The Review Of
DIABETIC
STUDIES OPEN ACCESS

Impact Of DPP4 Genotypes On GLP-1 Level And Response To Sitagliptin With Metformin Therapy In Iraqi T2DM Patients

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Abstract

Sitagliptin is one of numerous oral medications that cause the dipeptidyl peptidase-4 (DPP-4) activity inhibition. Crucially, the DPP-4 enzyme is accountable for rapidly degrading and rendering inactive the incretin hormones. The action of these circulating incretins is prolonged by DPP-4 Inhibition via raising their levels and extending their half-life. Genetics significantly influences the progression of diabetes and determines who are susceptible to it. Our study aims to identify the association of (rs6741949) polymorphism for the gene encoding DPP4 with serum GLP-1 level and glycemic response for type 2 diabetics treated with sitagliptin in combination with metformin. High-Resolution Melting (HRM) analysis successfully identified SNP (rs6741949) in the study population, yet it exhibited no significant effect neither on serum GLP-1 level nor on the treatment response. However, the (rs6741949) genotypes were significantly associated with serum insulin and cholesterol levels, suggesting the potential role of the (rs6741949) variant in dyslipidemia regulation.

Key words: DPP4, Genotypes, GLP-1, Sitagliptin, SNP .

Introduction:

Type 2 diabetes mellitus (T2DM) accounts for about 90% of all diabetes cases, primarily arising from the body's inadequate utilization of insulin and the pancreatic β cells' failure to meet the increased insulin demand, leading to dysregulated glucose homeostasis [1]. The prevalence of T2DM in Iraq has been reported to range from 8.5% to 13.9% [2]. Poor glycemic management over time damages many parts of the body, particularly the nerves and blood vessels, which promotes the progression and development of macrovascular and microvascular problems, neuropathies and early dying [3]. Achieving early glycemic control can prevent organs destruction and significantly lower the risk of microvascular and macrovascular complications [4][5]. Genetic heterogeneity contributes to interindividual differences in therapeutic response. The discipline that examines the influence of the entire genome on individual drug responses is pharmacogenomics, in more particularly, pharmacogenetics concetrates on genetic variability within a population and how these variations can influence the therapeutic responses and incidence of adverse effects [6]. Thus, pharmacogenetics has a crucial role in the efforts of translational medicine to close the gap between clinical application and genetic research.

Research in genetic has focused on assessing the interindividual differences in responses to injectable and oral antidiabetic agents, with numerous pharmacogenetic studies published in recent years examining the associations between genetic variants and responses to these drugs. The pharmacogenetic studies aimed to recognize individual subsets that were more or less presumably to respond therapeutically to the specific drug in study. In fact, the management of T2DM patients necessitates an individualized strategy because of the disease's heterogeneity, variations in pathophysiological and molecular pathways of glucose homeostasis among individuals, and the unpredictable effects of current therapies on individual responses to glucose-lowering drugs[7].

Distinctly, an individualized approach is essential due to the various clinical features that influence in making decision including body weight, age, duration of disease, expectancy of life, history of glycemic control, adverse effects of glucose- lowering drugs, risk of hypoglycemia, psycho- socio- economic factors and presence of complications and comorbid conditions[8].

Approximately 40% of individuals undergoing treatment for T2DM fail to reach the recommended target HbA1c of less than 7%[9]. Nonetheless, numerous patients, especially those with elevated baseline glycated hemoglobin (HbA1c) levels, may fail to attain their glycemic targets on metformin monotherapy, even after titration to the highest tolerable doses, necessitating the addition of other medications [10]. The first DPP-4 inhibitor (DPP-4i) to receive approval in the US, sitagliptin, has been utilized as an adjuvant to exercise and diet both alone and in conjugation with other types of oral antidiabetic agents [11].

Several studies have notified a correlation between particular genetic variations and glycaemic responses elicited by antidiabetic drugs. Pharmacogenetic studies on prevalent antidiabetic medications, including sulphonylurea and metformin, potential clinically related genetic modulators influencing their efficacy and safety has identified [10 -11].

Much less studies has been done in pharmacogenetic implications of DPP-4i. Nevertheless, the DPP-4i response varies among T2DM patients; the genetic factors are not entirely understood. DPP-4i, which impede enzymatic hydrolysis and thereby inhibit active the incretins degradation, are utilized to improve incretin-mediated glycaemic regulation. DPP-4i have been suggested as prospective therapeutic agents for T2DM [14].

A meta-analysis study demonstrated that DPP-4i significantly reduce level of HbA1c to a greater extent in Asians compared to non-Asians [15].

Therefore, as pharmacogenetic and pharmacogenomic studies have shown, genetic differences among various ethnic groups may change the therapeutic response and metabolism of DPP-4 inhibitors[16].

Thus, using case-control studies and clinical trials with a nominee genes approach, they have examined the genetic impacts of a number of genes, including GLP1R[15-16], DPP4[17-18] and TCF7L2[21], on the DPP-4i treatment response in T2DM patients. Recently, the DPP4 gene has attracted significant attention because of the glucose regulatory action of DPP-4 on incretin hormones (GIP and GLP-1), which are the major regulators of insulin secretion postprandially.

Because T2DM is a multifactorial illness having a distinct genetic constituent and in addition to the crucial role of DPP-4 in homeostasis of glucose via the controlling of levels circulating insulinotropic incretin [22], thus, our study aimed to ascertain the correlation of a genotype characterized by SNP (rs6741949) within the DPP4 gene (noncoding regions) with the fasting glycemic parameters among Iraqi T2DM samples, taking serum GLP-1 levels into account.

As part of the growing field of precision or personalized medicine, pharmacogenetic information may be beneficial patients' classification to determine response and to weigh the potential risks and benefits of antidiabetic drugs [23].

