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Evaluation of Inflammatory State in Diabetic Patients by Measuring of Interleukin-6 and Tumor Necrosis Factor-α in Obese and Non-Obese Type 2 Diabetes Mellitus Patients as Compared with Control Subjects

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Abstract

Background Inflammation was one of the most important events in the biology of obesity; the obese subjects were recognized recently as characterized by low-grade chronic inflammation. It was thought that the mild inflammation associated with obesity, and particularly the production of inflammatory adipocytokines like interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α), was important in the etiology of the diseases associated with obesity. In particular, insulin resistance (IR) and type 2 diabetes mellitus (T2DM).

Objective To investigate whether IL-6 and TNF-α play an important role in the etiology of IR and T2DM.

Methods This study enrolled 70 T2DM patients randomly assigned into two subgroups, 35 non-obese (body mass index (BMI) < 30) diabetic group 1 and 35 obese (BMI ≥ 30) diabetic group 2 with another 50 healthy control volunteers, divided into two subgroups, 25 non-obese (BMI < 30) control group 1 and 25 obese (BMI ≥ 30) control group 2. Levels of IL-6, TNF-α, fasting glucose, fasting insulin, HbA1c, homeostasis model assessment of IR (HOMA-IR), homeostasis model assessment of β-cell function (HOMA-B%) were examined.

Results The serum concentration of IL-6 of obese and non-obese diabetic patients was significantly ($p < 0.05$) lower as compared with obese and non-obese controls in contrast to the serum concentration of TNF-α, which was significantly ($p < 0.05$) higher in non-obese diabetic patients in comparison to non-obese controls. No significant correlation was observed for the levels of IL-6 and TNF-α with BMI of study population

Conclusion The proposed link between serum inflammatory cytokines (IL-6 and TNF-α) and T2DM was more related to insulin sensitivity, insulin secretion and/or glycemic control than to adiposity. Therefore, the inflammatory cytokines may play an important role in the etiology of IR and T2DM.

Keywords IL-6, TNF-α, type 2 diabetes mellitus, obesity, insulin resistance.

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List of abbreviation: BMI = Body mass index, CG1 = Control group 1, CG2 = Control group 2, DG1 = Diabetic group 1, DG2 = Diabetic group 2, HOMA-IR = Homeostasis model assessment of insulin resistance, HOMA-B% = Homeostasis model assessment of pancreatic function, IL-6 = Interleukin-6, IR = Insulin resistance, T2DM = Type 2 diabetes mellitus, TNF-α = Tumor necrosis factor-alpha, WHR = Waist to hip ratio.

Introduction

Type 2 diabetes mellitus (T2DM) is the most common metabolic disorder in man, affecting over 170 million individual over the world and, potentially, over 365 million in the year 2030 [(1)]. The etiology of T2DM involves abnormalities in both insulin action and secretion [(2)]. Although the precise pathological sequence which leads to insulin resistance (IR) was still unknown, sedentary lifestyle and excess nutrition leads to excessive lipid accumulation in adipose and peripheral tissues resulting in obesity.

Recent studies have shown that chronic low-grade inflammation is an important factor in the etiology of T2DM in humans [(3,4)]. Although liver and muscle show obesity-induced mild inflammation without significant changes of immune cells, adipose tissue was the most vulnerable target to mediate significant infiltration of the immune cells and inflammation contributing to systemic inflammatory response and IR in obese humans [(5)].

Several studies have observed the local expression of pro-inflammatory cytokines and activation of inflammatory cells in the liver and skeletal muscle [(5)]. Whether this results from local release of pro-inflammatory cytokines or systemic inflammation emanating from adipose tissue has yet to be established. Therefore, the aim of this study was to investigate the association of pro-inflammatory cytokines including interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) with the pathogenesis of IR and T2DM.

Methods

A case – control study was conducted at Diabetes Centre of Al-Mawana Hospital in Basra from April 2014 till March 2015. The selected patients were informed about the aims of study before their written informed consent was obtained. The study was approved by the medical ethical committee of College of Medicine, Al-Nahrain University.

This study enrolled 70 T2DM patients (already diagnosed) randomly assigned into two subgroups, 35 non-obese (BMI < 30) diabetic group 1 and 35 obese (BMI ≥ 30) diabetic group 2. Patients were selected according to the diabetes diagnostic criteria of WHO (2011); fasting glucose level ≥ 7 mmol/L (≥ 126 mg/dl) and glycosylated hemoglobin (HbA1c) ≥ 6.5%. All patients had no other disease than T2DM. Another 50 subjects were enrolled in this study as healthy control volunteers, divided into two subgroups, 25 non-obese (BMI < 30) control group 1 and 25 obese (BMI ≥ 30) control group 2, control subjects were selected with glycemic control inclusion criteria were fasting glucose < 7.0 mmol/L (< 126 mg/dl) and HbA1c < 6.5%.

