورا حاسما في التسبب بالاصابة في النوع الأول والنوع الثاني لداء السكري ، ولكن أهمية التغيرات الحاصلة لـ IL-12 في دم المرضى الذين يعانون من هذا المرض بنوعه الثاني مازالت غير واضحة لحد الان .ومع ذلك، من غير المعروف ما إذا كانت العوامل المتصلة أثناء الاصابة بداء السكري النوع الثاني كتعويض التمثيل الغذائي، ضعف دور خلايا بيتا، وتأثير مقاومة الأنسولين تؤثر على مستوى تركيز IL-12 . أجريت الدراسة لتحديد مستوى المصل من IL-12 في عينة من المرضى العراقيين المصابين بالسكري النوع الثاني T2DM. وكان التقييم في ضوء وجود السمنة (كدالة كتلة الجسمBMI) ومقاومة الانسولين(بنموذج التقييم الاستتبابي HOMA).

أجريت الدراسة على 50 شخص مصابين بداء السكري النوع الثاني (29 انثى و21 ذكر) والذين تراوحت اعمارهم بين(28-60)، ولغرض المقارنة اعتمد 30 شخص من الاصحاء (السيطرة)المتوافقين بالعمر (33-59 سنة)والجنس (16 انثى و 14 ذكر) مع المرضى المصابين بداء السكري النوع الثاني.قياس سكر بلازما الدم الصائم و المستوى المصلي لهرمون الانسولين قد اجري لتحديد مقاومة الانسولين بواسطة نموذج مقاومة الانسولين . المستوى المصلي لانترلوكين IL-12 و دالة كتلة الجسم قيست ايضا.

اظهر المستوى المصلي لهرمون الانسولين زيادة معنوية وهي (12.4 مقابل 8.3 مايكروووحدة عالمية/مليليتر) ($P\leq0.01$) ومقاومة الانسولين HOMA(5.5 مقابل 1.8) ($P\leq0.001$) في المرضى مقابل السيطرة.المستوى المصلي لانترلوكينIL-12 اظهر ارتفاعا معنويا (19.3 مقابل 6.0 بيكو غرام/مليليتر) ($P\leq0.001$) في المرضى مقابل السيطرة بينما لم تكون هناك اي فروق معنوية بين نسبة هذا الانترلوكين مع مجموعات الثلاثة من السمنة (عادي، زيادة الوزن والبدانة) بينما اظهر التأثير الصافي و المستقل لمقاومة الانسولينHOMA بعد ضبط فروقات المرضى و السيطرة على الناتج المنتقى(المستقل)، قيم معنوية لمستوى المصلي IL-12 . ان الزيادة في مؤشر مقاومة الانسولين HOMA مع زيادة في نسبة IL-12 كان قادرعلى شرح 26.7٪ من التباين الملحوظ في النتائج المتغيرة و بالتالي هذا النترلوكين قد يلعب دورا حاسما في التسبب بمضاعفات السكري النوع الثاني.

ABSTRACT

The IL-12 plays a critical role in the pathogenesis of type I and type II diabetes mellitus, but the significance of IL-12 changes in the blood of patients with type 2 diabetes mellitus T2DM remains unclear. However, it is not known whether factors related to the course of T2DM, such as metabolic compensation, beta cell secretary dysfunction, and insulin resistance affect IL-12 concentrations.

To determine the serum level of IL-12, in a sample of T2DM obese type 2 diabetic patients. This determination was interpreted on the ground of obesity (body mass index; BMI) and insulin resistance (homeostatic model assessment; HOMA).

The study was conducted on 50 type 2 diabetic patients, 29 females and 21 males, the age range within 28-60 years .For the purpose of comparisons, Thirty control subjects comparable to diabetic patients in respect to age (33-59 year) and gender (16 females and 14 males), were included in the study. Fasting plasma glucose, Insulin and serum IL12 was determined to assess Insulin resistance by using HOMA model. Also body mass index was measured.

