IL-6 and Type 2 Diabetes

Maha F.Almelan* VM, MSc

Summary:

Background: Interleukin -6 (IL-6) as the key mediator of the acute phase reaction is of interest .elevated protein concentrations of IL-6 in the blood have been shown in patients with type 2 diabetes. This study aimed to investigate the association of IL-6 and type 2 diabetes.

Materials and Methods: Blood samples were collected from 40 patients with type 2 diabetes and 40 person apparently healthy control were examined for IL-6 level by Enzyme Linked Immune Sorbent Assay. HbA1c determined by high pressure liquid chromatography .total cholesterol, HDL cholesterol and triglyceride were determined enzymatically. Other risk factors study like age, sex and BMI.

Results: results shows that IL-6 was higher in patients than in control ($p<0.006$) and there is significant increase in triglyceride. HbA1c shows high levels in diabetic patients (mean 6.855±1.57%) than the healthy control (mean 4.650±o.673) and when comparing the three diabetic BMI groups with healthy control a significant higher serum IL-6 level was found $p=0.008$ in over weight group .

Conclusion: there is a significant elevation in serum IL-6 in type 2 diabetics when compared to healthy controls and there is a significant elevation of IL-6 in over weight.

Key words: Interleukin -6, Type 2 Diabetes, Hemoglobin A1c, Body Mass Index.

J Fac Med Baghdad
Vol. 51, No.4, 2009
Received: July.2009
Accepted: July.2009

Introduction:

The pathogenesis of type 2 diabetes is characterized by a combination of insulin resistance at the level of skeletal muscle ,fat and failure of pancreatic β-cells to compensate for the enhanced insulin demand .(1,2) recently published cross sectional studies have provided support for the hypothesis that chronic sub clinical inflammation may be associated with insulin resistance and precede the development of clinically over type 2 diabetes (3,4 and 5).other studies have shown strong relationship between inflammatory markers and metabolic disturbances ,obesity and atherosclerosis where as inflammation has been considered as a (common soil)between these clinical entities type 2 diabetes. The accumulation of macrophages in adipose tissue, the common origin of macrophages and adipocytes , the prevalent presence of peripheral mononuclear cells and apoptotic beta cells by themselves seem to be the sources of inflammation present in type 2 diabetes since they generate the mediators of the inflammatory processes namely cytokines.(6) Cross sectional studies have shown that obesity and insulin resistance are associated with higher levels of markers of inflammation and endothelial function recent prospective studies have shown a relationship between various inflammatory markers specially sialic acid ,oroscomucoid C-Reactive protein and interleukin 6 (IL-6) and the risk of developing type 2 diabetes (7). IL-6 as the key mediator of the acute phase reaction is of interest. Elevated protein concentration of IL-6 in the blood have been shown to predict type 2 diabetes (8).low physical activity and hyperalimination are life style associate with an increased risk of type 2 diabetes. To gain insight into the sources of reported variation of the inflammation marker diabetes association we characterized the association with this score across sex, body mass index and smoking status as well as its independence of measures of obesity and fasting glucose (9).

Laboratory measurement: 40 patients (24 males and 16 females) with type 2 diabetes aged between (20- 65) years and their (mean ± SD) age were (44.05±10.41) year attended the national diabetes center, university of AL –Mustansiriya for treatment and research. A matching control group of healthy volunteer subjects (10 males and 30 females) with age range (20-66) years and their (mean±SD) age were (38.48±13.26) year. The patients were asked to fast twelve hours before measurement of their lipid profile .A list of questionnaire was designed to obtain information from both diabetic patients and control group.

Specimen's collection: venous blood samples were taken using plastic disposable syringes .The blood samples were separated by centrifugation at (3000 rpm) for 15 min. The sera were stored frozen at (-20 C°) until assayed(10). Each serum sample was analyzed for total cholesterol, triglyceride, high density lipoprotein-cholesterol, glucose, IL-6.Serum IL-6 was measured using an immunenzymometric assay for the quantitative measurement of human IL-6(11). Anthropometric measurement body mass index (body height and weight kilograms per meters squared) was used as indicator of obesity (12, 13). Total serum cholesterol ,triglyceride and HDL cholesterol were measured by the enzymatic method , serum low density lipoprotein –cholesterol can determined indirectly using Friedwal equation(14).determination of HbA1c depend on the principle of Bio Red VARIANT HbA1c program

**Department of Basic Sciences /College of Dentistry/University of Baghdad*

utilize principles of ion-exchange high performance liquid chromatography for automatic and accurate separation of HbA1c serum glucose was measured using kits (biocan Germany)(15)which based on the PAP enzymatic determination of glucose .
Anthropometric measurement BMI (body height and weight kilograms per meters squared) was used as indicator of obesity. information on life style characteristics were obtained from self administered questionnaires.
Statistical analysis: data presented in simple measures of mean, standard deviation and range (minimum-maximum values) and use SPSS.

