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Association of Transforming Growth Factor Beta1 Gene Polymorphism with Diabetes Mellitus Risk in Iraq Patients

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Abstract: Diabetes mellitus (DM) refers to a group of multifactorial metabolic disorders characterized by elevated blood glucose levels (hyperglycemia) that result from defects in the body's ability to produce and/or insufficiency of insulin action .This study was to investigate the correlation between TGFβ1 polymorphism and Diabetes Mellitus .The present study carried out in the labs of college of education for pure science and Center for Diabetes and Endocrinology of the Health Directorate in Thi- Qar province, the period of research was extended from January- July 2017. To test for the association of Polymorphisms in promoter region (G-800A) and (C-509T) of transforming growth factor- β 1 (TGF-β1) gene with diabetes mellitus in Iraqian patients. The study included a total of 120 patients with type I and II diabetes and their age between 1-51 years. in addition to 52 healthy controls . DNA has been isolated and RFLP-PCR was performed by using primers specific for genotypes of two region of the TGF β1 gene (C509T)and (G800A). The results showed that only C509T polymorphism of the TGFβ1 gene is significantly different in genotype distribution in allelic frequencies between DM patients and control subjects and association with clinical characteristics. Thus this SNP seems to be related to DM susceptibility. This study supports the involvement of TGFβ1 gene polymorphism in the incidence of DM in Thi-Qar population.

Keywords: Diabetes Mellitus, TGF-β1 , G800A, C509T.

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Introduction:

Diabetes mellitus (DM) refers to a group of multifactorial metabolic disorders characterized by elevated blood glucose levels that result from defects in the body's ability to produce and/or insufficiency of insulin action (1,2) Without enough insulin, the cells of the body cannot absorb sufficient glucose from the blood; hence blood glucose levels increase, which is termed as hyperglycemia. The problem of diabetes is not just a high blood sugar, it is a symptom of a disease that penetrates the human body silently and slowly, but it hits violently.

Type 1 diabetes mellitus (D1M) occurs when there is the autoimmune destruction of pancreatic beta cells leading to insufficient insulin and resulting hyperglycemia. Pathogenesis of type 1 diabetes mellitus is different from that of type 2 diabetes mellitus (D2M), where both insulin resistance and reduced secretion of insulin by the β cells which play a synergistic role (3) In both D1M and D2M, various genetic and environmental factors can result in the progressive loss of beta-cell mass and/or function that manifests clinically as hyperglycemia.

Transforming Growth Factor-Beta 1(TGF-β1) is a pleiotropic cytokine

plays a significant role in regulation of different cellular process, including tissue homeostasis, development, growth and regulation of the immune system (4) migration of various cell types and induces the production of extracellular matrix proteins (5,6,7) .It is synthesized by T regulation cells (Treg) (8) .Both arms of the immune system are inhibited by regulatory effects of this cytokine (9).

The human genome exhibits a large amount of diversity, within and between populations and individuals. This inherited variation or different genotypes/alleles of genes in the Deoxyribonucleic acid (DNA) sequence refers to the term "polymorphism" that occur in more than 1% of a population (10,11)

The smallest unit of genetic polymorphism and which represents the most common type of it in human is a single nucleotide polymorphisms (SNPs). The human genome are at least 3.1 million (SNPs), or about 1 SNP per kilobase of sequence (12). SNPs make up about 90% of all human gene differences About 12 million SNPs and a large amount of other types of genetic variation were known in the human genome (13). Various common single nucleotide polymorphisms (SNPs) in this gene can modify the protein expression of TGF-β1 (14).

The aim of study was to determination polymorphism of TGFβ1 gene in patients with diabetes type I(D1M) and type II(D2M).

Materials and Methods:

This study was performed on 172 Iraqi patients with diabetes mellitus D1M, D2M patients, who attended the consultant clinic for diabetes mellitus in endocrine and diabetic center in Al-Nasiriya city in the period from the beginning of (January 2017 to end in July in the same year). Blood samples were collected by venipuncture from 56 T1DM, 65 T2DM patients and 52 controls (Three milliliters of venous blood) were put directly in a sterile tube containing EDTA for DNA extraction.