The mean of insulin serum level (12.4 *vs*. 8.3 µIU/ml) or HOMA (5.5 *vs*. 1.8) was significantly increased in T2DM patients as compared to controls. The serum level of IL-12, (19.3 *vs*. 6.0 pg/ml) showed significant ($P \leq 0.001$) increase in

geometric mean in T2DM patients as compared to controls. IL-12 showed a non-significant variation between the three groups of obesity (normal, overweight and obese). The HOMA had the most important impact on the outcome variable, after adjusting for BMI and study group. An increase in HOMA index was associated with a proportional increase in serum IL-12 and able to explain 26.7% of the observed variation in the outcome variable therefore this cytokine may play a critical role in the pathogenesis and complication of T2DM.

INTRODUCTION

Inflammation is emerging as an important mechanism for micro- and macrovascular complication of diabetes. The macrophage plays a key role in the chronic inflammatory response in part by generating particular cytokines. IL-1ß, IL-6, IL12, IL-18, TNF-α, and interferon-γ are produced primarily in macrophages and have been associated with accelerated atherosclerosis and altered vascular wall function. Recent investigations have suggested the role in the aeteopathogenic mechanism of type 2 diabetes mellitus T2DM, directly or indirectly, or through their interaction with adipose-derived factors [1,2,3]. Interleukin-12, a cytokine produced by antigen presenting cells like dendritic cells (DC), macrophages also by natural killer (NK) cells. It plays a critical role in cell-mediated immunity [4]. A significance of IL-12 changes in the blood of patients with T2DM remains unclear. It has been observed that IL-12 plasma concentrations are elevated in T2DM [5], and that IL-12 contributes to the process of atherosclerotic plaque formation and probably accelerates the development of macro vascular complications in T2DM, as well [6]. Additionally, it has been noted that elevated glucose levels in diabetic animals stimulates inflammatory reactions related to IL-12 cytokine gene expression [7]. However, it is not known whether factors related to the course of T2DM, such as metabolic compensation, beta cell secretary dysfunction, and insulin resistance affect IL-12 concentrations [1]. Based on the forthcoming presentation, the present study was planned to determine the serum level of IL-12 and insulin in a sample of T2DM patients. These determinations were interpreted in the ground of obesity (body mass index; (BMI) and insulin resistance (homeostatic model assessment ;(HOMA).

MATERIALS AND METHODS

The study was conducted on 50 patients with T2DM, 29 females and 21 males, the age range within 28-60 years randomly selected from those attending the National Diabetes Center / Al-Mustansiriyah University between October 2010-March 2011. Patients were fully examined and were free of acute illness or infection at time of study and with no known diseases which are associated with disordered glucose metabolism. For the purpose of comparisons, 30 control subjects, age

(33-59 year) and gender (16 females and 14 males), were included in the study. The controls were selected among subjects who were healthy in terms of non-diabetic, non-hypertensive, no other endocrine disorders or metabolic kidney diseases and were free of acute illness or infection at time of sampling. Also, they had no history of smoking or alcohol drinking. The T2DM and controls were categorized in terms of age, gender, family history of diabetes, BMI and HOMA. The patients were also determined for duration of disease, fasting plasma glucose (FPG) and glycosylated hemoglobin (HbA_{1c}).HOMA was determined using the equation of Wallace *et al*. [8]: HOMA = (FPI × FPG)/22.5, Where FPI is fasting plasma insulin concentration (µIU/ml) and FPG is fasting plasma glucose (mmol/L). ELISA kits for quantitative determination of IL-12 (USBiological Company, USA) and Insulin level (Monobind Inc. Company, USA) in human serum were used.

The data were translated into a computerized database structure, and the statistical analyses were carried out using the computer programme SPSS version 13 (Statistical Package for Social Sciences).