Given the paucity of pharmacogenetics study on Iraqi population and the new perspective on T2DM treatment, with the hope that this study would help pave the road towered customized pharmacological therapy by providing information about genetic makeup of Iraqi population.

Patients &Methods

Patients: Iraqi T2DM patients who were diagnosed using the American. Diabetes. Association's. diagnostic. criteria participated in a cross-sectional study at The Diabetes and Endocrinology Unite/ Baghdad Teaching Hospital - Medical City in Baghdad, between March 15th, 2024 to August 22th, 2024. The research protocol was received approved from the College. of. Pharmacy Scientific. and. Ethics. Committee, University of Baghdad (RECAUBCP3112024) on Jan 30, 2024. Moreover, Informed consent was obtained in writing from each participant. Initially, the study enrolled 98 patients with T2DM. However, only 90 (35 male; 55 female) patients who matched the requirement of the study, after excluding eight patients (three patients because of non-valid samples and five patients because of insufficient patient information).

Data Collection

Researchers conducted interviews with all participants and collected demographic data, which were documented on a data collection sheet, encompassing gender, age, disease duration, treatment duration, waist circumference, body weight, and height.

Inclusion and Exclusion Criteria: The study employed specific inclusion criteria for participant selection, including patients should be between the ages of 40 and 65 years, patients were selected to be treated with sitagliptin (DPP-4i) in combination with metformin 50 mg/1000 mg taken once daily for at least 3 months and up to 12 months duration, duration of disease (type 2 diabetes) less than 10 years, absolutely no changes to any antidiabetic medications have occurred in the past 3 months, patients must adhere to the treatment completely (the assessment of adherence by the four item Morisky.Medication.Adherence.Scale(MMAS-4). While exclusion criteria patients were, including the patients on insulin therapy, who have HbA1c $\geq$ 13, with iron-deficiency anaemia, acute haemolysis or received blood transfusion, chronic (heart, liver, renal) disease, malignancies, or autoimmune disease, who have diabetes secondary to underlying endocrine diseases (such as cushing syndrome, acromegaly, pheochromocytoma).

Groups of Patients and Clinical Assessment

The participants would be categorized into two groups based on their responses, including (45 patients; 18 male and 27 female) who responded clinically and (45 patients; 17 male and 28 female) who failed to respond to the treatment. The response was assessed based on HbA1c level after 3 months of treatment, but not more than 12 month of continuous treatment with sitagliptin plus metformin. According to many studies that determined the diabetic patients who have an HbA1c value $\leq$ 7.0% were considered to have a good treatment response, whereas those who have HbA1c values > 7.0% were considered to have a poor treatment response[24] . Therefore, depending on these studies, we classified patients with HbA1c $\leq$ 7.0% clinical responders, while those with HbA1c > 7.0% non-responders (Figure 1).

