ARTICLE / INVESTIGACIÓN

The role of TNFα in type2 diabetes mellitus

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Abstract: Type2 Diabetes mellitus is a chronic and most common form of diabetes characterized by hyperglycemia arising from problems with the utilization or production of insulin. Various agents related to an increased risk of developing T2DM include pro-inflammatory cytokines that are critically associated with the progressive resistance of insulin and the pathogenesis of newly diagnosed T2DM. The study aimed to study a possible association of TNFα serum level in diabetes type2 initiation using enzyme-linked immunosorbent assay (ELISA). In this study, 50 newly diagnosed T2DM patients with an age range between 25 and 70 years were included; in addition to fifty healthy volunteers whose sex and age were matched with the T2DM patient's group to act as a control, serum samples were collected to estimate serum levels of TNFα by Enzyme-Linked Immunosorbent Assay (ELISA). The TNFα revealed a statistically significantly higher serum level in the newly diagnosed T2DM patients compared with the healthy control group. On the other hand, there is no statistically significant difference between obese and non-obese T2DM. The higher level of TNFα in T2DM patients compared to the control group may have a role in the development and pathogenesis of T2DM. The current study showed an association between the serum TNFα level and newly diagnosed type 2 DM, and this studied marker might help in the prediction of T2DM

Key words: T2DM, TNFα, ELISA.

Introduction

Type 2 diabetes is related to insulin resistance associated with metabolic stress and inflammation and other factors, such as genetic[1], that can lead to cardiovascular disease and atrophy[2]. Although type 2 diabetes (T2D) is characterized by insulin resistance which means that insulin responding to its target tissues (especially the liver, muscle, and fat) will be the decline, in this case, insulin inoperative with rising its generation to sustain glucose level as a consequence, blood sugar is unable to enter these cells and be used for energy storage. When sugar is unable to enter cells, the blood sugar level rises. This is referred to as hyperglycemia. The body is incapable of using glucose as an energy source. This results in type 2 diabetes symptoms[3-5]. Type 2 diabetes commonly advances misdiagnosed for several years due to slow of hyperglycemia and, through earlier stages, is mostly insufficient for the patient to observe the usual manifestations of diabetes. However, patients who are undiagnosed have rising danger progressing of microvascular and macrovascular problems[6]. Hence, due to the immediately developing frequency and ubiquity of T2DM rises necessary urgency to advance competent or powerful means being used for initial and earlier detection of the disease in order to avoid the development of T2DM and ameliorate clinical outcomes of patient[7]. Tumor necrosis factor-alpha (TNF-α) is one the most important pro-inflammatory mediator. It is yielded chiefly from adipocytes and/or peripheral tissues and activates inflammation of particular tissue via the production of reactive oxygen species and induction of diverse intermediate transcriptional pathways[8]. When TNF-α consistent elevated it will activate resistance of insulin in both peripheral tissues and adipocytes via hindering the insulin signaling through serine phosphorylation which promote arising of T2DM[9]. It is a cytokine that is secreted by chronically inflamed cells. The immune system produces it. Type 2 diabetes mellitus is believed to be connected with low-grade chronic pancreatic inflammation[10].

Materials and methods

This case-control study was conducted on 100 Iraqi adults who were divided into two groups

Group (1): includes 50 newly diagnosed patients with type2 diabetes mellitus as a case group.

Group (2): includes 50 healthy subjects as a control group.

Diabetic pregnancy, patients on insulin or any other drugs that would affect glucose homeostasis, smokers' and alcoholism patients were excluded from the study. This study was established in AL-Emamain AL-Kadhemain Medical City and Diabetes Disease center in Baghdad during the period between October 2020 till September 2021.

Blood sample collection

Five ml of venous blood from each subject were distributed into two tubes. The first 3ml was dispensed into a gel tube containing a clot activator, left at room temperature

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to clot for about (10) minutes, and then centrifuged for (10 min at 4000 rpm). In this study, the serum was isolated and utilized to assess glucose, cholesterol, and triglyceride. The residual serum was frozen at -20 °C till the quantified of TNFα in serum using a sandwich ELISA kit. The other 2ml was transmitted to the ethylenediaminetetraacetic Acid tube (EDTA) for routine work glycated Hemoglobin assessment.

HbA1C, cholesterol, TG, CRP

The healthy subjects and patients were investigated for the following parameters: hemoglobin A1c, fasting blood sugar FBS, TG, cholesterol, CRP was measured by Cobas c111 system. A ready commercial Kit Mybio-source Catalog No: MBS455676 / USA was used for the in vitro quantitative measurement of TNFa in human serum.

