Investigating the Role of TAFI Gene G>A rs3742264 Polymorphism in patients with Type 2 Diabetes Mellitus.

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Abstract

Background: Type 2 diabetes is one of the major public health problems worldwide. It ranks third in the world in the mortality rate from complications of type 2 diabetes. Thrombin-activated fibrinolysis inhibitor TAFI is enzyme play major role in fibrinolysis resistant. **Aim** The current study aimed to investigate correlation between TAFI and develop T2DM complication and e study association of TAFI G>A rs3742264 SNP ,study assumed that genetic variants that occur in the TAFI gene are associated with vascular damage patients. **Methods** case control study was performed 200 volunteers participated in this study, divided into two groups, 100 patients with type 2 diabetes and 100 healthy people, Genetic change and its relationship to biochemical variables were determined in this study. SNPs were genotyped by Tetra-Primer Amplification Refractory Mutation System-Polymerase Chain- PCR(TARMs-PCR) of TAFI G>A (rs3742264), **Results** Age showed a statistical significance when comparing healthy subjects and patients, as well as for BMI ($P<0.00$), TAFI show weak significant difference ($P<0.033$) , person correlation (cholesterol, triglycerides, -C, LDL-C, and VLDL-c) positive with TAFI, where as with HDL was negative. chi-square ($\chi 2$) value and the P-value, the results in the deviate from Hardy-Weinberg equation, and p-value of GG, AA, GA, (0.4573, 0.3362 0.6595) were no significant. **Conclusion** Results of this study indicated a positive relationship between TAFI, lipid profile, insulin level, HOMA-IR, and BMI, where the relationship was highly correlated. However, the results were not statistically significant TAFI G>A rs3742264 association with diabetic patients. However, we recommend conducting the study in a larger sample and different races to confirm the results that we have reach.

Keywords: T2DM, TAF G>A rs3742264, T-Arms, diabetes complication,

Introduction:

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both[1]. The chronic

hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels[2]. as aging have both led to a rise in instances[3]. Only 1-3% of cases of diabetes are caused by type 1 diabetes mellitus, while type 2 diabetes mellitus accounts for 90–95% of all occurrences of diabetes . Type 1 and type 2 diabetes mellitus' polygenic nature[4].. These range from autoimmune destruction of the β-cells of the pancreas with consequent insulin deficiency to abnormalities that result in resistance to insulin action[5]. Diabetes problems primarily affect the coronary arteries, peripheral arteries, and cerebrovasculature. Diabetes is a leading cause of morbidity and death since more than 50% of diabetic patients pass away from diabetic macrovascular complications (DMC), such as cardiovascular disease[6,7]. TFAI molecular link between processes of coagulation and fibrinolysis TAFI which is Thrombin activated platelets can promote fibrinolysis by binding plasminogen in a fibrinogen dependent manner and enhancing its activation by tissue type plasminogen activator (t-PA)[8, 9]. In addition, TAFIa has antiangiogenic effects on endothelial. TAFI is a zinc-dependent metallocarboxypeptidase, synthesised by the liver and megakaryocytes(MKs) as a propeptide consisting of 423 amino acids; when the 22 amino acid signal peptide is removed, the 56 kDa proenzyme containing 401 amino acids is secreted into the blood circulation[10]. Thrombin activatable fibrinolysis inhibitor TAFI, a proenzyme is stimulate decrease of the fibrinolytic system [11]. Thrombin plays a central role in this process, it not only forms the clot but it is also involved in stabilizing the clot by TAFI. Activated TAFI protects the fibrin clot against lysis [12]. activated platelets localize to the injury site where they become activated and aggregate to form a platelet plug. Tissue factor exposed at the site of injury triggers thrombin generation, which leads to fibrin formation. Fibrin stabilizes the platelet plug and renders it an efficient barrier for prevention of blood loss. CPB2, The Gene Encoding TAFI The human gene encoding TAFI is CPB2 (Carboxypeptidase B2 by the Human Genome Organization (HUGO), and is located on chromosome 13 (13q14.11. The gene spans 48 kb of genomic DNA, and has 11 exons and 10 introns . The position of the exon/intron boundaries established in CPB2 were found to be the same as in the genes that TAFI is evolutionarily related to, such as the pancreatic and mast cell carboxypeptidases. Two of nineteen identified single-nucleotide polymorphisms (SNPs) located in the coding region result in amino acid substitutions, which create four TAFI isoforms of which the 325 Thr/Ile polymorphism affects TAFIa stability and

antifibrinolytic activity. The G> A rs3742264 nonsynonymous (ns)-SNP which codes for the Ala147Thr amino acid substitution in TAFI, is linked to a lower risk of myocardial infarction and venous thrombosis. Interestingly in plasma, homozygosity for the A-allele (AA) of G>A rs3742264 is associated with increased levels of TAFI.

