Association of TNF-α 308 polymorphism with diabetes mellitus type 2

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Abstract:

Back ground: The association between tumors necrosis factor-alpha (TNF-α)308 polymorphism and type 2 diabetes mellitus (T2DM) remains controversial .The variation in ethnicity and life style play important role in these conflicting results.

Objective: To investigate association of TNF-α 308 polymorphism with T2DM,TNF level and body mass index in these patients.

Patients and methods: The current case control study included fifty patients with T2DM in addition to twenty five healthy controls. The fasting blood sugar (FBS)and fasting blood (cholesterol, triglyceride) were done by colorimetric methods .The body mass index (BMI) was calculated for each patients and healthy controls. The level TNF-α in serum was measured by ELISA method(Ray biotechnology/ USA, 46078). The TNF-α 308 polymorphism was done by restriction enzyme digestion after polymerase chain reaction (PCR).

Result: The age range for T2DM patients was (43.54±4.590) year while for control was (45.04±4.394) year. The T2DM patients whom carry AA alleles for TNF-α 308 polymorphism showed highly significant association with study parameter F.B.S, BMI, cholesterol, triglyceride and TNF-α level with (P<0.01).The T2DM patients with normal allele GG genotyping and GA genotyping of TNF-α 308 polymorphism also showed highly significant association with study parameter F.B.S,BMI,Cholesterol,Triglyecride and TNF- α with (P<0.01). The TNF- α level in serum of T2DM patients showed highly significant association with F.B.S, cholesterol and triglyceride with (P P<0.01), however the TNF- α level was nonsignificant with BMI in T2DM patients.

Conclusion: In the present study TNF-α 308 polymorphism allele (AA, GA) showed a statistically significant association with TNF-α level in serum of T2DM patients and BMI. The AA and GA alleles showed a statistically significant association with high fasting glucose level. The TNF- α level didn't show a statistically significant association with BMI.

Keywords: Type 2 diabetes mellitus, TNF-α 308 polymorphism, TNF-α level, BMI

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Introduction:

Type 2 diabetes mellitus is one of major health problem. This form of diabetes, which accounts for ~90–95% of those with diabetes, previously referred to as non–insulin-dependent diabetes, type 2 diabetes, or adult-onset diabetes, encompasses individuals who have insulin resistance and usually have relative (rather than absolute) insulin deficiency. At least initially, and often throughout their lifetime, these individuals do not need insulin treatment to survive. There are probably many different causes of this form of diabetes (1). T2DM is characterized by peripheral insulin resistance in the liver, skeletal muscle, and adipose tissues, as well as impaired insulin secretion from the pancreatic beta cells. Evidence has been presented that Type 2 diabetes mellitus (T2DM) drives from the coexistence of genetic and environmental factors .However, the molecular mechanisms underlying T2DM are poorly understood (2). Obesity is considered as a major cause of insulin resistance which is implicated in metabolic dysregualtion including hypertension and hyperlipidemia. Regarding lipid metabolism there are many articles on ssociation of TNF-α polymorphism with serum lipid especially level of cholesterol, the most important risk factor for cardiovascular diseases (3).

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Direct exposure of muscle cells to these fatty acids damage insulin mediated glucose up take, this may cause insulin resistance (4).Tumor necrosis factor alpha (TNF-α) is an adipose cytokine involved in systemic inflammation and stimulates the acute phase reaction . The TNF-α is firstly produced by macrophages as well as by many other cells as adipocytes (5). The TNF-α inhibits insulin transduction and disturbs glucose metabolism (6). Alteration in the TNF-α metabolism has a role in metabolic syndrome such as overweight and insulin resistance; this explains why perturbations of TNF-α metabolism may affect the occurrence of T2DM and development of the diseases (7). The TNF-α suppresses the expression of many proteins that are required for insulin-stimulated glucose uptake in adipocytes, such as the insulin receptor (IR), insulin receptor substrate-1 (IRS-1) and GLUT4 (8).