Results:
Table 1: shows the base line characteristics of the case and control subject. The mean age for diabetic patients was 44.05 ±10.41 years while in control mean 38.48±13.26 years (p<0.04).16(40%) diabetic were female and the rest were males compared to 30 (75%) female in healthy controls.
BMI is a significant p .0.14 were in diabetic patients and healthy control

Table (1): The characteristics of diabetic patients and healthy controls by age, sex, BMI, smoking status.

	Diabetic patient		healthy controls	
	No	%	No	%
Age group 20-30years	3	7.5	11	27.5
30-40 years	12	30	9	22.5
40-50 years	13	32.5	10	25
50-60 years	8	20	7	17.5
More than 60 years	4	10	3	7.5
Mean ± SD	44.05±10.41		38.48±13.26	
F-test	4.37, 0.04*			
Sex Female	16	40	30	75
Male	24	60	10	25
BMI Group 20-25 (Normal)	6	15	14	35
25-30 Over weight	16	40	12	30
30-35 (Obese)	14	35	11	27.5
More than 35 (Over Obese)	4	10	3	7.5
Mean ± SD	2.144, 0.147 NS			
F-test				
Smoking Yes	22	55	26	65
No	14	35	11	27.5
Quit	4	10	3	7.5

The F-test is significant at the 0.05 level or less

The mean of serum IL-6 of diabetic patients was 44.256±6.27 pg/ml ranging from 33.44 to 57.84 pg/ml which was significantly p, 0.006 that is higher than the healthy controls (60.25±31.75 pg/ml) and ranging from 28.50 to 92.12 pg/ml as it is shows in table 2.

Table (2): Serum IL-6 level for diabetic patients and healthy controls.

IL-6	Diabetic patient	healthy controls
Minimum	33.44	28.50
Maximum	57.84	92.12
Mean	44.256	60.25
Std. Deviation	6.27	31.75
F-test Equality of Means, df, P	8.477, 78, 0.006*	

The F-test is significant at the 0.05 level or less

Lipid profile is another risk factors in diabetic patients is compared to healthy the results in table(3) shows the level of cholesterol was not significant mean 222.93±45.07 mg/dl p.0.019 while triglyceride was showed significantly (p<0.004)higher levels in diabetics (195.50±98.50 mg/dl)compared to healthy control (132.16±76.64 mg/dl).HDL levels was (42.47±12.10mg/dl)in diabetic patients which was nearly to healthy control (42.13±12.36 mg/dl)that is not significant (p<0.907).the levels of LDL was (139±34.738mg/dl)and in healthy control (128.65±31.19 mg/dl)were not significant (p<0.206)while the levels of VLDL in diabetic patients (34.59±13.52 mg/dl)and in healthy control (30.74±22.96 mg/dl)that was not significant (p>0.409).

Table (3): Serum Ch., TG. HDL, LDL, VLDL level of diabetic patients and healthy controls

Cholestrol	Diabetic patient	healthy controls
Minimum	141	107
Maximum	360	339
Mean	222.93	196.63
Std. Deviation	45.073	47.106
F-test Equality of Means, df, P	5.815, 71, 0.019	
Triglyceride		
Minimum	64	56
Maximum	442	400
Mean	195.50	132.16
Std. Deviation	98.970	76.64
F-test Equality of Means, df, P	8.815, 71, 0.004	
HDL		
Minimum	23	23
Maximum	70	66
Mean	42.47	42.13
Std. Deviation	12.10	12.36
F-test Equality of Means, df, P	0.014, 67, 0.907(NS)	
LDL		
Minimum	59	57
Maximum	199	171
Mean	139.15	128.65
Std. Deviation	34.738	31.199
F-test Equality of Means, df, P	1.632, 64, 0.206(NS)	
VLDL		
Minimum	13	12
Maximum	67	107
Mean	34.59	30.74
Std. Deviation	13.527	22.961
F-test Equality of Means, df, P	0.692, 64, 0.409 (NS)	

The F-test is significant at the 0.05 level or less

The HbA1c level showed higher levels in the diabetic patients (mean 6.855±1.57%) than the

healthy control (mean 4.650±0.673%) depended on their HbA1c diabetic patients were subdivided in to four groups according to National Health for Services (NHS) (British 2003).