DNA was extracted from blood samples according to the leaflet attached with DNA Extraction Kit (Geneaid /Thailand).

PCR technique was used to amplify the TGFβ1 gene according to (15,16) and used the following Primers for the PCR technique. Primers (forward and reverse) as the (Table 1) the kit provide by Bioneer company.

Table (1): Oligonucleotide Primer Sequences used for Amplification

First promote region	**F**	5′CCCGGCTCCATTTCCAGGTG-3′
	R	5′GGTCACCAGAGAAAGAGGAC-3′
Second Promote region	**F**	5′GGCAGTTGGCGAGAACAGT-3′
	R	5′ACCCAGAACGGAAGGAGAGT3′

The PCR mixture (total, 20 µl) containing 5 µL DNA, 1 µl of each primer, 5 µl Taq master mix (containing Taq DNA Polymerase, dNTPs, MgCl2 and reaction buffers at optimal concentrations for efficient amplification of DNA templates by PCR) and 8 µLwater. The PCR mixture was incubated at 95˚C for 5 minutes, then 1 cycle 95˚C for 1 minutes to denature, 58˚C for 40 seconds to anneal the primers and 72˚C for 8 minute to elongate the strand PCR. Subsequently, the amplified products were digested by

the Bsu361, HpyCH41v restriction enzyme according to the manufacturer's instructions. Digested products were separated on 3% agarose gel stained with ethidium bromide.The products were transfered by the electrophoresis apparatus by dissolving (1.2) of the Agarose gel in 60 mL of the TBE to become the final concentration (2%) and Visualization of amplified product was conducted by U.V transilluminator (302nm). The size of amplified product was determined according to ladder marker and imaged by digital camera.

The PCR product was observed when the base pair (808bp and 600 bp)was observed indicating the presence of the TGFβ1gene after comparing it with DNA Marker (100-3000bp). After adding the interaction materials needed to cut the first region (808bp)of gene ,the TGFβ1gene was cut off using Bsu361, which recognizes the four nucleotide sequence, and according to the leaflet attached to the enzyme Company (Biolabs, UK). The second region of the gene, which appeared at (600bp) was cut off after adding its reaction materials of TGFβ1gene was cut off using HpyCH41v, which recognizes the four nucleotide sequence, and according to the leaflet attached to the enzyme (Biolabs UK).

Results and Discussion:

Genomic DAN was successfully extracted from all studied groups. Extracted by following the instructions of (Geneaid Kit), The presence of DNA bands was confirmed by agarose gel electrophoresis on a 0.8 % as shown in (Figure 1). The location of G-800A and C-509T polymorphisms in the *TGF-β1* promoter and the electrophoresis results of PCR-RFLP are shown in (Figure 2) and (4). For the G-800A polymorphism genotyping, the PCR products, 808 bp in length, were digested with Bsu361, Two fragments of 617 and 191 bp were shown if the product was digestible and categorized as homozygous CC genotype.808 bp fragment was categorized as homozygous TT genotype .Three fragments of 808, 617 and 191 bp were categorized as the heterozygous CT genotype, (Figure 3). For the C-509T polymorphism genotyping, the PCR products, 600 bp in length, were digested with HpyCH4IV**,** Two fragments of 401 and 199 bp were shown if the product was digestible and categorized as homozygous GG genotype. 600 bp fragment was categorized as homozygous AA genotype. Three fragments of 600, 401 and 199 bp were categorized as the heterozygous GA , (Figure 5).