Subjects were excluded if they have: (1) macrovascular complications such as angina pectoris, myocardial infarction and peripheral vascular diseases. (2) history of hypertension or systolic blood pressure (SBP) > 140 mmHg and/or diastolic blood pressure (DBP) > 85 mmHg. (3) pregnancy.

Five ml blood samples were collected by venipuncture after overnight fasting (10-12 hr) and were divided into two portions, 2 ml in EDTA tube for measurement of HbA1c and 3 ml in plain tube, centrifuged for separation of serum, which was divided into two portions, one portion for assay of fasting serum glucose and the second portion of serum sample was frozen and stored at (–20 °C) for assay of IL-6, TNF-α and insulin.

Statistical analysis

All data were expressed as mean ± standard deviation. Student's t test was used to analyze sample averages. One-way analysis of variance (ANOVA) was used to evaluate differences of means between groups. Ratio was compared by the chi square test. Correlations between Homeostasis model assessment of insulin resistance (HOMA-IR), other parameters were analyzed by Pearson's correlation. $P < 0.05$ was accepted as statistical difference.

Results

The general characteristic of all study groups including age, age range, men/women, address, smoking, BMI, WHR, and physical activity were listed in table (1). All groups were matched for age, sex and number of smokers.

Table 1. General characteristics of control and diabetic groups

Parameter	Non-obese Control Group1 (CG1) n=25	Obese Control Group2 (CG2) n=25	Non-obese Diabetic Group1 (DG1) n=35	Obese Diabetic Group2 (DG2) n=35	P value
Age (years)	36.4±10.9	38.4±8.5	39.0±8.8	40.2±7.3	0.37
Age Range	23 - 58	28 - 58	26 - 62	29 - 54	
Men/women	13/12	13/12	18/17	17/18	0.9908
Address (center/rural)	13/12	23/2 a	22/13 b	20/15b	0.0055
Smoker/Non-smoker	4/21	9/16	11/24	7/28	0.20541
Body mass index (BMI)	27±2.1	37.4± 3.4 a	27±1.8b	36.7±3.4ac	< 0.001
Waist to hip ratio (WHR)	0.93±0.085	0.98±0.074 a	0.98±0.046 a	1±0.045 a	0.0005
Physical activity (min/wk)	456.4±77.2	290.0±164.8 a	239.7±92.8a	193.9±87.9abc	< 0.0001

All values were expressed as mean±SD
a Significant ($p < 0.05$) as compared with control group 1
b Significant ($p < 0.05$) as compared with control group 2
c Significant ($p < 0.05$) as compared with diabetic group 1

Group comparison using student t-test, as shown in table (2), the serum concentration of IL-6 of obese and non-obese diabetic patients was significantly ($p < 0.05$) lower as compared with obese and non-obese controls in contrast to the serum concentration of TNF-α, which was significantly ($p < 0.05$) higher in non-obese diabetic pt. in comparison to non-obese controls.

The serum insulin concentration of diabetic groups was significantly ($p < 0.05$) lower than control group 2 but no significant difference as compared with control group 1. Also, the serum insulin conc. of control group 2 was significantly ($p < 0.05$) higher than that of control group 1 as shown in table (3). The pancreatic β-cell function (HOMA-B%) of diabetic patients' groups was significantly ($p < 0.05$) lower than that of control subject groups with non-significant difference between diabetic groups (diabetic group 1 and diabetic group 2) while there was a significant ($p < 0.05$) difference between control subject groups as shown in table (3). The insulin sensitivity (HOMA-IR) of diabetic patients was significantly ($p < 0.05$) lower than that of control subjects (control group 1) with non-significant difference between diabetic groups (diabetic group 1 and diabetic group 2), and significant ($p < 0.05$) difference between control groups (control group 1 and control group 2) as shown in table (3).