Materials and Methods:

Samples were collected from the period February 20, 2022, to augustes 15 , 2022. As well as the practical aspect of the study was carried. Include 100 individual as healthy subject and 100 T2DM patients, Five milliliters of blood taken from a vein were collected using two types of collection tubes, blood were collated from vein puncture and blood was divided into two parts, 3 ml of blood were placed in gel tubes for biochemical examination and 2 ml of blood that had been placed in an EDTA tube and freezing to deep freeze for genotyping analysis. Weight and height were estimated using anthropometric measures according to established body mass index (BMI), Estimation of Serum fasting glucose level(FBS), Insulin, HOMA-IR, and evaluated Lipid Profile (Cholesterol, Triglyceride, LDL, vLDL, HDL). Isolation DNA and Genotyping: 2 cm3 blood from vein puncture was collected in EDTA tubes. and genomic DNA was isolated using an extraction (FavorPrep Blood/ Cultured Cells Genomic DNA Extraction Mini Kit). Estimation of DNA Purity and Concentration 1.788 ±0.067 and 88.5 ± 10.6 respectively. Sequence primers F-5′-CTTCCACATGCAGCTCTGAC-3′ R-5′-ATAGCCCAGTTGAGTCTGACAC-3′, and F-5′-GGTTTCTGGAAAAGAACTAG 3′

R-5′-CATATGGCATTTTTGGCCGT-3′. Extracted DNA using agarose gel electrophoresis TAFI rs3742264 polymorphism was genotyped using T- ARMs PCR designed primer based on [13] The DNA amplification work was carried out using (GoTaq® G2 Green Master) Mix12.5 µl Forward-primer 1.5 µl, Reverse-primer1.5 µl, Genomic DNA 5µl Nuclease free water 4.5 µl. program information for PCR condition Initial Denaturation95 C°5 min 1 cycle, Denaturation 95 C°, Annealing 55 C°, Extension72 C° for 3.5 min with 36 cycles and Final Extension 72 C° for 3.5 min

Statistical Study

case-control study was performed statistically on the data obtained using (IBM Spss statistics version 26) using the T-test to study the relationship between biochemical measurements among(BMI , FBS,Fasting Insulin, HOMA-IR, Cholesterol, Triglyceride, LDL, vLDL, HDL A logistic regression model was used to calculate Chi-Squair order ratio OR .and confidence interval 95% CI as an estimate of the ratios risk of complications from type 2 diabetes. To find out the extent to which the results shift or fit to the TAFI + rs3742264 polymorphism, Hardy-Weinberg equilibrium distributions were used. All regression analyzes are presented between healthy subjects and patients. Statistical significance was defined as a two-tailed p <0.05 For the statistical analysis.

Results

The present study includes 200 volunteers separated into two groups. The first group comprised of 100 volunteers patient with T2M aged (29-66 years) and the second group consisted of 100 volunteers as a healthy control group (30-64). The anthropometric and biochemical results for each groups were given in Table 1. Results revealed significant differences for all parameters (except the age and Height) between T2DM group and the healthy control group. The obtained data showed statistically significant relationships, and it is one of the signs associated with the signs of diabetes2 type, as it showed a statistical significance that was less than (P<0.001) for each Age and Weight respectively.

Table1: Results of phenotyping for T2DM patients and the control group

Parameter	Control Min-Mix	Patient Min-Mix	P-Value
NO. of F/M	**100(48/52)**	**100(58/ 42)**	**0.144**
Age/Y	**35.5000±9.85384** **30-64**	**48.0300±10.4793** **29-66**	**0.000**
Height (m2)	**1.6858±.09843**	**2.7111±.27046** **2.10- 3.46**	**0.342**
Wight(Kg)	**76.2347±12.97484**	**71.9300±10.99518** **49-98.**	**0.000**

BMI Kg/ m^2	**26.7353 ± 3.54722**	**26.53705±3.152116 19.879-35.201**	**0.000**
onset diseases (Y)	----------	**2.9293±1.59721 1.00-5.00**	----------

Comparison between patient group and control healthy group. The mean difference is significant at p<0.05 level, F female, M male, BMI body mass index

Glucose metabolic properties (fasting blood glucose and HbA1c%) were among the parameters measured. Additionally, evaluating the effect of diabetes on the metabolism of lipid profile (cholesterol, triglycerides, HDL-C, LDL-C, and VLDL-c) and TAFI show weak correlation (P<0.033) table 2.