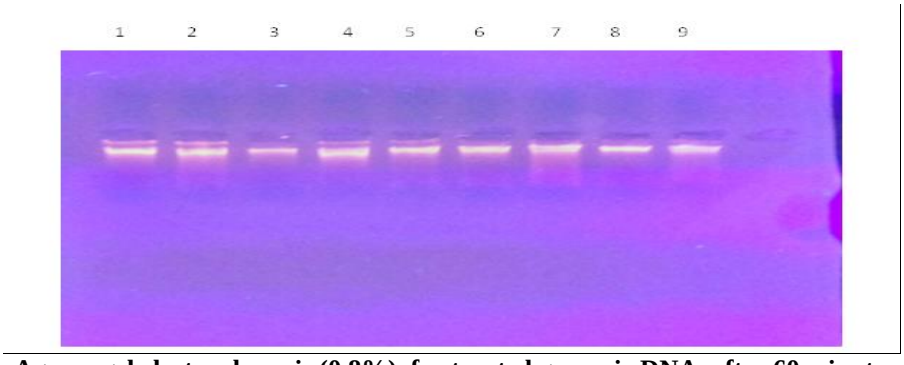

Figure (1): Agaros gel electrophoresis (0.8%)of extracted genomic DNA, after 60 minutes at 100 V, stained with ethidum bromide and visualized by a UV transilluminator. Lanes 1-9: Genomic DNA .

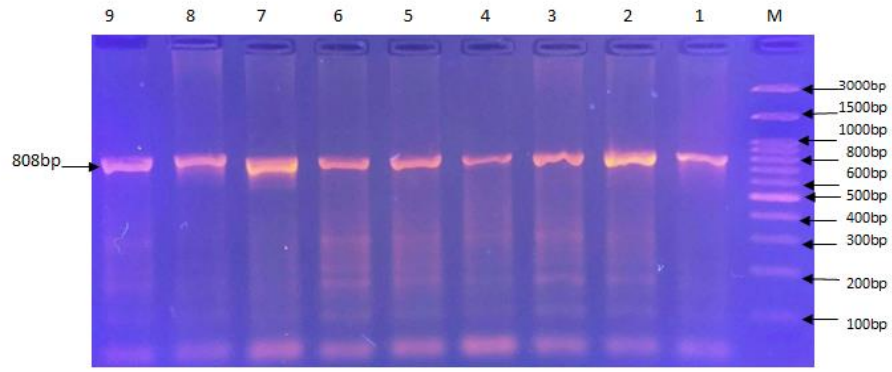

Figure (2): Agarose gel electrophoresis for amplified TGFβ1gene of DM patients. Bands were fractionated by electrophoresis on a 2 % agarose gel (1 h., 80V/cm) and visualized under U.V. light after staining with ethidium bromide staining. (L:100 - 3000bp M: ladder) Lane: 1,2,3,4,5,6,7,8 and 9: positive results.

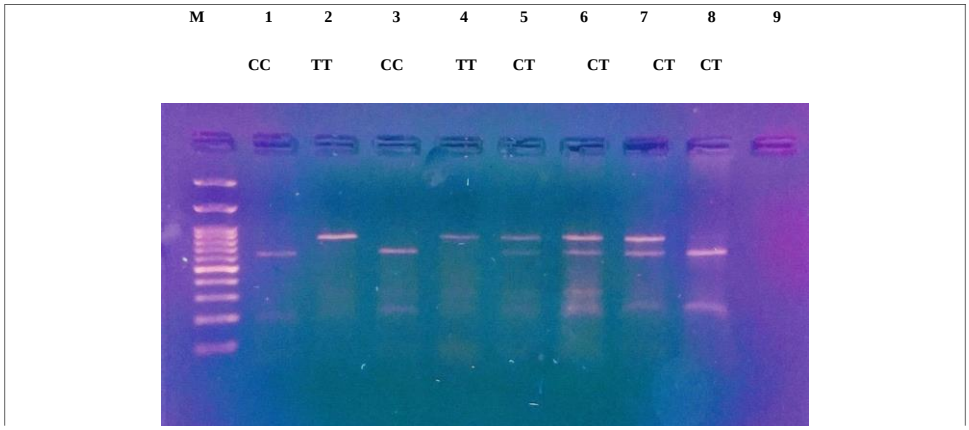

Figure (3): Agarose gel electrophoresis for amplified TGFβ1gene of DM patients. Bands were fractionated by electrophoresis on a 3% agarose gel (1 h., 80V/cm) and visualized under U.V. light after staining with ethidium bromide staining.(L :100 - 3000bp M.ladder) using Bsu361. Lane: 1, 3, 8 (Intact sample C Allele 617,191 bp fragment or wild homozygous CC); Lane: 2, 4 (Mutant sample T Allele 808 bp fragment or mutant homozygous TT); Lane: 5,6,7 (mutant sample C/T or mutant heterozygous CT).