Table 2. Results of serum pro-inflammatory cytokines (IL-6 and TNF-α) of control and diabetic groups

Parameter	Non-obese Control Group1 (CG1) n=25	Obese Control Group2 (CG2) n=25	Non-obese Diabetic Group1 (DG1) n=35	Obese Diabetic Group2 (DG2) n=35	P value
IL6 (pg/ml)	28.5±16.3	20.1±11.67 a	11.5±4.87ab	6.5±5.1abc	< 0.0001
TNF-α (pg/ml)	152.9±64.0	154.2±84.0	221.9±131.9ab	197.4±117.7	0.03345

All values were expressed as mean±SD
a Significant ($p < 0.05$) as compared with control group 1
b Significant ($p < 0.05$) as compared with control group 2
c Significant ($p < 0.05$) as compared with diabetic group 1

The correlation analysis revealed a significant negative correlation between IL-6 and fasting glucose, HbA1c and HOMA-IR with significant positive correlation between IL-6 and HOMA-B% in study population. Also, there was a significant positive correlation between TNF-α and fasting glucose and HbA1c with significant negative correlation between TNF-α and HOMA-B% in the study population as shown in figure (1), figure (2) and figure (3) respectively.

Table 3. Results of Glycaemic Control parameters and HOMA-indexes of control and diabetic groups

Parameter	Non-obese Control Group1 (CG1) n=25	Obese Control Group2 (CG2) n=25	Non-obese Diabetic Group1 (DG1) n=35	Obese Diabetic Group2 (DG2) n=35	P value
Fasting serum glucose (mg/dl)	99.1±8.4	99.6±9.3	218.7±72.7ab	234.5±90.1ab	< 0.0001
Disease duration (years)	-	-	4.8±3.9	2.8±2.2c	0.011
Family History (positive/negative)	17/8	13/12a	28/7b	27/8b	0.03
Family History Average scores (mean±SD)	0.7±0.48	0.5±0.51	1.6±1.5ab	1.6±1.36ab	< 0.0001
Insulin (µU/ml)	15.3±6.37	40.4±25.81a	14.5±12.28b	20.5±15.14b	< 0.0001
HOMA-IR	3.7±1.5	10.2±7.38 a	8.7±8.24a	12±11.07a	0.0021
HOMA-B%	164.4±94.7	395.1± 226 a	43.3±34.2ab	52.6±39.15ab	< 0.0001
HbA1c%	5.9±0.35	6.0±0.36	9.7±2.03ab	9.0±1.97ab	< 0.0001

All values were expressed as mean±SD
a Significant ($p < 0.05$) as compared with control group 1
b Significant ($p < 0.05$) as compared with control group 2
c Significant ($p < 0.05$) as compared with diabetic group 1

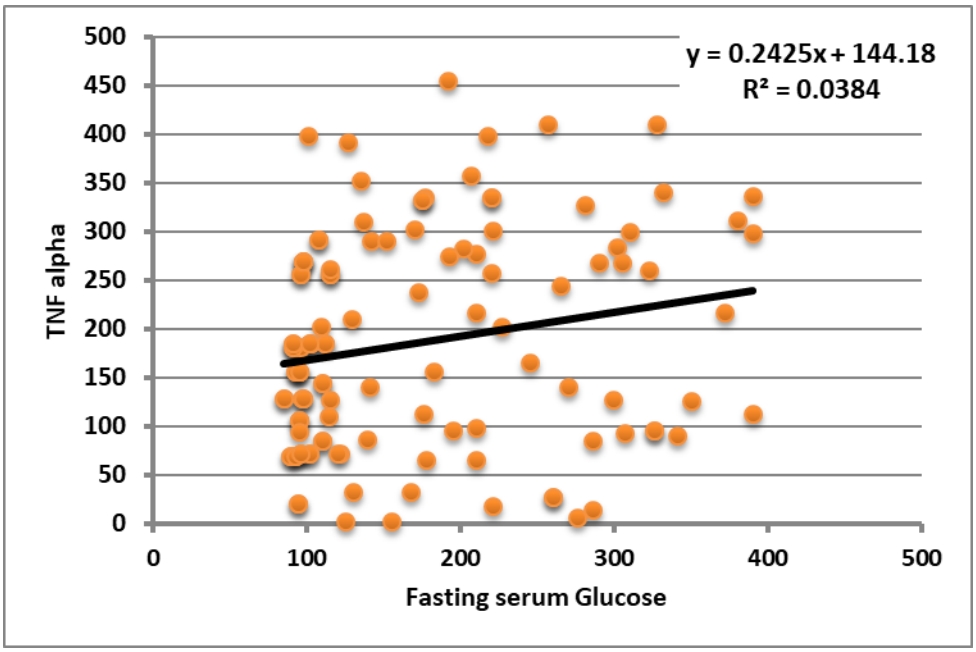

Figure 1. Correlation of TNF-α with fasting glucose of control and diabetic groups