Material and methods:

This case control study included fifty T2DM patients with twenty five persons as healthy controls. The T2DM patients attending center of endocrinology and diabetes /Al-Russafa Directorate for period from October 2016 to February 2017. The consent form was taken from each unrelated T2DM patients and unrelated healthy controls. The body mass index was done for each T2DM patient and healthy control by measuring body weight in kilogram divided by height in squared meters(9).

Blood samples

In this study 5 ml of blood were taken from each patient and healthy control by venipuncture after fasting for 12 hours ,Then 3 ml of blood were separated by centrifuge and serum samples were used for detection (F.B.S,cholesterol,triglceride).The remaining serum samples were isolated and stored at (-20Ċ)until used while the other 2 ml of blood was kept in EDTA tubes and stored at(-20Ċ) until used.

Fasting blood sugar test: The fasting blood sugar test was done by colorimetric methods according to instructions manual by Human company / Germany.

Serum cholesterol test: The fasting Cholesterol level was done by colorimetric methods according to instructions manual by Human company/Germany.

Serum Triglyceride test: The fasting triglceride level was done by colorimetric methods according to instructions manual by Human company/Germany.

Tumors necrosis factor alpha: The TNF-α level where measured by ELISA technique according to instructions manual by Ray Biotechnology company / USA. Tumors necrosis factor alpha 308 Genotyping: DNA extraction from blood samples

Reliaprep blood gDNA(miniprep system) provided by promeqa/USA and extraction of DNA was done according to manufacture instructions manual. PCR for TNF-α -308 G>A. Tumor necrosis factor alpha was done as proposed by Dalziel et al. Amplification primer for 308 tumor necrosis factor polymorphism.

Forward primer: AGGCAATAGGTTTTGAGGGCCAT

Reverse ward primer: ACACTCCCCATCCTCCCTG CT

Restriction fragment length polymorphism was done on gel by addition NCOI enzymes to PCR product which give three alleles GG alleles represent wild type while AA and GA represent mutated type(10).

Statistical analysis:

The statistical analysis of this case control study was performed with the statistical package for social sciences (SPSS)21.0 and Microsoft excel 2013.Categorial data formulated as count percentage .T- test was used at 5%,1% level of significance to describe the association of these data (11).

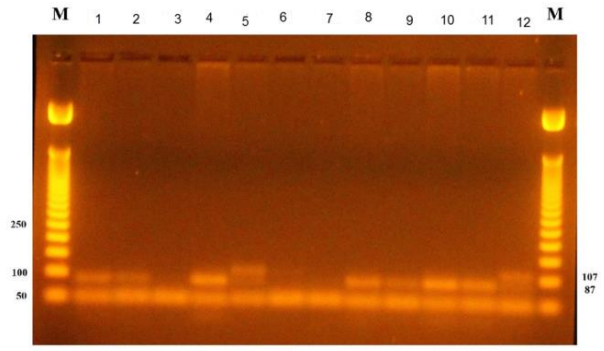
Result:

Table (1) compared between healthy controls and study group (T2DM patients) .it shows highly significant association between them regarding the fasting blood sugar, BMI, cholesterol, triglyceride ,TNF-α level , while age shows a significant association between healthy control and T2DM patients (P<0.05). Table (2) shows the association between TNF-α 308 polymorphism (AA allele) for T2DM patients with study parameters (age, F.B.S, BMI, cholesterol, Triglyceride and TNF- α level), there is sixteen T2DM patients carrying AA alleles, they showed highly significant association (p<0.01) with mean for all parameters more than healthy controls.

Table (1): Comparison between healthy controls and type 2 diabetes mellitus patients with study parameters by T-test.