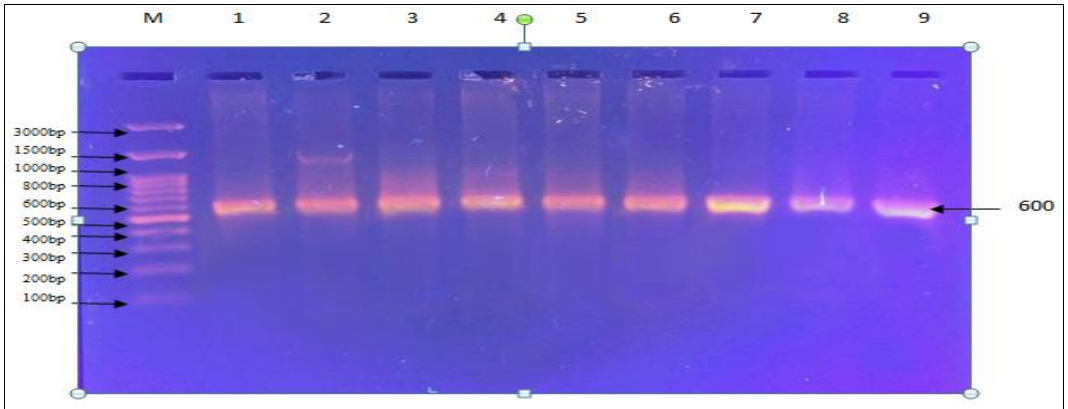

Figure (4) Agarose gel electrophoresis for amplified TGFβ1gene of DM patients. Bands were fractionated by electrophoresis on a 2 % agarose gel (1 h., 80V/cm) and visualized under U.V. light after staining with ethidium bromide staining. (L:100 - 3000bp M: ladder,); Lane: 1,2,3,4,5,6,7,8 and 9. positive results.

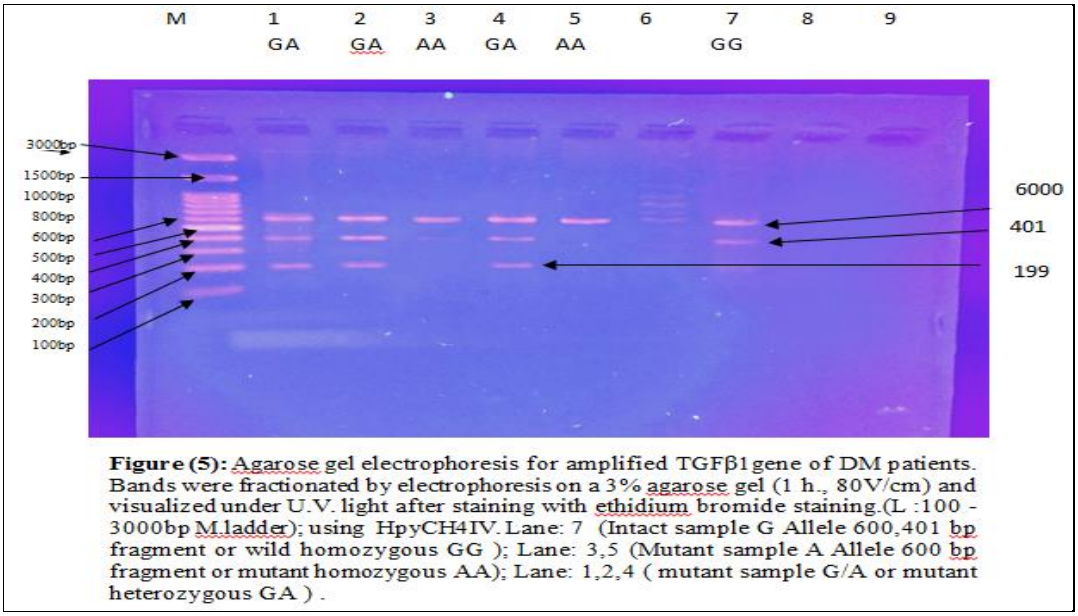

Figure (5): Agarose gel electrophoresis for amplified TGFβ1 gene of DM patients. Bands were fractionated by electrophoresis on a 3% agarose gel (1 h., 80V/cm) and visualized under U.V. light after staining with ethidium bromide staining.(L :100 - 3000bp M.ladder); using HpyCH4IV. Lane: 7 (Intact sample G Allele 600,401 bp fragment or wild homozygous GG); Lane: 3,5 (Mutant sample A Allele 600 bp fragment or mutant homozygous AA); Lane: 1,2,4 (mutant sample G/A or mutant heterozygous GA) .