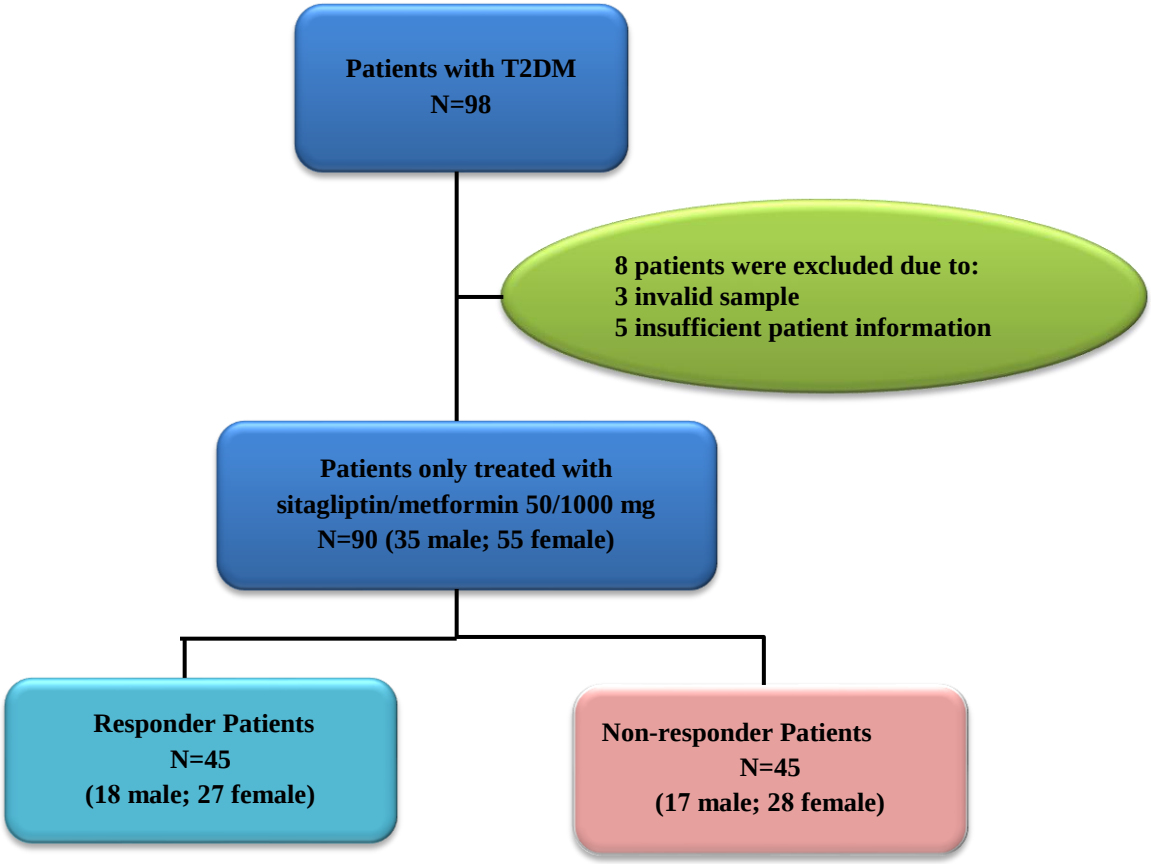

Figure 1: Distribution of Subjects Participated in the Study

Sample collection and preparation:
Sample of eight milliliters (mL) of venous blood were collected from each participant via venipuncture following a minimum fasting period of eight hours. Transfer five mL of whole blood into a gel tube; leave it for about half an hour to clot. Then, centrifuging at 3000 rpm at room temperature for 10 minutes to extract the serum. On the same day of collection, the hospital's laboratory utilized a portion of the serum to measure the fasting serum glucose (ELItech, France, Catalogue No: GPSL0250), lipid profile (ELItech, France, Catalogue No:CHSL0250&TGML0250), urea (ELItech, France, Catalogue No:URSL0250), creatinine (ELItech, France, Catalogue No:CRSL) levels by an enzymatic colorimetric technique. Aliquoting the residual serum into 0.5 ml Eppendorf tubes, it was stored at -20ºC till the assay time for insulin (DRG Instruments GmbH, Ltd.Germany, Cat.No: YHB2553Hu) and GLP-1 (BT LAB, China, Cat.No: E0022Hu) levels using ELISA kits. One milliliter of the whole blood was shipped to (EDTA) tube for analysis of HbA1c at the same center. For DNA extraction, another one milliliter of the whole blood was shipped to separate (EDTA) tube and stored at temperature ($^{+}2$- $^{+}8$) ºC until the extraction process.

DNA Extraction
The Promega. ReliaPrepTM. Blood. gDNA. Miniprep. System facilitates genomic DNA extraction from peripheral white blood cells (WBCs) utilizing EDTA blood samples which stored and the Easy Pure Blood Genomic DNA Kit (TransGen Biotech, China, Catalogue No: EE121) offering an efficient method for DNA purification.

The Primer

The primer was designed utilizing the. Primer. 3. plus,.V4, and double checked by the University Code of Student Conduct (UCSC) programs, and with their reference sequences in the National Center for Biotechnology Information (NCBI) database (Table I). Synthesis and lyophilization were conducted by Alpha DNA Ltd. (Canada)

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Table I: The Primer Designed for DPP4 SNP (rs6741949)

Primer	Sequence (5′→3′ direction)	Size of primer bp	Size of Product bp	Ta °C
DPP4 (SNP Genotyping) rs6741949				
Forward	CTCAGCTGCCACTCTAGCAT	20	67	58
Reverse	TGCAGAATTAGCACCACATGA	21		

bp: base pair; Ta: annealing temperature.

Primer Preparation:

Preparation of the required primer, as specified in (Table I), is as follows: Following the dissolution of the lyophilized sample in nuclease-free water according to the manufacturer's guidelines, a stock solution was generated with a concentration of 100 pmol/µL and stored at -20°C. Subsequently, 10 µL of the primer stock solution was diluted in 90 µL of nuclease-free water, yielding a working solution with a concentration of 10 pmol/µL, which was stored at -20°C until required.

Primer Optimization

By amplifying the DNA template using the same primer pair (Forward and Reverse) at annealing temperatures of 55, 58, 60, 63, and 65°C, the optimum annealing temperature (Ta) of the primer was determined. The optimum (Ta) was 58°C for the primer to produce clear and sharp band; hence, it was used in the current study.

High-Resolution Melting (HRM) Analysis

HRM was chosen for DPP4 SNP detections (rs6741949), the DPP4 genetic variations were used to investigate their relationships with the sitagliptin response in Iraqi type 2 diabetic patients.

HRM Real Time PCR runs:

HRM is a post-PCR technique employed for the identification of polymorphisms, mutations and epigenetic variations in samples that contain of DNA. HRM is performed following real-time PCR amplification using fluorescent DNA-binding dyes such as Eva Green or SYBR Green. After amplification, the temperature is gradually increased, and the melting behavior of the PCR products is monitored in real-time based on fluorescence changes. Different DNA sequences exhibit distinct melting curves due to variations in their nucleotide composition, length, and GC content. This technique is highly sensitive, cost-effective, and does not require post-PCR processing, making it suitable for rapid genotyping and mutation screening [25].

A Rotor-Gene Q Real-Time PCR System (QIAGEN, Germany) was utilized to conduct qPCR-HRM, succeeded by high-resolution melting analyses with a temperature rose from 55°C to 95°C by 0.2°C.The reaction was performed using 2× TransStart® Tip Green qPCR SuperMix (TransGen Biotech, China) (Table II). Synthetic DNA sequences including SNPs were assessed in duplicates. Allelic variants were identified by qPCR-HRM utilizing duplicate synthetic controls and both normalized melting curves (NMCs) and difference curves (DCs) were generated utilizing the integrated HRM tool in Rotor-Gene Q