		Mean± Std.	t-Test	P-Value	C.S
Age/Control	*Vs*	45.04±4.394	2.064	.044	P<0.05
Age/Study		43.54±4.590			(S)
*SUG.(mg/dl)/Control		90.40±9.138	-	.000	P< 0.01
Vs			19.166		(HS)
SUG.(mg/dl)/Study		09.20±43.347			
*BMI(kg/m^2)/Control	*Vs*	18.12±2.237	-	.000	P< 0.01
BMI(kg/m^2)/ Study		25.30±4.195	10.203		(HS)
* Chol.(mg/dl)/Control			-8.272	.000	P< 0.01
Vs		62.00±13.248			(HS)
Chol.(mg/dl)/Study		51.70±76.296			
* Trig.(mg/dl)/Control	*Vs*		-7.208	.000	P<0.01
Trig.(mg/dl)/Study		106.80±9.885			(HS)
		06.40±96.347			
TNF-α		4.40±2.539	- 4.448	.000	P<0.01
level(pg/ml)/Control *Vs*					(HS)
TNF-α level(pg/ml)//Study		40.54±57.026			

*SUG=sugar,BMI=bodymassindex,Chol=Cholesterol,Trig.=Triglyceride,TNF-α=tumornecroticfactorealpha,S=siginificant,HS= highly significant.



Figure(1-2) Gel electrophoresis for PCR product of TNF-α after digestion with restriction enzyme NCOI(AA allele is 107bp ,87 and 107bp is GA allele ,87 bp is GG allele),M:1000 bp marker,(90 mint.,100 volt).

Table (2): Comparison between genotyping (AA) of TNF-α 308 polymorphism for type 2 diabetes mellitus patients and study parameters by T-test.

items	N	Mean± Std.	T-test	P-Value	C.S.
Age	16	45.563±5.1377	35.473	.000	P< 0.01 (HS)
*SUG.(mg/dl)	16	205.000±62.902	13.036	.000	P< 0.01 (HS)
*BMI(kg/m^2)	16	28.750±3.416	33.669	.000	P< 0.01 (HS)
Chol.(mg/dl)	16	310.938±90.189	13.790	.000	P< 0.01 (HS)
Trig.(mg/dl)	16	48.125±118.643	8.365	.000	P< 0.01 (HS)
TNF-α level(pg/ml)	16	89.063±76.983	4.628	.000	P< 0.01 (HS)

*SUG= sugar,BMI= body mass index, Chol.=cholesterol,Trig.= triglyceride,TNF-α= Tumor necrosis factor alpha.

Table (3) shows the association of heterozygote genotyping (GA) of TNF-α 308 polymorphism with all parameters of study (age, F.B.S, BMI, cholesterol, triglyceride, TNF-α level), there is highly significant association (P<0.01) with mean for all parameters in patients more than healthy controls.

Table (3): Comparison between genotyping (GA) of TNF-α 308 polymorphism for the T2DM patients and study parameters by T-test.

items	N	Mean± Std.	T-test	P-Value	C.S.
Age	8	43.000±3.891	31.254	.000	P< 0.01 (HS)
SUG.(mg/dl)	8	203.750±29.970	19.229	.000	P< 0.01 (HS)
BMI(kg/m^2)	8	27.250±3.059	25.196	.000	P< 0.01 (HS)
Chol.(mg/dl)	8	230.625±58.459	11.158	.000	P< 0.01 (HS)
Trig.(mg/dl)	8	228.750±81.843	7.905	.000	P< 0.01 (HS)
TNFαlevel (pg/ml)	8	48.375±26.699	5.125	.001	P< 0.01 (HS)

Table (4): Comparison between genotyping (GG) of the TNF-α 308 polymorphism for the T2DM patients and items by T-test.

items	N	Mean± Std.	t-test	P-Value	C.S.
Age	26	42.462±4.159	52.057	.000	P< 0.01 (HS)
SUG.(mg/dl)	26	213.462±31.899	34.122	.000	P< 0.01 (HS)
BMI (kg/m^2)	26	22.577±2.887	39.878	.000	P< 0.01 (HS)
Chol.(mg/dl)	26	221.731±47.433	23.836	.000	P< 0.01 (HS)
Trig.(mg/dl)	26	173.846±74.140	11.957	.000	P< 0.01 (HS)
TNF-α level (pg/ml)	26	8.269±2.987	14.114	.000	P< 0.01 (HS)

Table (4) shows the relation between wild allele GG of TNF-α 308 polymorphism for T2DM patients with study parameters (age, F.B.S,BMI, Cholesterol, Triglyceride, TNF-α level), there is a highly significant association (P<0.01) but less than T2DM patients with AA and GA allele.