1-group Ⅰ: HbA1c level less than (7%) which the goal of treatment.

2-group Ⅱ: HbA1c level between (7-7.9%) clinical suggested recommended.

3-group Ⅲ: HbA1c level between (8-8.9%) clinical recommended.

4-groupⅣ: HbA1c level ≥ 9% which is the poor control of the treatment.

When categorizing the HbA1c according to the NHS classification all healthy control were of the HbA1c (<7%) 100% while in the diabetic <7%65.8%,

7-7.9 %(13.2%), 8-8.9 %(10.5%) and in ≥ 9.0 %(10.5%)

As well as when comparing the three diabetic BMI groups with healthy controls a significant higher serum IL-6 level was found p=0.008 in obese which is clearly identified in table (4).

Table (4): Serum IL-6 level for diabetic BMI groups and healthy controls.

IL-6	Diabetic patient	Healthy controls	The F-test is significant at the 0.05 level or less
	mean ± SD	mean ± SD	
BMI Group 20-<25(Normal)	43.68±6.85	60.27±11.72	0.075 NS
25-<30 Over weight	42.64±6.23	70.83±16.66	0.008*
30-35 (Obese)	46.67±6.34	49.66±21.66	0.761NS
> 35 (Over Obese)	42.89±4.83		
The F-test is significant at the 0.05 level or less	0.006*		

The F-test is significant at the 0.05 level or less

Discussion:

In this study we studided the association of IL-6 and risk factors in diabetic patients .As shown in the result of this study, there is strong association of IL-6 and type 2 diabetes p<0.006, as well as other study have strong relationship between inflammatory markers and type 2 diabetes (16).Herder(17) preview the immunological analysis of the KORA Survey S 4 (1999/2001) that show the levels of circulating acute phase proteins like IL-6 are highly correlated and associated not only with over type 2 diabetes but already with impaired glucose tolerance (IGT)pointing out a role of the mediator in the pathogenesis of type 2 diabetes .2 In addition to previous studies this study found the possible role for inflammation in diabetogensis (11) that compatible with hypothesis originated by Pickup and Crook.(18) According to the previous results the explanation of the effect of IL-6 that the accumulation of macrophages in adipose tissue the common origin of macrophage and adipocytes the prevalent presence of peripheral mononuclear cells and apoptotic beta cells by themselves seem to be the source of inflammation present in type 2 diabetes (6).other paper explain IL-6 has in addition to its immunoregulatory actions been proposed to affect glucose homeostasis and metabolism directly and indirectly by action on skeletal muscle cells ,adipocytes ,hepatocytes ,pancreatic β cells and neuroendocrine cells (2).lipid profile in our study is one of the risk factors that compare in diabetic patients to healthy that seen in table (3) that shown triglyceride was significantly(p<0.004) the dyslipidaemia common in type 2 diabetes (hypertriglceridaemia)is also a feature of natural and experimental acute phase reactions .that compatible with pickup that found the dyslipidaemia common in type 2 diabetes. In addition to dyslipidaemia, the other risk factor is obesity. The obesity mediate cytokine production is another important and perhaps central mechanism for systemic elevation of IL-6.Abdominal obesity and the subsequent secretion of pro-inflammatory cytokines and acute phase reactant may contribute to the relationship between chronic inflammation and type 2 diabetes (19)

We use HbA1c assay to diagnosis diabetes because an expert committee recommended it. Due to relationship between long term, glysemic exposure and complications. HbA1c which measure average blood glucose over a period of two to three months may serve as a better marker and diagnostic test for diabetes than fasting plasma glucose or oral glucose tolerance test .also testing for diabetes using HbA1c is more convenient and easier for patient who will no longer be required to perform a fasting or oral glucose tolerance test (20).

Conclusion:

There is a significant elevation in serum IL-6 in type 2 diabetics when compared to healthy controls and there is a significant elevation of IL-6 in over weight group.

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