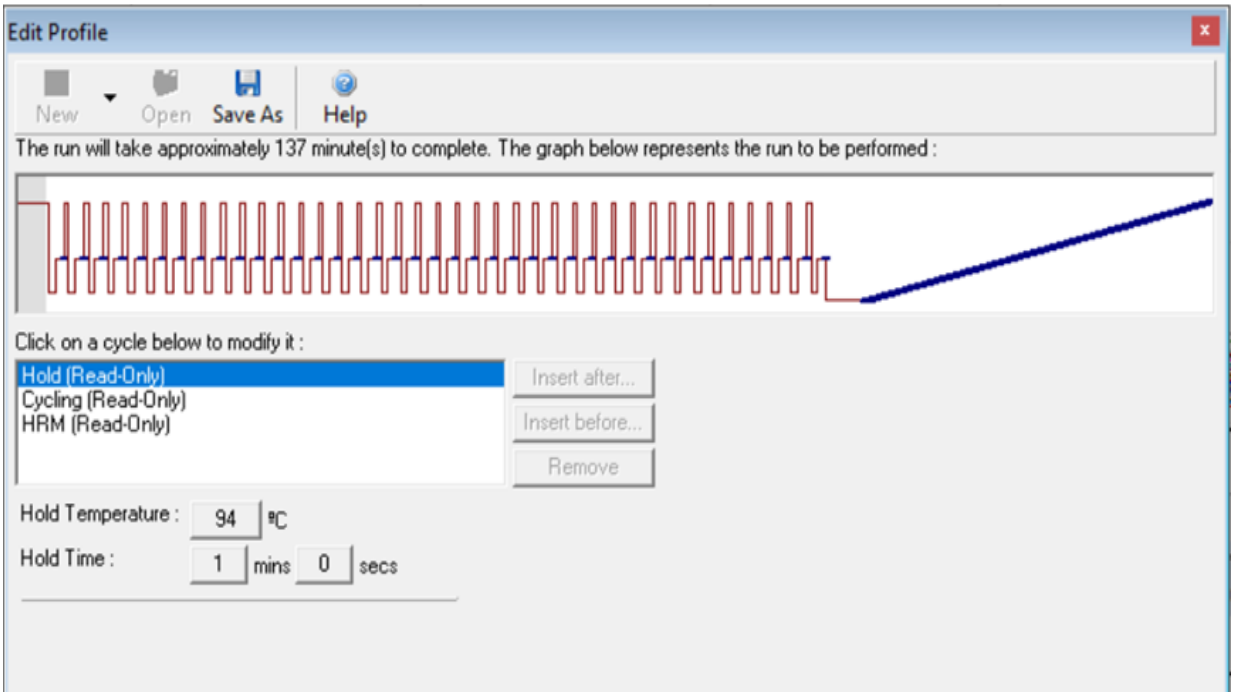
software version 4.4. The cycling protocol was set up for the following optimal cycles based on the thermal profile, as shown in table III & figure 2:

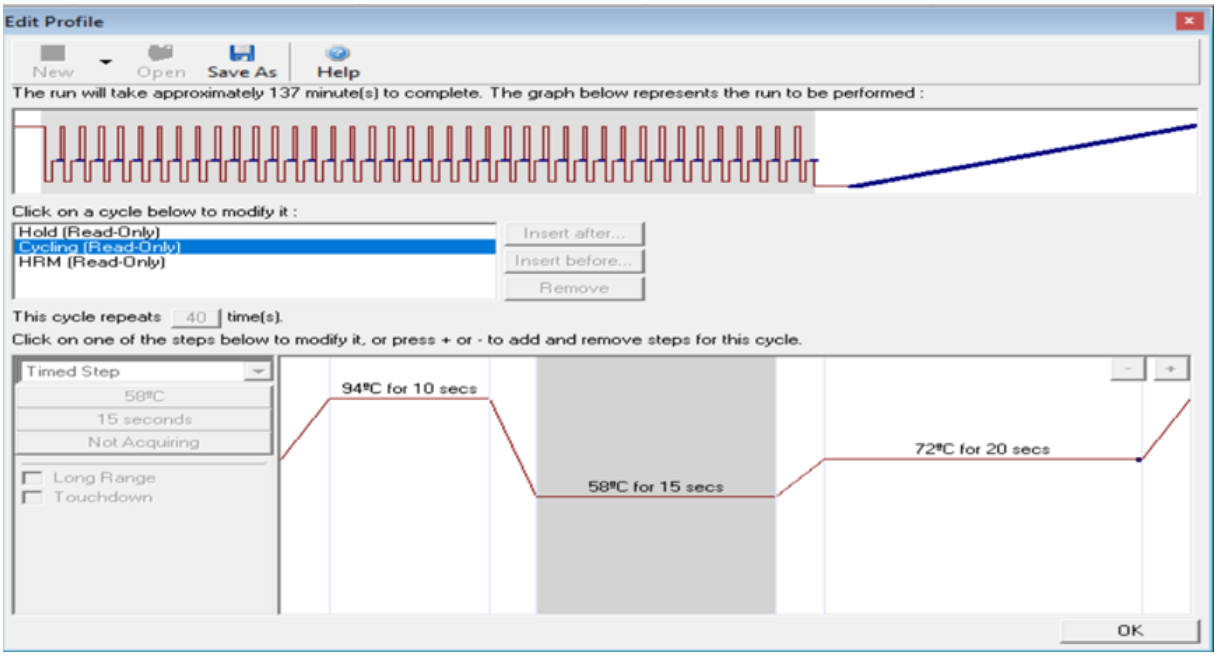
Table II: Components of the Quantitative HRM Real-Time PCR

components	20 µl rxn
Forward Primer (10 µM)	1
Reverse Primer (10 µM)	1
DNA	4
Nuclease free water	4
2xTransStart® Tip Green qPCR Super Mix	10

Table III: The HRM Thermal Profile

Step	Temperature (°C)	Time (sec.)	Cycles
Enzyme activation	94	30	1
Denaturation	94	5	40
Annealing	60	15	
Extension	72	20	
HRM	55-95	0.2sec for 1 degree	





