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ASSOCIATION BETWEEN DIPEPTIDYL PEPTIDASE-4 POLYMORPHISM (RS1861978) AND INCRETINS LEVELS IN TYPE 2 DIABETIC PATIENTS

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ABSTRACT

Introduction: Type2 Diabetes mellitus (DM) was associated with progressively increasing morbidity and mortality. It is a complex multigenic disease with severe consequences. Single Nucleotide Polymorphism (SNP rs1861978 T>G) in dipeptidyl peptidase-4 (DPP-4) and Incretin hormones like Glucose-Dependent Insulinotropic Polypeptide (GIP) have been suggested to be associated in Type2Diabetes mellitus. **Aim:** To estimate the serum levels of Glucose-Dependent Insulinotropic Polypeptide (GIP) and study if there is an association between its levels and dipeptidyl peptidase-4 (DPP-4) gene polymorphism (rs1861978 T>G) in T2DM group and compare their levels with healthy control group and study their correlations with other biochemical parameters. **Materials and Methods:** The circulating GIP and DPP-4 gene polymorphism (rs1861978 T>G) were measured in 120 patients (63 male and 57 female), and 120 healthy control (65 male and 55female) without a known family history of diabetes with matched age (age range 44-55years) they were attending Al-Hassan diabetic and endocrinology care center in Imam Hussein Medical City / Kerbala - Iraq during the period from Jan. , 2018 to Nov. 2018. ELISA kit was used to measure S.GIP, DNA extraction kit and arms polymerase chain reaction (PCR) was used for DPP-4 gene polymorphism (rs1861978 T>G) Polymerase Chain Reaction (PCR) technique and the PCR product was visualized by agarose gel electrophoresis, and enzymatic methods were used for blood sugar, and lipid profile were measured for each subject. **Results:** The clinical characteristics showed a significant increase ($p<0.05$) in the patients group compared to the control group. DPP-4polymorphism analysis showed a higher frequency of T/T genotype in control group than patients group (78% versus 59.1%), while the analysis of the allele impact, including the genotypes that contain the G allele(G/G genotype) showed significant association in patient group compared with control and (G/G genotype) association with GIP level in patient group. **Conclusion:** The results of the present study showed that control group had a higher frequency of the T/T genotype regarding the patients group, and that the G allele of the DPP-4 (rs1861978 T>G) single nucleotide polymorphism has more role in the pathogenesis of T2DM than the T allele, and could be considered as a risk factor for T2DM in Iraqi population, as well as the association of the G/G genotype with the incidence of T2DM in Iraqi patients. DPP-4 (rs1861978 T>G) single nucleotide polymorphism was significantly associated with GIP level in T2DM group than control.

KEYWORDS: DPP-4 gene polymorphism rs1861978, GIP, T2DM.

INTRODUCTION

Diabetes mellitus is a metabolic disorder that is characterized by high blood glucose levels for prolonged period of time, which might be associated with long term damage and failure of many body organs; like eyes, kidneys, heart and nerves.[1,2]

Millions of people are affected with diabetes mellitus over world, making it a famous global health problem. There are two well-known types of diabetes mellitus: Type 1 Diabetes Mellitus (T1DM), which occur mostly among children and young people and considered to be an autoimmune disease, while T2DM (T2DM) is a multifactorial disease that is closely related with life style and obesity.[2] Several studies have shown significant association between various biochemical and molecular parameters with type 2 diabetes mellitus with / without other diseases such as hypertension, cardiovascular diseases in obese and non-obese patients.[3,4]

Various pathophysiological factors were related to the development of macrovascular and microvascular complications in patients with T2DM, suggesting that T2DM may be the result of an inflammation that reflects innate and acquired immunity responses.[5]

The importance of DPP4 for the scientific and medical community raised substantially since the approval of DPP4 inhibitors for the treatment of type 2 diabetes mellitus (T2DM). These so-called gliptins increase the incretin levels and therefore prolong the post-prandial insulin action. Since soluble DPP4 is characterized as an adipokine.[6]