RESULTS AND DISCUSSIONS

The mean age of diabetic patients was (49.48±1.03). While that of controls was (45.40±1.31). According to BMI, Obese individuals accounted for 66.0% of T2DM patients compared to 50.0% of controls, the rest were overweight (16.0% *vs.* 30.0%) and normal weight (18.0% *vs.* 20.0%) in diabetics and controls respectively (P=0.277). The duration of disease in T2DM patients was 10 years and more in 22% of patients while rest were 20, 48 & 10% for 5-9 years, 1-4 years & less than 1 year respectively. The T2DM patients showed a slightly increased mean of BMI in comparison with controls (31.80 *vs*. 29.70 kg/m^2) and the difference was not significant (Table 1).

The FPG showed a significant ($P \leq 0.001$) increased mean in patients as compared to controls (191.50 *vs*. 88.50 mg/dL). The HbA_{1c} also showed a significant ($P \leq 0.001$) increased percentage mean (9.50 *vs*. 5.80%)(Table 1).

Table-1: means of body mass index, fasting plasma glucose and HbA_{1c} in T2DM patients and controls.

Parameter	Mean±SE (Range)		P value ≤
	Patients (n=50)	Controls (n=30)	
Body Mass Index (kg/m^2)	31.80 ± 0.80	29.70 ± 0.71	N.S.
Fasting Plasma Glucose (mg/dL) (Range)	191.50 ± 9.37 (77.0-347.0)	88.50 ± 1.92 (72.0-115.0)	0.001
HbA_{1c} (%) (Range)	9.50 ± 0.25 (6.0-13.2)	5.80 ± 0.12 (4.4-6.7)	0.001

N.S.: Not significant (P > 0.05).

The mean of insulin serum level (12.4 *vs*. 8.3 µIU/ml) and HOMA (5.5 *vs*. 1.8) were significantly increased in T2DM patients as compared to controls (Table 2).

The serum level of IL-12, (19.3 *vs*. 6.0 pg/ml) showed significant ($P \leq 0.001$) increase in geometric mean in T2DM patients as compared to controls (Table 2).

Table-2: medians (geometric means) of serum insulin level, HOMA and IL-12 in T2DM patients and controls.

Parameter	Patients (n=50)	Controls (n=30)	P value ≤
Serum Insulin (µIU/ml) Mean±SE (Range) Median (Geometric mean)	 14.65±1.51 (4.8-65.0) 11.3 (12.4)	 9.75±1.15 (3.5-32.5) 7.5 (8.3)	0.01
HOMA Mean±SE (Range) Median (Geometric mean)	 6.66±0.71 (2.02-31.66) 5.35 (5.5)	 2.14±0.27 (0.73-7.22) 1.65 (1.8)	0.001
IL-12 (pg/ml) Median (Geometric mean)	18.2(19.3)	5.3(6)	<0.001

Obesity (BMI) Impact on Investigated Parameters:-

To investigate the impact of obesity, as defined by BMI, on the investigated parameters (FPG, HOMA, insulin, IL-12), statistical differences between means or medians of these parameters were assessed among the three groups of obesity; normal (< 25 Kg/m^2), overweight (25 - 29.9 Kg/m^2) and obese (30 - 40 Kg/m^2). Such differences were assessed independently for T2DM patients and control subjects. IL-12 (Table 3) showed a non-significant variation between the three groups of obesity (normal, overweight and obese). The HOMA means of T2DM did not show a significant variation or correlation in

the three groups of obesity (normal, overweight and obese),. In contrast, it showed different means in the control groups, and the difference was significant ($P \leq 0.001$) (Table 4).

Table-3: Interleukin-12 serum level in diabetic patients and controls defined by obesity (body mass index).