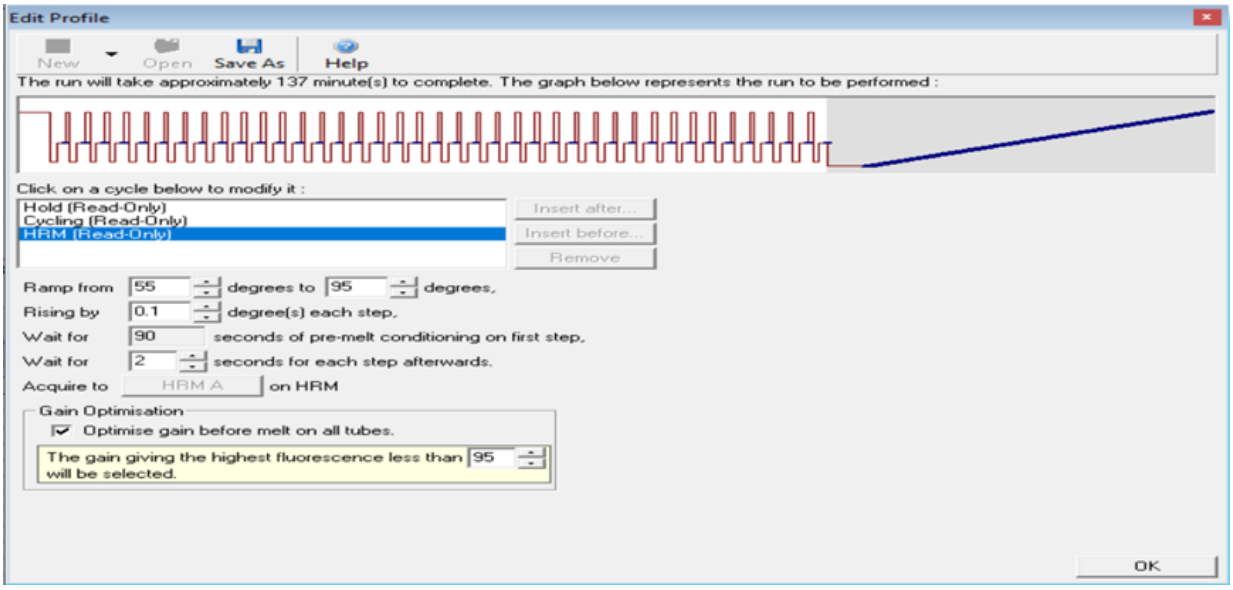


Figure 2: Thermal Profile Used for HRM Genotyping. The Images were directly taken from the qPCR Machine

Statistical Analysis:

The analysis was performed using SPSS for Windows, version 29 (SPSS Inc., Chicago, Illinois, United States). Examining whether the study parameters followed a Gaussian distribution was done using the Shapiro-Wilk normality test.

The mean ± standard deviation (SD) is used to express the results.

T-test and One-way ANOVA were employed to significantly compare means. The Hardy-Weinberg equilibrium (HWE) was employed to examine frequency of alleles and genotypes. The results were computed using a web-based tool. The frequency of alleles and genotypes in the responders and non-responders groups was compared using the Chi-square test. The strength of the association between the

investigated gene (DPP4) SNPs and the treatment response was evaluated by calculating odds ratios (ORs) with a 95% confidence interval (CI).

The SPSS software and WINPEPI were used to identify the genotypes. For all tests, a P-value of less than 0.05 was considered significant.

Results and Discussion:

Demographic and Baseline Clinical Characteristics

Table IV illustrates the demographic and clinical data of the participants, where the responder group in the current study was matched with the non-responder group regarding the gender, age, BMI, serum GLP-1, serum urea levels and GFR as these parameters did not differ significantly. Whereas the disease & treatment duration, waist circumference, HbA1c, FBS, HOMA-IR, serum insulin, serum creatinine, serum triglyceride and serum total cholesterol levels mean values differ significantly between the groups.

In type 2 diabetes, gender has been shown to affect treatment outcomes. For example, women who had never taken insulin before starting a basal insulin regimen demonstrated a lower improvement in HbA1c, which was linked to a higher incidence of hypoglycemia than men[26]. A meta-analysis by Guh et al. indicates that the incidence rate ratios of diabetes are elevated in females compared to males [27]. Unlike our study, the distribution of gender was comparable across both groups, with females outnumbering males in each. This suggests that gender did not influence the treatment response.

Concerning BMI, the values were comparable between the two groups. In two Japanese studies, a BMI was identified as an independent predictor of a poor response to DPP-4 inhibitor [28]. Lim et al. [29] did not indicate any association between them either. As in our study, no significant correlation was detected between BMI and responsiveness to DPP-4 inhibitors

The two groups differ significantly in terms of disease and therapy duration, this implies that the response to treatment improves as the disease duration decreases, while it also enhances with an increase in therapy duration.

Furthermore, regarding the waist circumference, its values were higher the group of non-responders because of increment insulin level (High levels of insulin promote fat storage, especially in the abdominal region) led to an increase in waist circumference. In general, the prevalence of obesity in Iraqi community is substantial and necessitates urgent attention from health officials and public health experts to devise effective preventative measures to avert severe health complications [30].

The glycemic parameters of included patients, HbA1c, FBS, serum insulin level and HOMA-IR, shows there is significant difference (p value 0.001 for all glycemic the parameters except for serum insulin level p value 0.023) between responder and non-responders groups, Our findings indicate that marker of elevated insulin resistance are consistently linked to diminished glycaemic response to DPP-4 inhibitor treatment, Consistent with research indicating that T2DM patients exhibit significant insulin resistance while potentially maintaining normal or elevated insulin levels, the increased serum glucose in these individuals could result in further elevated levels of insulin, assuming the normal functionality of their β-cells [31].