Genetic factors and lifestyle play a critical role in the development of T2DM.[6] The Asian population has strong genetic susceptibility to T2DM, developing diabetes at younger ages and at a lower degree of obesity.[7] Despite knowledge of the critical role of genetic factors, these have not been incorporated into the clinical assessment of T2DM risk.[6,7]

The DPP4 gene encoding for dipeptidyl peptidase IV (DPP-IV), also known as cell surface antigen CD26, is located on chromosome 2q24.3.[8] Polymorphism in the DPP4 gene was a risk factor for a myocardial infarction (MI) in patients of European ancestry with atherosclerosis.[9]

Dipeptidyl-peptidase (DPP) 4, which is also known as CD26, is a ubiquitously expressed glycoprotein of 110 kDa, which was first characterized by Hopsu-Havu and Glenner [10]. DPP4 is a type II transmembrane protein, which is also cleaved off the membrane and released into the circulation by a process called shedding.[11]

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Nonetheless, genetic polymorphisms of DPP4 and their association with T2DM have rarely been investigated.[12] Moreover, a review of the scientific literature revealed that the association of the DPP4 genetic polymorphisms gene with T2DM among Asian populations has not been published. To the best of our knowledge, no such study has been initiated in Asian populations.

Hence, this study was performed to investigate whether DPP4 gene polymorphism are associated with T2DM in Iraqi subjects and to determine SNP within the DPP4 gene, which could be associated with serum GIP levels.

MATERIALS AND METHODS

This retrospective case control study was conducted on 240 Iraqi subjects, 120 patients (63 males and 57 female) that were diagnosed with T2DM, who were periodic patients at the Al-Hassan diabetic and endocrinology care center in Imam Hussein medical city/Holy Kerbala city/Iraq, from January 2018 to April 2018. The patients had been instructed about the purpose of the study and interested volunteers have been enrolled along with 120healthy subjects (65 males and 55 female) that were taken as a control group. Diabetic patients were selected according to the World Health Organization 2016 guideline:

Fasting Blood Sugar (FBS) ≥126 mg/dL (7.0 mmol/L), or Glycohemoglobin (HbA1c) ≥ 6.5% (48 mmol/mol).[2]

Diabetic patients younger than 18-year-old, those with less than six months of follow up, or pregnant women were excluded. Also, subjects with the history of Hypertension, Coronary Artery Disease (CAD), Endocrinopathy or those taking any lipid altering medication were excluded from the study, while control group consisted of non-diabetic healthy individuals according to the laboratory finding of FBS value <90 mg/dL.

Venous blood samples (7 mL) were divided into two aliquots; one for biochemical tests and the other for DNA genotyping. Fasting Blood Sugar (FBS), Glycohemoglobin (HbA1c) and Lipid profile (Total cholesterol, Triglyceride (TG), high density lipoprotein (HDL-C), low density lipoprotein (LDL-C), very low density lipoprotein (VLDL-C) were measured by enzymatic colorimetric methods with commercially available kits (Human, Germany and SPINREACT Company, USA). Body mass index weight/height (kg/m^2) was calculated for both groups.

Genomic DNA was extracted using Relia prep DNA purification Kit (Promega/USA), the amplification of rs1861978DPP4SNP was done by designing sequence specific primers using PRIMER3PLUS software, the two Inner forward and Inner Reverse were different only with the targeted allele (Inner forward -185 T allele and Inner Reverse -350 G allele) and a common two Outer forward were used with both forward primers to obtain a PCR product size of 490 bp [Table-1].

Table-1: Primers of DPP4 Gene rs1861978.