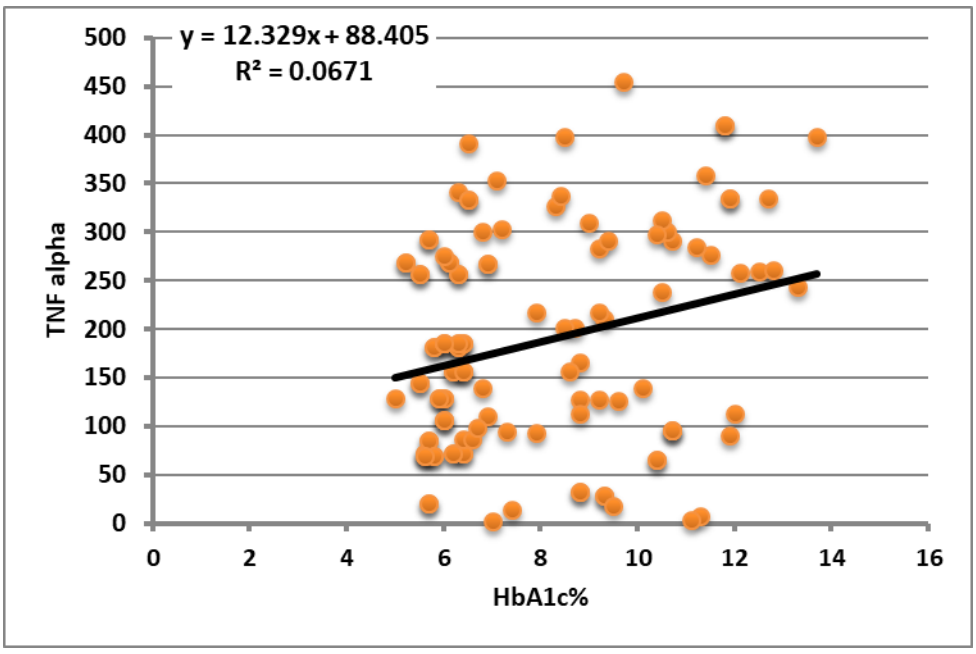

Figure 2. Correlation of TNF-α with HbA1c of control and diabetic groups

Discussion

The data of this study showed decreased levels of interleukin-6 and increased levels of TNF-α in type 2 diabetes mellitus compared to healthy controls. Decreased level of pro-inflammatory cytokine IL-6 was in agreement with Al-Shukaili et al. 2013 [6] who found that the levels of IL-6 were decreased in T2DM and were in disagreement with previous findings by Marques-Vidal et al. 2013 [7], Vidhate et al. 2013 [8], Al-Dahhan and Al-Dahhan 2015 [9],

those found that patients with T2DM had increased levels of IL-6 as compared to healthy control subjects and thus they suggest that lL-6 being a pro-inflammatory mediator might be responsible for some underline changes, which may contribute for the development of T2DM. This disagreement with the current study results of IL-6 could be attributed to the (I) duration of the disease (II) small sample size, and (III) the differences in age and sex of the studied groups Al-Shukaili et al. 2013 [6].