The concentration of serum creatinine is a straightforward, cost-effective, and often utilized measure for assessing renal function and serves as a risk indicator for diabetes [32]. A cross-sectional study was undertaken involving 100 patients with impaired glucose tolerance at the Amin Hayat Diabetic Centre in Lahore, Pakistan. The results of this study showed HbA1c had a significant association with creatinine, but not with blood urea and uric acid in prediabetes [33]. Our findings are in accordance with the former study in Pakistan [33], which reported no significant difference between the groups in serum urea level and eGFR whereas serum creatinine level does. Despite these results, we noticed higher urea and

creatinine levels and lower eGFR values in the non-responders than in the responders. So, when HbA1c increase, serum creatinine level increase

A clinical trial identified T2DM patients who were non-responders to sitagliptin treatment; the fasting active form of GLP-1 is linked to lowering HbA1c and is independent of the previously identified factors associated with non-responders, such as high body mass index or low baseline HbA1c [34]. In our study, we found no significant association in fasting serum levels of GLP-1 between the groups, but the levels of GLP-1 were slightly higher in the responders. This indicates GLP-1 is not related to HbA1c level. Thus, our findings do not agree with those of a previous study [34], in which fasting serum GLP-1 is not considered a predictive marker for the efficacy of Sitagliptin.

Some studies indicated the impact of DPP-4i therapy affects lipid metrics [35][36][29][37] and identify a relationship between DPP-4i therapy and diminished total cholesterol levels. Another study was conduct to compare the impact of vildagliptin and sitagliptin on metabolic markers. The effectiveness of these medications was compared directly using various lipid and glucose metrics across a 24-week therapy period. No significant alterations in lipid indices were observed after sitagliptin administration [38]. Whereas our findings reported a significant effect in the serum triglyceride and total cholesterol levels between the groups.

Table IV: Parameters of Demographic and Clinical Characteristics

Criterion		Responders(n=45)		Non-Responders(n=45)		p-value
		Mean	SD	Mean	SD	
Gender, n(%)	Male	17(37.8%)	-	18(40%)	-	0.8 ns
	Female	28(62.2%)	-	27(60%)	-	
Age (years)		56.57	7.64	55.57	8.40	0.5 ns
BMI (kg/m^2)		28.49	4.75	29.85	5.18	0.2 ns
Duration of disease (years)		4.71	3.66	6.37	3.81	0.03*
Duration of treatment (months)		6.98	3.78	5.40	3.55	0.04*
Waist Circumference (cm)		95.17	12.55	100.89	13.42	0.03*
HbA1c		6.08	0.69	9.52	1.52	0.001**
FBS mg/dl		124.8	21.87	215.55	80.79	0.001**
S. insulin (mIU/ml)		21.41	8.94	28.07	7.19	0.023*
HOMA-IR		6.67	2.05	15.93	5.65	0.001**
S. GLP-1 (Pmole/L)		27.01	6.79	26.21	7.06	0.6 ns
Urea mg/dl		37.68	8.86	41.34	10.71	0.1 ns
Creatinine mg/dl		0.69	0.13	0.79	0.25	0.01*
GFR		110.63	12.12	110.67	11.14	0.9 ns
TG mg/dl		160.8	47.55	278.33	90.24	0.01*

TC mg/dl	184.5	41.53	229.91	71.82	**0.01***

Data were expressed as mean ± SD; Statistical analyses were performed by T-test. SD: standard deviation, BMI: body mass index, FBS: fasting blood sugar, HOMA-IR: Homeostatic Model Assessment for Insulin Resistance, GLP-1: Glucagon-like peptide-1, GFR: glomerular filtration rate, TG: Triglyceride, TC: Total cholesterol, ns: no significant difference, **significant at the 0.01 level (2-tailed), * significant at the 0.05 level (2-tailed).

Receiver Operating Characteristic Curve (ROC) for GLP-1

ROC curve is utilized to determine the diagnostic accuracy of the involved biomarker (GLP-1) by identifying specificity and sensitivity to specify the appropriate cut-off value for a biomarker (GLP-1). A promising biomarker or indicator is indicated by a higher area under the ROC curve (AUC) score, while a low score indicates a poor indicator. The optimum diagnostic cut-off for GLP-1 is recorded in (Table V) and (Figure 2). The ROC curve assessed the functional significance of the above biomarker in different studied groups and evaluated its validity as a marker for diabetic patients.

According to table V data (AUC was 0.52, a p-value 0.71), indicating that GLP-1 has very poor discriminatory power and is not statistically significant as a predictor of treatment response. Additionally, the sensitivity (66%) and specificity (48%) were both relatively low, suggesting limited clinical usefulness. Consequently, our research indicates that GLP-1 does not significantly influence the response to treatment, and it cannot be considered a reliable biomarker for predicting responders versus non-responders in this context.

Table V: Receiver Operating Characteristic Curve Data for GLP-1

AUC	Standard Error	Asymptotic Significant	Asymptotic 95% Confidence Interval		The best Cut off	Sensitivity %	Specificity %
			Lower Bound	Upper Bound			
.52	.06	.71	.40	.64	22.92	66	48

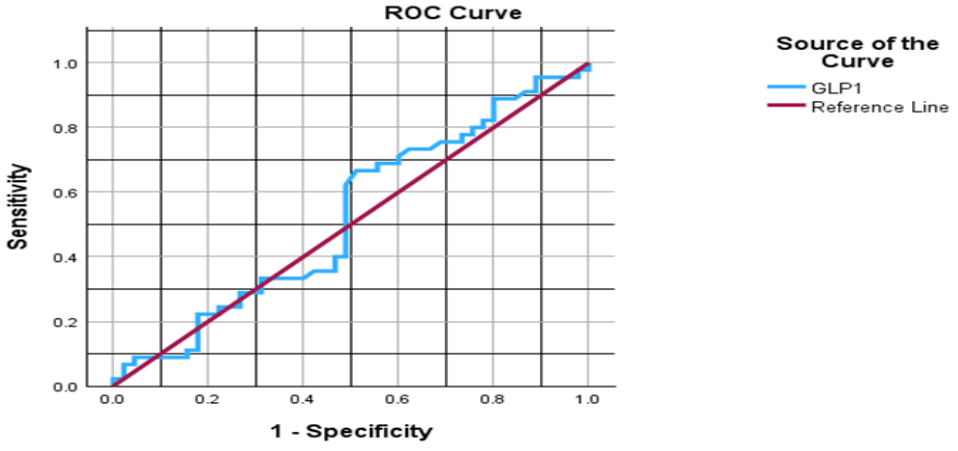


Figure 2: Receiver Operating Characteristic Curve for GLP-1

Correlations of the Included Parameters of Whole Participants:

(Table VI) shows a significant positive correlation between the serum GLP-1 level with both HOMA-IR and the serum insulin level, while there is no significant correlation was observed with HbA1c. A

significant positive correlation was observed between HOMA-IR and serum insulin levels. Also, HbA1c levels were found to have a significantly positive correlation with both HOMA-IR and serum insulin level.

Table VI: Pearson's Correlation Analysis of the Glycemic Parameters of the Whole Participants

parameters		GLP-1	HOMA-1R	Insulin
GLP-1	r	1	.288**	.340**
	p		.006	.001
HbA1c	r	-.038	.492**	.206*
	p	.725	<.001	.049
Insulin	r		.813**	1
	p		<.001	

HOMA-IR: Homeostatic Model Assessment for Insulin Resistance, GLP-1: Glucagon-like peptide-1, ** Significant at the 0.01 level (2-tailed).*significant at the 0.05 level (2-tailed)

DPP4 gene polymorphism rs6741949

DPP4 gene was selected to investigate their association with the Sitagliptin response Iraqi T2DM patients. The resulting output of qPCR machine (Rotor-Gene® Q) of the analysis process for SNP (rs6741949) of DPP4 gene by HRM-qPCR is shown in the figure 4 & 5.

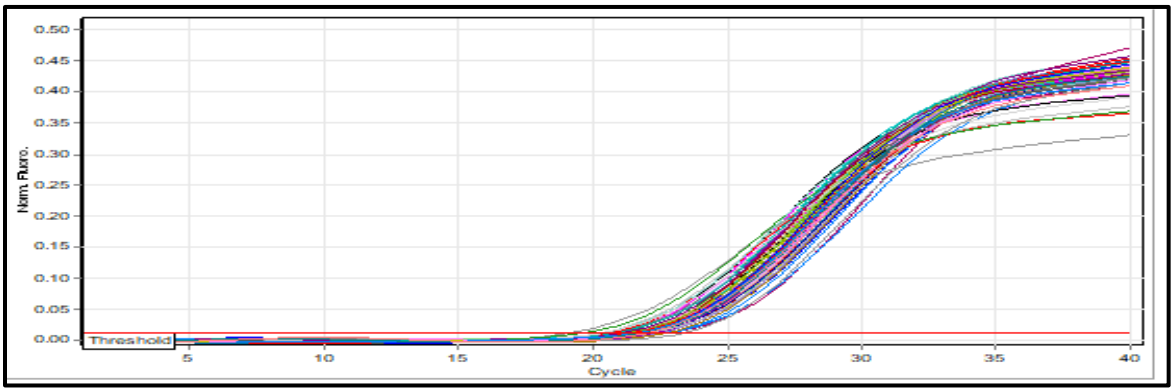


Figure 4:The Amplification Plot of DPP4 Gene in All Patients

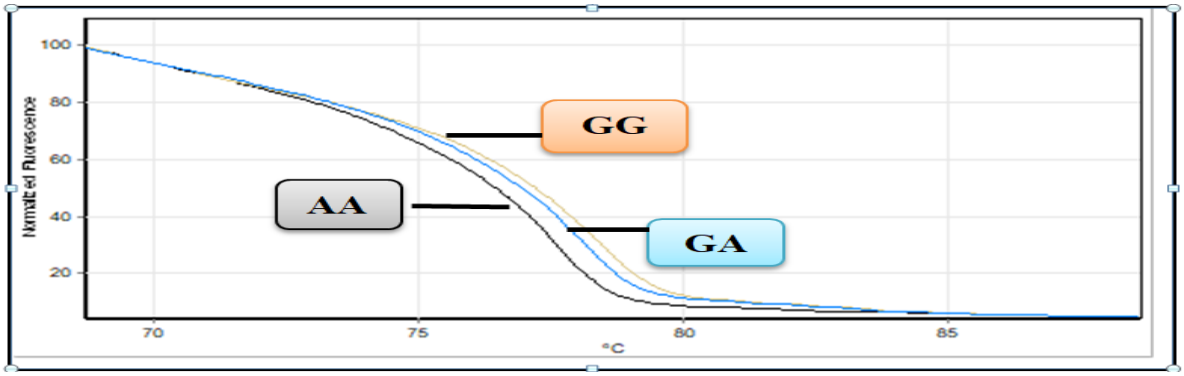


Figure 5: The Result of HRM-qPCR for the Genotypes (GG Wild, GA Heterozygous and AA Mutant) of SNP (rs6741949) of DPP4 Gene

Analysis of Hardy-Weinberg equilibrium (HWE) in the responders group revealed a significant deviation from equilibrium, with a significant effect observed between the observed and expected frequencies of genotypes. In contrast, the genotypes distribution of the non-responder group was consistent with HWE, revealing no significant difference between the frequencies of observed and expected in blood samples (Table VII).
Table VIII presents the analysis of genotype and allele frequency distributions for the rs6741949 SNP among the responder and non-responder groups. The allele and genotype of the wild type have been utilized as a reference.