Inner forward	5’-CGTGAAAGCCGCAAGAGTTT-3’
Outer forward	5’-CCTCCCCACCCTCCAACT-3’
Inner Reverse	5’-GGTCTTCAGTGTTTAGGCTGC-3’
Outer reverse	5’-TGGAGCTCAGGTCAGGCT-3’

Lyophilized primers were supplied by Alpha DNA (Canada) and were suspended according to the manufacturer's instructions. PCR reaction was performed using Go Taq ® green master mix (2X) of Promega (USA) instructions for a final volume of 50 µL (two tubes for each sample: one for Inner forward and the second for Inner Reverse, the two reverse primer was added to each tube), as following: 25 µL of 2X Go Taq ® green master mix, 1.5 µL of 10M of each primer (forward and reverse), 3 µL of genomic DNA and the volume was completed to 50 µL with nuclease free water, positive and negative controls were made with each run. Reaction was carried out with Gene Amp ® PCR System 9700 thermocycler with denaturation for 5 minute at 95°C; followed by 45 cycles of 95°C for 30 seconds, 55°C for 45 seconds, and 72°C for 45 seconds. Final extension was carried out at 72°C for 5 min. The PCR product (490 bp fragment) was visualized by 2% agarose gel electrophoresis (stained by 2 µL ethidium bromide).

Statistical analysis was performed using the SPSS software version 25.00 (SPSS Inc., Chicago, USA). A two-tailed p-value < 0.05 was considered statistically significant. Statistical significance of differences between the means for normally distributed variables (FBS, HbA1c-, HDL-C, LDL-C, VLD-CL, TG as well as BMI and the Abdominal Circumference) was assessed using student's t-test.[13]

RESULTS

Clinical Features

The tests result for both patients and control group are listed in [Table-2].

Table-2: Clinical characteristics of patients and control group. Student t-test between Diabetic patients and control group, * p<0.05, ** p<0.001, SD=standard deviation, TG = triglycerides, TC = total cholesterol, HDL-C = high density lipoprotein- cholesterol, LDL-C = low density lipoprotein-cholesterol, VLDL-C = very low density lipoprotein cholesterol, GIP = Glucose-Dependent Insulinotropic Polypeptide, FBS = Fasting Serum Glucose , BMI = body mass index.

Parameter	Diabetic	Control	P. Value
	Mean ± SD	Mean ± SD	
Age (years)	49.26± 4.75	49.16± 4.66	p>0.05
BMI (kg/m2)	28.95± 4.02	28.69± 3.47	p>0.05
FBS (mg/dl)	235.70± 105.59	126.89± 58.69	<0.001
HbA1c %	8.15± 2.14	5.72± 1.01	<0.001
Cholesterol (mg/dl) **(200-239)**	203.37± 53.45	160.85± 41.28	<0.001
TG(mg/dl) **<180**	230.55± 96.07	171.34± 77.57	<0.001
HDL-C(mg/dl) **(30-60)**	36.72± 10.67	45.88± 11.97	<0.001
LDL-C(mg/dl) **(100-190)**	120.53± 48.06	80.70± 43.40	<0.001
VLDL-C (mg/dl) **(2-30)**	46.11± 19.21	34.26± 15.51	<0.001
GIP (pg/ml)	3384.09± 1521.07	2330.19± 1443.94	<0.001

These results showed significant differences in biochemical tests (FBS- Fasting blood sugar, HbA1c- Glycohemoglobin, HDL- high density lipoproteins, LDL-low density lipoproteins, VLDL- very low density lipoproteins, TG- triglycerides and GIP) as well as Body Mass Index (BMI) and gender showed no significant differences in patients group compared with control group.

Table-3, shows significant differences ($p \leq 0.05$) between the patients and control in all tested clinical features (FBS, HbA1c, HDL, LDL, VLDL, TG and GIP) according to the genotype results, as well as a significant difference between G/G genotype patients group and the G/G genotype patients group ($p \leq 0.05$) in all clinical tests results. As noticed from the present data, healthier individuals own just the G/G genotypes.

Table-3: Comparison of the clinical features according to the genotypes. P value derived from ANOVA test,*Significant: p<0.05, ** highly significant: p<0.001, No significant: p>0.05, n.=number, SD=standard deviation, BMI=body mass index, TG=triglycerides, TC=total cholesterol, HDL-C=high density lipoprotein-cholesterol, LDL-C=low density lipoprotein-cholesterol, VLDL-C=very low density lipoprotein cholesterol, GIP= Glucose-Dependent Insulinotropic Polypeptide, FBS= Fasting Serum Glucose, [a] ANOVA test= G/G vs. G/T Diabetic patients, [b] ANOVA test= G/G vs. T/T Diabetic patients, [c] ANOVA test= G/G vs. G/G Diabetic patients vs. control.