Statistical analysis

To do statistical analysis, SPSS 20.0 and Microsoft Excel 2013 were used. The Shapiro-Wilk test was used to examine the normality of numerical data. The Mann–Whitney U test was employed to compare the median and 25-75 confidence intervals of the data for comparing two groups. Percentage of the total number of categories in the data set. The use of the chi-square test estimated the connection between variables. Below or equal to 0.05 is the minimum statistical significance level that may be acceptable.

Results

The mean age of patients was 46.00± 10.42 years, and there was female predominance among patients; there were 52.0% females, while 48.0 % were males. There was no statistically significant difference between patients and the control group (p> 0.05) in age or sex present. According to the (BMI) The results in this study demonstrated a statistically significant difference in (BMI) between the studied groups (p ≤ 0.001).

Fasting Blood sugar and HbA1c

The results in table 3 illustrated a highly significant difference in median FBS and HbA1c among study groups (P ≤ 0.001).

		Study groups	
		T2DM	Control
Age/years	Mean	46.00	45.64
	SD	10.42	9.73
P value	0.909 NS		

NS= nonstatistical significant difference (p>0.05)

Table 1. The age distribution of study groups.

Sex	Study groups	
	T2DM	Control
Female	26	26
%	52.0%	52.0%
Male	24	24
%	48.0%	48.0%
Total	50	50
P value	0.841 NS	

NS= nonstatistical significant difference (p>0.05)

Table 2. Sex type distribution in T2DM patients and control.

Regarding TG and cholesterol, LDL, AI with median value (177.50 Mg/dl), (200.00 Mg/dl), (137.00 Mg/dl) (0.64) respectively, was higher in T2DM than in control group (102.94 mg/dl), (164.69 Mg/dl), (94.99 Mg/dl), (0.30). with p-value =< 0.001, While the HDL results were shown that the median of patients' T2DM was lower than the control group (38.84, 51.18 Mg/dl) respectively, with p-value = < 0.001.(table 4). On the other hand, concerning CRP, the median value for T2DM was (4.47 Mg/L) higher than the median of the control group (1.36 Mg/L). So, there was a significant difference between the T2DM patients and control groups shown in table 5. Also, The current result revealed a higher concentration in the serum of patients TNFα compared with controls, as shown in Table 6.

The results of the current study concerning TNFα concentration between obese and non-obese patients showed a non-significant difference, as revealed in Table (7). Recent study results revealed a non-significant association between TNF alpha and HbA1C in patients with T2DM, as shown in Table 8.

Level of HbA1c, CRP, TNFα in obese and non-obese T2DM

According to the results demonstrated in Table (9), there was no statistically significant difference between obese and non-obese regarding HbA1c, and TNFα in patients with T2DM, except CRP. There was a statistically significant difference between obese and non-obese T2DM with a p-value (0.020). Also, this study revealed no statistically significant correlation between TNFα and group of demographic and laboratory findings (Age, BMI, FBS, HbA1c, Lipid profile, AIP and CRP) as in Table 10.

Discussion

This study revealed a significant difference in TG levels between T2DM patients and control groups, similar to research 11that established a link between high TG and type 2 diabetes. There is a significant difference in cholesterol levels between the patients and the control group. This is consistent with the findings[12], which showed that diabetes individuals had considerably greater CHO levels than non-diabetic individuals. The present results on TG and Cholesterol levels disprove those of other studies[13], which found no significant difference between T2DM and healthy controls. The rise in cholesterol levels appears to be due to increased cholesterol synthesis due to a lack of control or poor management of hyperglycemia, which returns

		Study groups		P-value
		T2DM	Control	
FBS (Mg/dl)	Median	182.50	86.56	<0.001**
	Percentile 25	131.00	78.22	
	Percentile 75	265.00	94.34	
HbA1C%	Median	9.05	4.90	<0.001**
	Percentile 25	7.00	4.69	
	Percentile 75	11.80	5.20	

**= highly statistical significant difference (p<0.001)

Table 3. Serum laboratory findings distribution in both T2DM patients and control.

		Study group		P-value
		T2DM	Control	
TG (Mg/dl)	Median	177.50	102.94	<0.001**
	Percentile 25	129.50	75.40	
	Percentile 75	255.00	118.09	
Cholesterol (Mg/dl)	Median	200.00	164.69	<0.001**
	Percentile 25	182.00	147.19	
	Percentile 75	231.00	180.00	
HDL (Mg/dl)	Median	38.84	51.18	<0.001**
	Percentile 25	34.00	46.64	
	Percentile 75	44.00	58.23	
LDL (Mg/dl)	Median	137.00	94.99	<0.001**
	Percentile 25	115.20	78.49	
	Percentile 75	167.00	110.24	
AI	Median	0.64	0.30	<0.001**
	Percentile 25	0.53	0.17	
	Percentile 75	0.79	0.36	

**= highly statistical significant difference (p<0.001)

Table 4. Lipid profile and Atherogenic index distribution in T2DM and control groups.