Table 2. Relationship between Biomarker in the Groups under Study

Parameters	***Control Group Mean ±STD Min-Mix***	***Patient Group Mean±STD Min-Mix***	***P-Value***
Fasting Blood Sugar(mg⁄dl)	94.2600 ±8.06491 83.00-94.26	275.2100±75.04831 97-421	0.000
HbA1c%	4.50±.5.73 4.8640- 0.28833	11.4550±2.40191 5.0-16.2	0.000
Triglyceride mg⁄dL	98.7800±39.45963 45.00-195.00	138.0600±59.09688 55-300	0.000
Cholesterol mg⁄dL	158.4540 ±28.33096 106.60-217.00	241.8600±88.48506 75-699	0.000
HDL-c mg⁄dl	48.4300±4.52659 39.00-55.02	33.5400±9.06254 15-35	0.000
LDL-c mg⁄dl	95.3200±20.37521 35-130	144.4200±73.94458 36-366	0.000
vLDL-c mg⁄dl	94.2600±8.06491 9.06-39.00	274.3100±75.32350 12.00-60	0.000

Insulin (µU/ml)	27.5452 ± 5.09261 20-36	37.2196± 5.02913 27- 40	0.000
HOMA-IR	6.4095±1.31751 4.61-12.24	23.8639±6.38859 12.33-42.35	0.000
TAFI	376.4727±150.64994 94.63-676.37	507.1451±590.24592 103.72- 2760.98	0.033

The current study focuses on the correlation between levels of TAFI in Diabetic patients and compared to the same biomarkers in the healthy controls. Use Pearson correlation to find out the correlation and relationships between biomarkers and their correlation with TAFI concentration, based on the results in Table 3.10 , levels of Thrombin Activatable Fibrinolysis Inhibitor Diabetes patients were significantly negatively correlated ($p < 0.01$) to, , FBS, BMI, HbA1c, HOMA-IR, whereas, in contrast to the levels of these biomarkers in the control group which was no significant statistically correlation, and was shown positive correlate with lipid profile marker except with HDL-c was negative relationship.

Table 3. Pearson Correlation of TAFI Levels to The Biomarkers in Studied Groups

Parameters		***Control***	***Patient***
Age	r	-.022	.117
	p	.826	.247
Insulin	r	-.049	.762**
	p	.627	.000
FBS	r	.084	-.891**
	p	.407	.000
HbA1C	r	-.061	0.584**
	p	.547	.000
HOMA-IR	r	-.102	-.672**
	p	.312	.000
BMI	r	.074	-.451**
	p	.463	.000
Cholesterol	r	.097	.781**

	p	.336	0.000
TG	r	-.086	.693**
	p	.398	.000
HDL-c	r	-.055	-.532**
	p	.588	.000
LDL-c	r	-.085	.740**
	p	.401	.000
vLDL-c	r	-.086	.697**
	p	.398	.000

****Correlation is significant at the 0.01 level, *Correlation is significant at the 0.05 level.**

Detection of TAFI Gene G>A (rs3742264) Polymorphism PCR products of TAFI gene. SNPs The polymorphisms of TAFI Ala147Thr or G>A (rs3742264). results of detection polymorphism in TAFI, locus shows there are three genotypes AA, GA, and GG with different bands GG : 520 bp , AA: 183 bp, GA:183, 520 bp, and 520 bp,

Table 4: TAFI Gene rs3742264G/APolymorphism Genotype and Allele Frequency in T2DM and Healthy Subject

G>A rs3742264	Cases N=100	Controls N=100	OR	95%CI	P-value
GG	32 (32%)	37(37%)	0.8013	0.4467 to 1.4372	0.4573
AA	3(3%)	1(1%)	3.0619	0.3130 to 29.9488	0.3362
GA	65 (65%)	62(62%)	1.1382	0.6398 to 2.0251	0.6595
Allele Frequency					
G Allele	129	136	0.8417	0.5552 to 1.2759	0.4167
A Allele	71	63			

*(P<0.05): significant , **or***(P<0.05) higher significant OR : Odds Ratio , CI: Confidence Interval

Table5: Apply HW.E for Polymorphism TAFI Gene rs3742264 G/A(rs3742264) to Calculate the Expected Frequencies

Genotype 505 G/A	GG	GA		AA
Observed genotype	**37**	**62**		**1**
Expected genotype	**46.24**	**43.52**		**10.24**
p-value= 0.0001 chi-Square =18.0312				

results of detection polymorphism in TAFI, locus shows there are three genotypes AA, GA, and GG with different bands , : GG 520 bp , AA: 183 bp, GA:183 bp and 520 bp. TAFI polymorphisms were found to be predominant in T2DM patients: GG (32%), GA (69%), and AA (3%), whereas in the healthy control group, GG(37%), GA (62%), and AA (1%), respectively as shown in Table 6. this result shows no statistically significant difference between T2DM and the healthy control group (p>0.05).