Subjects	Serum Level of IL-12 (pg/ml)	Obesity by BMI (kg/m^2)		
		Normal <25	Overweight 25 25 - 29.9	Obese 30-40
Patients	Number	9	8	33
	Range	4.1-79	2.9-504.1	0.1-427.6
	Median(geometric mean)	14.9 (15.9)	20.9 (27.38)	18.5 (18.46)
	Kruskal-Wallis Probability > 0.05 (not significant)			
	r = -0.02 ; P > 0.05 (not significant)			
Controls	Number	6	9	15
	Range	(2.9-9.3)	(4-648.1)	(1.3-19.3)
	Median(geometric mean)	3.1(4.6)	8.9(13.38)	4.6(4.13)
	Kruskal-Wallis Probability > 0.05 (not significant)			
	r =-0.151 ; P > 0.05 (not significant)			

Table-4: homeostatic model assessment in diabetic patients and controls defined by obesity (body mass index).

Subjects	Homeostatic Model Assessment	Obesity by BMI (kg/m^2)		
		Normal <25	Overweight 25 - 29.9	Obese 30-40
Patients	Number	9	8	33
	Range	2.3-10.6	2.4-14.0	2.0-31.7
	Median (geometric mean)	7.15 (6.18)	7.10 (6.20)	4.40 (5.20)
	Kruskal-Wallis Probability > 0.05 (not significant)			
	r = -0.057; P > 0.05 (not significant)			
Controls	Number	6	9	15
	Range	0.9-1.5	0.8-4.3	1.1-7.2
	Median (geometric mean)	1 (1.06)	1.5 (1.78)	2 (2.32)
	Kruskal-Wallis Probability ≤ 0.01			
	r = 0.568 ; P ≤ 0.001			

Parameter Impact on HOMA:-

To evaluate the impact of IL-12 on the median or mean of HOMA, each parameter was first classified into ordered categories (quartiles) based on the cut-off value of each parameter in the patients and controls. Accordingly, each parameter was classified into three categories; lowest quartile (the subjects had the lowest data values), inter-quartile (the average range or central 50% of data values), and highest quartile (the subjects had the highest data values). Based on this classification, comparisons were made between the three quartiles of T2DM patients and control subjects independently for each parameter to assess statistical differences between medians or geometric means of HOMA, and at the same time the correlation was also assessed. The lowest quartile of serum IL-12 level recorded the lowest HOMA mean in T2DM, while it was leveled at the range 5.6-5.8 in the inter-quartile and highest quartile groups. Such variation failed to reach a significant level, and the r value also did not score a significant correlation. In controls, the HOMA mean showed a gradual increase (1.5, 2.1 and 3.1, respectively) in the three quartiles of IL-12, but also without a significant difference. However, r value contradicted the picture, and a significant ($P \leq 0.01$) correlation between these means was recorded in the controls (Table 5).

Table-5: homeostatic model assessment (HOMA) in diabetic patients and controls divided by serum IL-12 quartiles.

Subjects	Homeostatic Model Assessment	Serum IL-12 quartiles (pg/ml)		
		Lowest	Inter-quartile	Highest
Patients	Number	5	26	19
	Range	2.7-8.4	2.3-16.9	2.0-31.7
	Median (geometric mean)	3.9 (4.0)	5.8 (5.8)	5.7 (5.6)
	Kruskal-Wallis Probability > 0.05 (not significant)			
	r = 0.086 ; P > 0.05 (not significant)			
Controls	Number	15	14	1
	Range	0.7-4.9	1-7.2	3.1-3.1
	Median (geometric mean)	1.3 (1.5)	1.9 (2.1)	3.1 (3.1)
	Kruskal-Wallis Probability > 0.05 (not significant)			
	r = 0.462 ; P ≤ 0.01			

Adjusted Effect of BMI and HOMA:-

Using serum IL-12 as the dependent outcome variable, it was shown that being a diabetic is associated with an increase (although not significant statistically) in serum IL-12 level compared to controls, after

adjusting for BMI and HOMA (SPRC = 0.193). An increase in BMI was expected to be associated with a slight and a non-significant reduction in IL-12 after adjusting for HOMA and study group. The HOMA had the most important impact on the outcome variable, after adjusting for BMI and study group. An increase in HOMA index was associated with a proportional increase in serum IL-12 (SPRC = 0.250). The regression model was statistically significant and able to explain 26.7% of the observed variation in the outcome variable (serum IL-12) (Table 6).