Parameter	Diabetic (n.=120) mean ± SD			Control (n.=120) mean ± SD		
	G/G (N=11)	G/T (N= 50)	T/T (N= 59)	G/G (N= 6)	G/T (N= 36)	T/T (N= 78)
Age (years)	48.81±4.33	49.26±4.93	49.35±4.75	49.16±4.53	48.88±4.77	49.29±4.67
BMI (kg/m^2)	29.06±3.90	29.67±4.30	28.32±3.77	28.18±4.90	29.27±3.81	28.46±3.20
FBS (mg/dl)	262.1±120.48 [c*]	247.7±108.11	220.53±100.05	102.1±11.86	104.88±29.88	138.96±66.99
HbA1c %	8.27±1.95 [c*]	8.46±2.10	7.86±2.20	5.83±0.98	5.75±1.11	5.78±0.99
TC (mg/dl)	203.51±61.90 [c*]	205.68±49.50	201.39±55.90	129.7±15.49	173.19±48.83	157.56±37.11
HDL-C (mg/dl)	34.40±6.88 [c*]	36.41±10.12	37.42±11.74	48.16±14.11	42.36±11.45	47.34±11.87
LDL-C (mg/dl)	126.28±56.27 [c*]	121.41±47.87	118.74±47.42	51.87±12.78	95.57±52.04	76.06±38.47
VLDL-C (mg/dl)	42.84±16.24 [c*]	47.86±20.80	45.24±18.47	29.74±14.93	35.26±15.59	34.16±15.65
TG (mg/dl)	214.20±81.19	239.3±104.01	226.19±92.33	148.7±74.65	176.31±77.93	170.80±78.27
GIP (pg/ml)	4571.3±842.5[a,b,c*]	3589.3±1607.8[a,b,c*]	2988.8±1406.4[a,b,c*]	1949.4±734.9	2376.9±1413.5	2337.9±1505.5

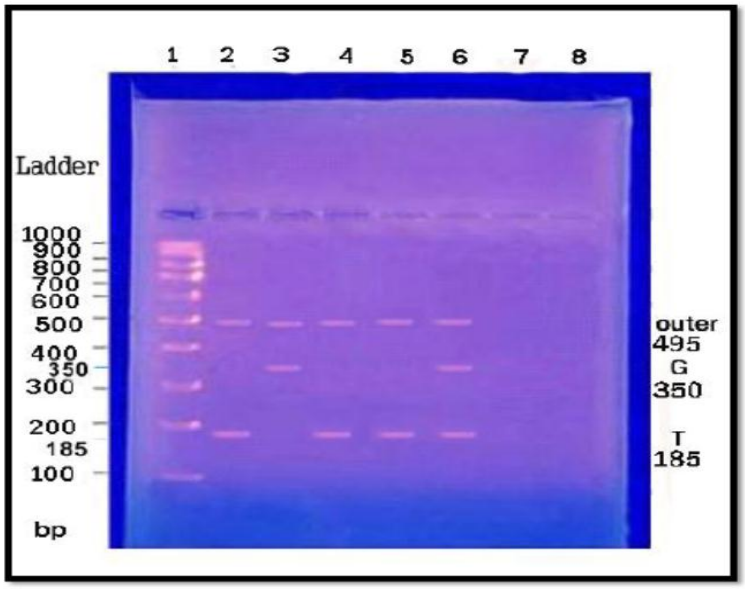

Fig. 1: ARMS-PCR product of DPP4 gene. Lane 1 is size marker, lanes 2, 4, 5 are the T/T genotype (homozygous), Lane 3 is the GG genotype (homozygous wild type) and lane 6 is the G/T genotype (heterozygous).