Based on the chi-square($\chi 2$) value and the P-value, the results in the deviate from thr Hardy-Weinberg equation, deviation was observed between the expected and observed results. Therefore, the allele GG is the common allele in Iraqi society, and it is the type that prevailed in the control group..This study was conducted within a limited number of participants, and the results could be more accurate if it was conducted on a larger number of participants who cover a larger geographical area, and we recommend that it include diverse races

Discussion

Age, weight gain and genetic factor are among the most important risk factors that increase the possibility of developing diabetes or diabetes in control on blood sugar lead to development diabetes in table1 showed P<0.001 between patient group compare with control group and statistically significant relationships[14], it is one of the signs associated with the signs of T2DM, for each Age , Weight and the BMI respectively. BMI confirmed higher risks of diabetes with overweight and higher BMI values among patients compared to healthy subjects[15-16]. The present results are in accordance with the study which discussed the fact that the prevalence of T2DM rises with age, reaching peak levels.while gender non statistical differences (p=0.144)[.

Parameters HbA1c, Fasting Blood Sugar , insulin, total cholesterol, TG, vLDL-c, and LDL-c values in type 2 diabetes patients were compared to those in controls, a significant relationship (p< 0.001) with TAFI and shown positive correlate with LDL[17, 18]. Positive correlate with lipid profile marker except with HDL-c was negative correlation with TAFI value[19] . Hyperlipidaemia (high serum triglyceride or total cholesterol concentrations or both) is increased frequency in diabetic type 2 patients[20]. Thus, it seems to be a substantial overlap of disturbances of carbohydrate and lipid metabolism and of blood pressure in the general population. Insulin is the central regulator of glucose and lipid homeostasis[21] it lowers blood glucose concentrations by inhibiting hepatic gluconeogenesis and glycogenolysis as well as increasing glucose uptake into striated muscles and adipocytes; it also increases triglyceride synthesis in liver and adipose tissues, increases the breakdown of circulating lipoproteins by stimulating lipoprotein lipase activity in adipocytes. When adipose, muscle, and liver cells do not react correctly to insulin, The most obvious finding in the current study was a substantial rise in HOMA-IR in patients with T2DM when compared to the healthy control groups; this finding suggests the pathogenic influence of IR, especially when all indications of T2DM in a person are present. Coagulation and fibrinolysis disorders are well-known consequences of type 2 diabetes mellitus. They lead to an increase in the incidence of macrovascular complications and microvascular complication such myocardial infarction and ischemic stroke, The high level of glucose in the blood caused about 20% of deaths from cardiovascular diseases, This study was consistent with previous studies, which were conducted on patients with type 2 diabetes, and results of a previous study conducted of diabetic patients with albuminuria indicated the involvement of TAFI in the mechanism of fibrinolysis in diabetes. It is important that the results

of the current study are consistent with the above study, and the significant increase in plasma TAFI levels in patients with diabetes type2 [21]. . However, our current study is not compatible with a study conducted on diabetic patients in Guinea, where TAFI did not have a significant difference between patients and healthy subjects[22] . increase levels of TAFI in patient compare to control were associated with a higher risk of developing cardiovascular disease [23], as high readings indicate a higher risk of developing blood clots in the futureas . volunteers patients in this study did not have any history of cardiovascular disease and did not have any previous strokes[24]. The results of the current study are consistent with previous studies conducted on patients with type 2 diabetes[25] . The correlation between cardiovascular disease and plasma TAFI levels is widely established. TAFI levels have been linked to an increase in ischemic stroke [26].

Increase of TAFI and LDL that song evidence linkage between TAFI and stock[27]. Where LDL is considered the most important marker for the high probability of stroke or diabetes complications, as a precedent was conducted between them and its results were consistent with the results of the current study [28].

TAFI levels in plasma have been shown to practically quadruple the risk of acute coronary artery disease (CAD) and to rise in individuals undergoing coronary artery bypass grafting (CABG)[24]. On other hand result of genome study in table 4and 5 shown wasn't relationship between TAFI rs37422640G>A and increase risk of stock and diabetes type 2 complication in Iraqi patient, odds ratio of 0.3266 suggests a lower odd of having the AA+AG genotype in cases compared to controls the confidence interval (0.0334 to 3.1946) includes the value of 1 indicating that the difference is not statistically significant p-value of 0.3362, Individuals carrying the 505G allele, which is linked to lower levels of TAFI effect compared to the 505A allele, demonstrated a higher susceptibility to deep vein thrombosis (DVT) [28]. This finding suggests that the connection between TAFI and the development of venous thrombosis is more intricate than what was previously believed

Present results are in accordance with the study which discussed the fact that the prevalence of T2DM rises with age, reaching peak levels. The current study found gender non statistical differences in the healthy and patient groups, however there are no significant differences (p=0.144) between males and females in the T2DM.

Conclusion

In the conclusion of the study, TAFI has a significant relationship with the deterioration and development of diabetes, and the current study did not find a relationship between the genetic variation TAF G>A rs3742264 under study and the possibility of developing heart and arterial diseases in T2DM patient

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