		Study groups	
		T2DM	Control
CRP (Mg/L)	Median	4.47	1.36
	Percentile 25	2.60	.74
	Percentile 75	9.60	2.98
P value	<0.001**		

**= highly statistical significant difference (p<0.001)

Table 5. CRP parameter distribution in T2DM and control group

		Study groups	
		T2DM	Control
TNF-alpha (pg/ml)	Median	90.57	26.53
	Percentile 25	65.39	18.81
	Percentile 75	129.52	41.75
P value	<0.001**		

**= highly statistical significant difference (p<0.001)

Table 6. The concentration of TNFα in T2DM and control group.

		BMI	
		non-obese	obese
TNF-alpha (pg/ml)	Median	90.71	90.44
	Percentile 25	66.64	65.39
	Percentile 75	120.54	129.52
P value	0.976 NS		

NS=none statistical significance (p>0.05)

Table 7. The concentration of TNFα between obese and non-obese patients.

		HbA1C in T2DM	HbA1C in control
TNF-alpha (pg/ml)	Median	103.63	83.24
	Percentile 25	90.17	61.84
	Percentile 75	140.07	129.52
P value	0.426NS		

No statistical significance (p>0.05)

Table 8. Association between TNF-alpha and HbA1C in T2DM.

		T2DM	
		non-obese	obese
HbA1c	Median	11.00	8.80
	Percentile 25	7.70	6.90
	Percentile 75	11.80	10.50
P value	0.162		
CRP	Median	2.80	6.45
	Percentile 25	2.40	3.20
	Percentile 75	4.90	14.80
P-value	*0.020		
TNFα	Median	90.71	90.44
	Percentile 25	66.64	65.39
	Percentile 75	120.54	129.52
P value	0.976 NS		

Table 9. Level of HbA1c, CRP, TNFα in obese and non-obese T2DM.

	TNF-alpha (pg/ml)	
	r	p
Age (years)	0.045	0.754
BMI	-0.066	0.650
FBS	-0.136	0.347
HbA1c	-0.219	0.126
TG	-0.007	0.960
Cholesterol	-0.240	0.093
HDL	-0.024	0.868
LDL	-0.061	0.675
AIP	-0.042	0.774
CRP	0.255	0.074

Table 10. Correlation of TNFα with demographic and laboratory findings among the studied groups.

to standard or near-normal following adequate and suitable diabetic control[14,15]. In concerning to LDL and HDL current study revealed a significant difference in LDL levels of T2DM compared to healthy control subjects. Even though newly diagnosed diabetic patients show a significant decrease in high-density lipoprotein (HDL) compared to the healthy control group, this is consistent with a study[16] that found a significant difference ($p<0.001$) in the newly diagnosed diabetic group as compared to healthy control group regarding LDL and a negative correlation of T2DM with the control group in HDL Present study revealed a significant difference between the T2DM in compared to control groups regarding AI and this agrees with (17) studies which shown also AI in T2DM group was significantly higher than in the healthy control group. Study[16] reported a significant increase in levels AI in T2DM compared to the control group. AI is a good predictor and indicator for follow-up monitoring in managing patients with high-risk type 2 diabetes because it is a clinically convenient indicator for detecting T2DM with a high risk of complications and related disorders[18]. Sugar, cholesterol, and triacylglycerol levels were more elevated in patients with type 2 diabetes than those with normal blood sugar levels and lower serum levels of high-density lipoprotein cholesterol compared to those without type 2 diabetes. Diabetic people are more prone than non-diabetic patients to develop dyslipidemia, a key risk factor for atherosclerosis and coronary heart disease[19]. This study found that the CRP concentration was substantially more significant in the newly diagnosed T2DM group than in the healthy control group. This finding is consistent with research 20, which found that the serum CRP concentration was significantly higher in the IGT and type 2 DM groups (p 0.001). Some researchers believe that type 2 diabetes is a symptom of a persistent acute-phase response defined by changes in the levels of so-called acute-phase proteins, such as the inflammatory protein C-reactive protein (CRP) 21,22 Furthermore, according to the findings of the current investigation, the blood TNF- level was considerably greater in newly diagnosed T2DM patients. Compared to obese non-diabetic patients and non-obese diabetic patients, there was no significant difference in TNF- α levels between obese diabetic patients and obese non-diabetic healthy subjects. Study (10) did not confirm this association.