DISCUSSION

T2DM is associated with; first: abnormal carbohydrate, lipid and protein metabolism, second with insulin resistance and impaired insulin secretion.[2] In this case-control study, clinical features of the patients showed elevated levels of fasting blood sugar, HbA1c, total cholesterol, triglyceride, HDL-C and VLDL-C (with significant differences p<0.05) compared with controls. Previous studies mentioned that both male and female diabetic patients have elevated levels of FBS, HbA1c, and lipid profile.[14-16] These studies in addition to the current study results suggest the use of FBS, HbA1c, and lipid profile tests for T2DM diagnosis confirmation and they might be helpful in risk assessment of coronary heart disease in T2DM patients. The HbA1c test was suggested to be useful for screening diabetic patient for risk of cardiovascular complications and for intervention with lipid lowering drugs.[16] which is compatible with the results of another study which mentioned that HbA1c can be helpful as a suggested biomarker for predicting dyslipidemia in diabetic patients.[17]

All the patients in this study were diagnosed by specialist physicians; their elevated tests results indicated that high FBS and HbAIc levels might be the reason for the increase in their lipid profile, this goes with the finding of a previous study.[17]

So FBS, HbA1c could be used as an indicator for complications (including elevated levels of lipid profile) in Iraqi patients with T2DM.

DPP4 activity is subject to regulation at many levels, including control of gene and protein expression, interaction with binding partners, and modulation of enzyme activity. The *DPP4* gene does not contain conventional TATAA or CCAAT promoter sequences but is characterized by a cytosine/guanine-rich promoter region.[18]

The present study investigated the genotype of DPP-4 promoter gene polymorphism (rs1861978 T>G) SNP in a sample of T2DM Iraqi patients. Statistical analysis results showed that the G/G genotype has a significant association with T2DM in the patient group as well as higher frequency of the G allele, Fig. 1.

The genetic contribution to T2DM susceptibility is well accepted in previous studies.[19]

DNA variations can have a major impact on how humans respond to diseases, environmental insults, drugs, and other therapies. This makes SNP analysis of great importance or biomedical research and for developing pharmaceutical industry or medical diagnostics; it can also help identify multiple genes associated with complex diseases such as cancer and diabetes.[20]

Many SNPs in different genes were studied and found to be associated with the occurrence and development of T2DM.[21,22] Which support our finding that Dpp-4 promoter variation might have an effect in increasing the incidence of diabetes?

General analyses showed that (rs1861978 G/T genotype) was associated with the plasma DPP-4 levels (P = 0.034), total cholesterol (P = 0.006), triglyceride (P = 0.041). Additionally, the pairwise comparisons showed that the subjects with G-T had higher DPP-4 (P = 0.011) than those with T/T genotype, whereas the subjects with T/T had higher total cholesterol (P = 0.002) and triglyceride (P = 0.012) than those with G/T genotype.[23]

This study show that the genotypes that contain the G allele (G/G genotype) showed significant association in patient group compared with control and (G/G genotype) association with GIP level in patient group.

There are many good points that give strength to this study. To the best of our knowledge this study is the first population based study that investigated the Dpp-4 gene promoter (rs1861978 T>G) SNP in Iraqi patients with T2DM, all the Iraqi participants were from the Arab race and had similar genetic backgrounds, which minimized the potential confounding due to ethnicity.

Because of the time limit and the precarious security situation the study was conducted on a small size sample of population, a further limitation of the study is that only one polymorphism was examined, the positive association was found only in group. Also, due to the relatively small sample size, the frequencies of some homozygous variants were low or absent.

CONCLUSION

It was concluded that (G allele) at the DPP-4 gene promoter (rs1861978 T>G) SNP could be considered as risk factors and it has more role in the pathogenesis of T2DM, while the (T allele) might have a protective effect; also there was an association for the T/G genotype with all clinical parameters in T2DM patients. These results shed a light on the importance of the clinical factors in avoidance of diabetes, which might motivate people to improve their lifestyle into a healthier way leading to reduce their chances in developing diabetes and cardiovascular diseases. Further studies are needed with larger sample size to determine the role of DPP-4 SNPs in diabetes occurrence and complication; this could be helpful for better understanding of the genetic-environmental interactions and would assist in designing optimal measures for diagnosis, treatment and prevention of this disease.

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