Frequency of genotypes for the TGF β1 gene samples of D1M patients and healthy controls:

The results of the current study showed a significant difference between patients with type I diabetes and healthy controls genotypes of two region of the TGF β1 gene (C509T) when CT (OR =1.71) is more than one and half , (G 800 A) when genotype AA(OR= 1.30) is more than one time, but the other genotypes were TT(OR=0.49), GA(OR=0.83) as the (Table 2).

Table (2): Distribution of genotypes for the TGF β1 gene samples of D1M patients and healthy Control.

Gene	Genotype	Control	%	D_1M	%	OR	95%CI
C 509 T	CC	17	70.8	40	71.42	1	
	CT	3	12.5	11	19.64	1.71	0.4315 - 6.7859
	TT	4	16.66	5	8.92	0.49	0.1193 - 2.0135
G 800 A	GG	19	79.16	45	80.35	1	
	GA	4	16.66	8	14.28	0.83	0.2251-3.0845
	AA	1	4.16	3	5.35	1.30	0.1285-13.188

Frequency of genotypes for the TGF β1 gene samples of patients D_2M and healthy controls:

The results of (Table 3) showed the genotype CT of (C509T) TGF β_1 gene a high significant difference by more than one and a half times (OR=1.89).While other genotypes of (C509T) TT (OR=0.45) and GA (OR=0.84), AA(OR=0.81) of (G 800A) does not showed any significant difference among samples of patients and healthy controls of D2M.

Table (3): Distribution of genotypes for the TGF β1 gene samples of D_2M patients and healthy Control.

Gene	Genotype	Control	%	D_2M	%	OR	95%CI
C 509 T	CC	6	22.22	14	21.53	1	
	CT	12	44.44	39	60	1.89	0.7571 - 4.6438
	TT	9	33.33	12	18.46	0.45	o.1639 - 1.2512
G 800 A	GG	16	59.25	42	64.61	1	
	GA	8	29.62	17	26.15	0.84	0.312 - 2.2732
	AA	3	11.11	6	9.23	0.81	0.1880 -3.5204

To our knowledge, The present study is the first analysis of promoter region of TGF-β1 gene (C509T) and (G 800 A) polymorphism and gene expression related to Type1and type 2 of DM.

Genetic Polymorphism A difference in DNA sequence among individuals, groups, or populations. Sources of genetic Polymorphism include SNPs, sequence repeats, insertions, deletions and recombination. SNPs are the most simple form and most common source of genetic polymorphism in the human genome(17).

There are two types of nucleotide base substitutions resulting in SNPs:

1- A transition substitution occurs between purines (A, G) or between pyrimidines (C,T). This type of substitution constitutes two thirds of all SNPs.
2- A transversion substitution occurs between a purine and a pyrimidine (17,18). These results reveal that there is a significant difference between D1M patients and healthy controls genotypes of the TGF - β1 gene (C 509 T), as the results show the significant difference between patients and healthy controls genotypes of the TGF β1 gene (G 800 A) . Also in D2M patient of present study showed a high significant difference of genotype CT of the TGF - β1 gene (C509T). These results agree with (19) who found that a haplotype C-T was observed to confer nearly a 2–3-fold risk towards T2D. (20,21) reported a higher frequency of TGF- β1 CC/CT genotypes of Diabetic Nephropathy patients . These results indicate that there is a correlation between polymorphism of TGF β1 gene as risk factor of diabetes mellitus . The TGF- β1 gene polymorphism has also been found to be associated with disease states such as osteoporosis and myocardial infarction (22,23) .The study of (24) suggest that gene polymorphisms in TGF- β1 is a risk factor of genetic susceptibility to cerebral infarction in the Chinese population. Research of (25,26) showed that TGF β1 gene polymorphisms relate to many disease especially diabetes mellitus.

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