In this current study, the absence of correlation concerning TNF α concentration in obese and non-obese diabetic patients could be related to the duration of the disease. All patients present in the study were selected orderly as newly diagnosed T2DM. Most of the diabetic patients in this study didn't have a BMI greater than 30kg. Could be linked to the small sample size of obese patients in the current study. Furthermore, neither the waist-to-hip ratio nor the quantity of visceral fat was measured in this research. The inability to find any link between BMI and serum TNF- α may also be explained by several sources of circulating TNF- α and the failure to account for the independent impacts of obesity and diabetes. Multiple studies have shown that blood TNF levels were linked with abdominal obesity[23] or visceral fat[24], but not with subcutaneous fat or BMI. These findings are consistent with previous findings.

Additionally, TNF- α is produced and released by muscle[25] and lymphocytes and macrophages[26]. It has been proven *in vitro*[25] People with insulin resistance, and type 2 diabetes have higher levels of TNF- α in their muscles. Also, the generation of TNF- α by monocytes and macrophages is boosted by hyperglycemia[27] and hyperinsulinemia[28]. TNF- α levels seem to rise with aging in certain studies[29]. However, It was shown that even after adjusting for age, the diabetic group's TNF-a concentration increased. Meta-analysis of 19 studies shows that elevated TNF- levels are strongly associated with an increased risk of developing Type 2 Diabetes Mellitus (T2DM)[30]. TNF- α is a crucial modulator of inflammatory responses.

The role of cytokines in promoting the production of acute-phase proteins is well-known. Increases in inflammatory biomarkers are a result of both obesity and hyperglycemia. After neutralizing TNF- α, obese rats showed increased glucose absorption in the peripheral bloodstream, indicating that TNF- α plays a critical role in developing insulin resistance and diabetes[31]. TNF- α has previously been linked to insulin resistance and type 2 diabetes (T2DM) associated with obesity[32,33]; however, another investigation did not support this link[34]. The current study demonstrated no significant association between TNFα and HbA1C in T2DM. The duration of the diseases could cause this, the limited sample size or some of those patients had insulin sensitivity. High levels of inflammatory cytokines that emerged in the early stages of T2DM could predict the development of type 2 diabetes by lowering insulin sensitivity[35]. Current findings contribute to a more significant number of studies indicating that certain inflammatory factors play a vital role in the pathophysiology of T2DM. They also provide insights on the potential clinical utility of certain inflammatory factors as biomarkers for early T2DM detection and treatments[36]. According to another study, people with diabetes were shown to have considerably raised levels of TNF-α, which was most pronounced in those with HbA1c values of more than 6.5 %[37]. In this study, HbA1c levels were not linked to TNF- α in this group of T2DM patients, whose mean HbA1c was 9.49%. Because the glycation rate and the turnover of erythrocytes aren't directly related to blood glucose levels, the amount of HbA1c might fluctuate for reasons unrelated to blood glucose levels[37]. The negative relationship between TNFα levels and insulin sensitivity in persons with Normal Glucose Tolerance (NGT) and Impaired Glucose Tolerance (IGT) or prediabetics, regardless of age, sex or obesity, supports circulating TNFα playing a role in insulin action control[15]. Activated macrophages and lymphocytes were observed to produce and release tumor necrosis factor[26] because type 2 diabetes is thought to result from a constant state of inflammation[38]. A soluble TNF receptor-IgG fusion protein has been shown to promote insulin-stimulated autophosphorylation of insulin receptor and insulin receptor substrate-1 in muscle and fat, supporting this hypothesis[39]. Similarly, Obesity-induced suppression of TNF - α activity by an adenovirus vector with impaired replication By increasing insulin receptor signaling, the Zucker rat enhances hepatic and muscular sensitivity [23,40]. Neutralizing circulating TNF- α levels did not improve glycemic control or insulin sensitivity in obese, insulin-resistant diabetic and non-diabetic adults.

Conclusions

TNF- produced by fat cells and macrophages/lymphocytes may have interfered with the paracrine effects of TNF-neutralizing antibodies on the local level.

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Informed Consent Statement

Al-Nahrain University's College of Medicine's ethics committee authorized this research. All samples were taken with informed permission to comply with the Al-Imamain Al-Kadimain Medical City's policies on informed consent and informed consent forms.

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Author conflict

No conflict.

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