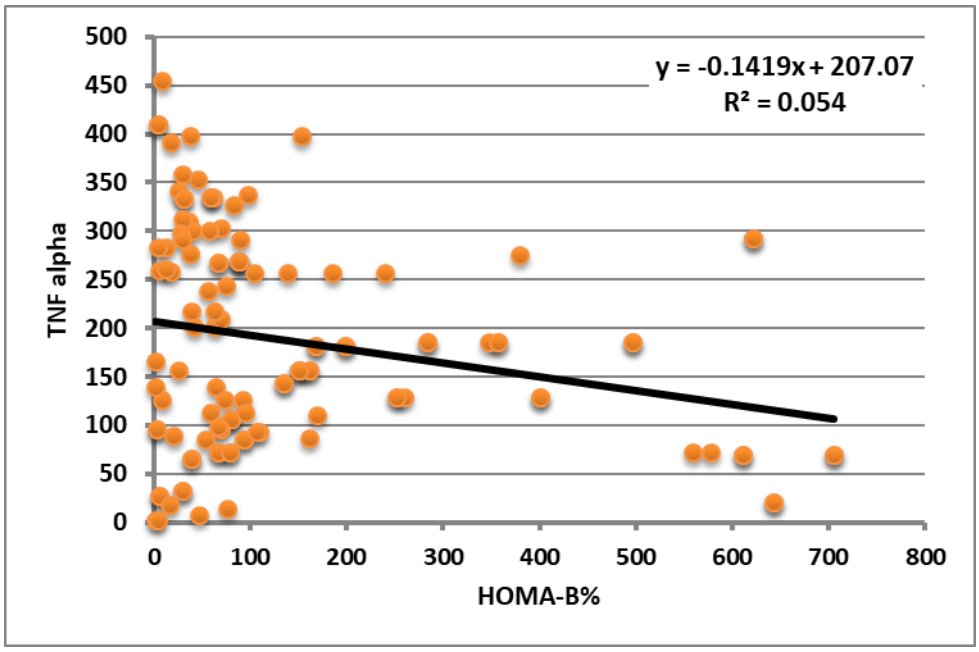

Figure 3. Correlation of TNF-α with HOMA-B% of control and diabetic groups

The role of IL-6 in insulin resistance is controversial with many findings suggesting a causative role in reduced glucose disposal, and others supporting the hypothesis that IL-6 might improve insulin sensitivity but the bulk of the previous findings suggest an insulin resistance inducing action of IL-6 [10].

Correlation and regression analysis of this study showed a significant negative correlation of IL-6 with fasting glucose, glycosylated hemoglobin and HOMA-IR and positive correlation with HOMA-B %, this may indicate a beneficial effect of IL-6 on insulin sensitivity and secretion in contrast to Hansen et al. 2010 [11] who found a significant positive correlation of IL-6 with HOMA-IR, fasting glucose and HbA1c indicating a negative effect on insulin sensitivity and glycemic control parameters and consistent with study done by Suzuki al. 2011 [12] who found that IL-6 acts directly on pancreatic β-cells and enhances glucose-stimulated insulin secretion.

The results of this study showed non-significant correlation between IL-6 and BMI of study population may be due to limited sample size, Darko et al. 2015 (13) observed a negative correlation between IL-6 and BMI of diabetic patients while Vozarova et al. 2001 [14] found positive correlation between them.

The current study revealed a significant elevation of TNF-α in diabetic patients as compared to controls consistent with Goyal al. 2012 [15] and Al-Dahhan and Al-Dahhan 2015 [9]; those observed an increase of TNF-α levels in T2DM patients as compared to controls. In obese individuals, the adipocytes enlarge, adipose tissue undergoes cellular alterations affecting systemic metabolism. First, increase in macrophage numbers in adipose tissue in obesity [16] where they apparently function to scavenge older adipocytes. Second, several

proinflammatory factors are produced in adipose tissue macrophages with obesity. In fact, almost all adipose tissue TNF-α expression originated from adipose tissue macrophages [17]. Concentrations of inflammatory markers such as TNF-α are also raised in diabetic patients. This finding has led to the proposal that elevated concentrations of pro-inflammatory cytokines may trigger much of the metabolic abnormalities due to obesity and diabetes mellitus. Therefore, it could be predicted that inflammation is thought to contribute to the development of insulin resistance, a significant outcome of obesity and is evident by studies describing role of TNF-α in mediating insulin resistance in obese patients [18].

Correlation and regression analysis of this study discovered a significant positive correlation between TNF-α and glycemic control parameters including fasting serum glucose and HbA1c indicating a probable role of TNF-α in impairment of insulin signaling and decrease cellular glucose uptake, which was consistent with Mahmoud et al. 2004 [19] and also there was a significant negative correlation of TNF-α with HOMA-B%, which indicate the inhibitory effect of TNF-α on islet β-cell secretory function consistent with Chen et al. 2007 [20]. The non-significant correlation of TNF-α with BMI and HOMA-IR might be due to limited sample size and was inconsistent with Swaroop et al. 2012 [21] who found a strong correlation between them indicating the association of elevated TNF-α with increasing adiposity and the role of pro-inflammatory cytokines in pathophysiology of insulin resistance. It has been demonstrated that TNF-α could inhibit insulin-responsive glucose uptake through a decrease in glucose transporter 4 gene transcription [22] and decrease tyrosine kinase activity of the insulin receptor [23].

This study concluded that the significant alterations in sera levels of IL-6 and TNF-α of diabetic patients as compared to controls were suggested a possible role of these pro-inflammatory cytokines with the presence of obesity, IR and T2DM and the assay and assessment of sera levels of IL-6 and TNF-α could be beneficial in early detection of T2DM and prevention of its unfavorable consequences especially the cardiovascular complications and atherosclerosis. It was still unclear whether altered inflammatory cytokines were a cause or compensatory mechanism to IR and T2DM.

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Author contribution

Dr. Fareed conceived and designed the study and preliminary analysis. Qasim collected, analyzed and interpreted the data and wrote the manuscript. Dr. Hassan did the statistical analysis of study.

Conflict of interest

None.

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