Table-6: multiple linear regression models with investigated variables (Log^{10}) as the dependent (response) variable and age, gender, HOMA, BMI and case-control group membership as the independent variables.

Parameter-Log^{10}	Standardized Partial Regression Coefficient (SPRC)			R^2 (Model)
	Being a Case with T2DM Compared to Controls	HOMA	BMI	
IL-12 (pg/ml)	0.193	0.250*	-0.077	0.267*

* Significant ($P \leq 0.05$) correlation

Inflammation is emerging as an important mechanism for micro- and macro vascular complication of diabetes. The macrophage plays a key role in the chronic inflammatory response in part by generating particular cytokines. IL-1ß, IL-6, IL12, IL-18, TNF-α are produced primarily in macrophages and have been associated with accelerated atherosclerosis and thought to be the major cause of vascular disease as well as hyperglycemia[9,10].

Obesity is associated with moderate but chronic increase of many inflammatory markers including IL12 which is an important factor, the observation that many contradict the thought that IL12 is involved with other inflammatory markers in pathogenesis of T2DM as pro inflammatory marker[11].

In present study IL12 in controls showed positive correlation with HOMA suggesting that an inflammatory state may lead to impairment of Insulin signaling and then Insulin resistance which both are involved in the pathogenesis of T2DM.

This study showed a significant increased level in IL-12 in T2DM patients as compared to control and the multiple linear regression models revealed that the HOMA had the most important impact on IL-12. An increase in HOMA index was associated with proportional increase in serum IL-12. Wegner *et al*., [1] demonstrated that elevated serum IL-12 levels in T2DM was related to the high fasting insulin

concentration in serum, high HOMA and c-peptide serum concentration, therefore, this cytokine may play a critical role in the pathogenesis of T2DM, but the significance of IL-12 changes in the blood of patients with T2DM remains unclear. However, it has been observed that IL-12 plasma concentrations were elevated in T2DM [5,12], and the IL-12 contributes to the process of atherosclerotic plaque formation and probably accelerates the development of macro vascular complications in the disease, as well [6,13]. Additionally, it has been noted that elevated glucose levels in diabetic animals stimulates inflammatory reactions related to IL-12 cytokine gene expression [7]. However, it is not known whether factors related to the course of T2DM, 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 T2DM primarily was dependent upon fasting pro-insulin concentration [1]. Their results demonstrated that elevated IL-12 serum levels in T2DM treated with sulphonylureas were induced especially by peripheral insulin resistance and beta cells dysfunction, as expressed by fasting serum pro-insulin levels. This finding gave the hope that treatment to decrease peripheral insulin resistance and to avoid excessive pro-insulin secretion might be successful in the prevention of IL-12-induced atherosclerosis. The study clarifies the non existence of any correlation between obesity and IL-12 in both diabetics and controls suggesting its relation to the disease process per se. However other worker demonstrated higher serum level of IL-12 in obese T2DM [14]. Additionally,it has been noted that increase level of IL-12 in DM is due to complicate interaction of insulin resistance ,hsCRP ,LDL& HDL-c from visceral fat and the adipose tissue are known for production of different pro-inflammatory cytokines [11].It seems that the release of IL-12 and other inflametory cytokines from various cell types which may activate the production of hsCRP from liver consequently these may further enhance the progression of T2DM and cardiovascular disease.

CONCLUSIONS

Considering the BMI and HOMA in the studied groups showed that an increase in only HOMA index was associated with a proportional increase in serum IL-12 and able to explain 26.7% of the observed variation in the outcome variable therefore further considerations have to be defined to include the clinical course of disease, response to medications and nutrition regimen, as well as, the complications of T2DM; for instance, retinopathy, nephropathy, hypertension and atherosclerosis. These estimations can be further explored in metabolic syndrome. Furthermore, it will be

fruitful to interpret these augmentations in the ground of other inflammatory cytokines.

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