Table (5): Comparison between the TNF-α level of T2DM patients and **items** by **t-Test**

items	Mean± Std.	T-test	P-Value	C.S.
TNF-α level(pg/ml)/Study ***Vs*** SUG.(mg/dl) /Study	40.54±57.026 209.20±43.347	- 15.792	.000	P< 0.01 (HS)
TNF-αlevel(pg/ml) /Study ***Vs*** Chol. (mg/dl)/Study	40.54±57.026 251.70±76.296	- 19.200	.000	P< 0.01 (HS)
TNF-αlevel(pg/ml) /Study ***Vs*** Trig.(mg/dl)/Study	40.54±57.026 206.40±96.347	- 11.353	.000	P< 0.01 (HS)
TNF-αlevel(pg/ml)/ Study ***Vs*** BMI(kg/m^2) /Study	40.54±57.026 25.30±4.195	1.953	.056	P> 0.05 (NS)

Table (5) shows the association between TNF-α level in patients with T2DM with study parameters which was highly significant association but BMI did not show a significant association with TNF- α level.

Discussion:

The association between the T2DM patients and TNF-α 308 polymorphism which effect on level of TNF-α in serum of patients. The genetic change in promoter region lead to increase expression of TNF-α gene as well as transcription and translation this will lead to increase TNF-α level.Higher level of TNF-α in serum of T2DM patients will effect on insulin action in addition to disorder in lipid metabolism (12). This study found a highly significant association between TNF-α 308 polymorphism with AA and GA alleles with serum level of TNF-α in T2DM patients as when compared with healthy controls, this result agree with Groop LC, et al. which proposed that increase expression of TNF-α in adipocyte may influence on insulin sensitivity , thus insulin resistance is one of the major critical factors in the pathogenesis of T2DM(13),However this finding was not consistent with Rfeng et al., they proposed lack of association between TNF-α 308 polymorphism and T2DM (14) . In our study ,Patients with T2DM carrying GA and AA alleles of TNF-α 308 polymorphism have significantly higher fasting blood sugar levels as when compared with T2DM patients carrying GG genotype and this result agree with H.Li et al ,who found that individual with AA allele of TNF-α 308 polymorphism (hyperproducer) have 4-6 times greater risk of presenting with T2DM than individual with GG alleles (hypoproducer) with low risk to presenting with T2DM(15),while this result was not correlated with Altshuler D *et al.* who found lack of association between TNF-α polymorphism and T2DM (16).This variation between these two studies may be due to difference in size of data and genetic background in addition to ethnicity variation. The TNF-α, is proinflammatory cytokine that exert numerous effects on adipose tissue including lipid metabolism and insulin signaling whose serum level are increased with obesity and decreased with weight loss(17) .In the current study, we found an association between polymorphism 308 in promoter region of TNF-α(type of allele) and level of each cholesterol and triglyceride in addition to BMI and this result agrees with Sookoian et al. who performed metanalysis of 315 studies involving about 3500 subjects on the association between 308 G/A polymorphism of the TNF-α gene and the component of metabolic syndrome. They indicated that subjects carrying AA alleles were at 23% higher risk of developing obesity ,had higher systolic blood pressure and insulin levels as when compared with subjects not carrying AA alleles (18), alternatively this finding was not in consistent with N.Ranjith,et al , who explained that polymorphism of TNF-α 308 was not associated with any components of metabolic syndrome in young Asian Indians woman with myocardial infraction in South Africa(19) ,this variation between two studies may be attributed to size of samples, genetic back ground and life style.

Conclusions:

The present study found a highly significant association between AA and GA alleles of TNF-α 308 polymorphism alleles with high level TNF- α level. The AA and GA alleles showed a highly significant association with BMI in addition to high level of cholesterol and triglyceride in serum of T2DM patients.

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