Out of 45 responders group, 19 were homozygous (GG) giving a total frequency of 42.2%, 26 were heterozygous (GA) with a frequency of 57.8% and homozygous mutant (AA) were absent in this group. While 45 non-responders group, 16 were homozygous (GG) giving a total frequency of 35.6%, 26 were heterozygous (GA) with a frequency of 57.8% and 3 were homozygous mutant (AA) with a frequency of 6.7%. Homozygous (GG) was used as a reference. There were no significant variations in the frequencies of homozygous (AA) and heterozygous (GA) genotypes between the groups. With respect to allele's frequencies, the G allele was present in 64 individuals (71%) in the responders group and 58 individuals (64.4%) in the non-responders group, and was used as the reference. The A allele was observed in 26 individuals (29%) among responders and 32 individuals (35.6%) among non-responders, indicating did not significantly differ in alleles frequencies between the groups. These findings imply that neither the GA and AA genotypes nor the A allele of the DPP4 Rs6741949 SNP are significantly associated with treatment response, indicating a lack of predictive or contributory role in therapeutic outcomes.

Table VII: Genotypes Frequencies of rs6741949 SNP in dpp4 Gene according to Hardy-Weinberg Equilibrium (HWE) of the Responder and Non-responder Groups

SNP rs6741949	**Responders N=45**		**Non-responders N=45**	
	Observed	**Expected**	**Observed**	**Expected**
GG	19	22.76	16	18.69
GA	26	18.49	26	20.62
AA	0	3.76	3	5.69
Total	45	45	45	45
p-value	**0.006****		**0.08**	

Statistical analyses performed by the Chi-squared test. N: number, ** highly significant at the 0.01 level (2-tailed)

Table VIII: Comparison of Genotypes and Alleles Frequencies of rs6741949 SNP in dpp4 Gene between Responder and Non-responder Groups

Genotype rs6741949	Responders n=45	Non- responders n=45	P-value	OR	CI 95%
GG	19 (42.2%)	16 (35.6%)	--	1.00	(Reference)
GA	26 (57.8%)	26 (57.8%)	0.6	0.8	0.36 to 1.99

AA	0 (0%)	3 (6.7%)	0.2	0.1	0.01 to 2.51
Total	45	45	--	--	--
Allele Frequency					
G	64 (71%)	58 (64.4%)	--	1.00	(Reference)
A	26 (29%)	32 (35.6%)	0.3	0.7	0.39 to 1.38

Statistical analyses were performed by chi-square test, OR: odd ratio, CI 95%: confidence interval, n: number

Effects of Alleles of rs6741949 Genotypes on the on the Studied Parameters for All Participants:
In the (Table Ⅸ) shows the effect of alleles of rs6741949 genotypes on all parameters of the total participants (n=90), there is a significant effect with the serum insulin level and the serum total cholesterol level (p value 0.02 for both) while a serum GLP-1 level and other parameters were not affected. Suggesting the highest value of serum insulin and total cholesterol levels were in the AA allele carriers while the lowest value were in the GG allele carriers.

Table Ⅸ: Association of rs6741949 SNP Genotypes with All Parameters

Parameters		**rs6741949 A/G**						
		GG (35)		**GA (52)**		**AA (3)**		**p-value**
		Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	
GLP-1		25.35	9.15	27.45	9.60	26.88	10.12	**0.6**
Insulin		24.19 **b**	14.79	24.39 **b**	11.91	45.93 **a**	29.98	**0.02***
Age		56.08	7.51	56.04	8.59	56.66	2.88	0.9
BMI		28.44	5.44	29.61	4.80	29.99	1.07	0.5
HbA1c		7.65	2.12	7.83	2.11	8.96	1.11	0.5
FBS		169.54	80.95	169.34	72.29	192.00	36.37	0.9
Urea		36.96	9.20	40.67	12.91	48.69	7.08	0.1
Creatinine		0.72	0.14	0.76	0.23	0.67	0.11	0.5
GFR		112.32	12.52	109.90	11.11	104.00	2.00	0.4
TG		198.90	92.33	235.96	133.31	238.33	78.17	0.2
TC		185.82 **b**	56.37	189.63 **b**	63.74	222.00 **a**	47.75	**0.02***
Gender	**Male,n=35**	16		18		1		0.6
	Female,n=55	19		34		2		

Data were expressed as mean ± SD; Statistical analyses were performed by ANOVA. ANOVA significance test (2-tailed). SD: standard deviation, GLP-1:BMI: body mass index, FBS: fasting blood

sugar, HOMA-IR: Homeostatic Model Assessment for Insulin Resistance, GLP-1: Glucagon-like peptide-1, GFR: glomerular filtration rate, n: number, TG: Triglyceride, TC: Total cholesterol, significant values are bolded. a and b: Different letters mean there is significant difference. Means followed by the same letter are not significantly different. ** Significant at the 0.01 level (2-tailed).*significant at the 0.05 level (2-tailed).

Discussion:

Type 2 diabetes mellitus (T2DM) results from a complex interaction of genetic and environmental variables, with common genetic differences associated with glucose metabolism disorders and the pathogenesis of T2DM[22]. There are several genetic differences that can lead to the development of diabetes complications, especially single nucleotide polymorphisms (SNPs). Variants in the induction of nucleotide substitution at particular gene locations are linked to differences in disease susceptibility [39]. In this context, interest variation is present also in the DPP4 gene, which have a pivotal role in glucose homeostasis; however, its relationship with T2DM and glycemic traits remains ambiguous[40]. Moreover, DPP4, GLP1R, KCNQ1, KCNJ11, CDKAL1, and other genes have variations that change the responsive of individuals with T2DM to DPP-4i [41]. The substrate for DPP-4 inhibitors is DPP-4, which binds to it thus preventing GLP-1 inactivation[42].

The approval of DPP-4i for the treatment of T2DM markedly increased the role of DPP-4 for the medical and scientific communities. The DPP-4i or so-called gliptins elevate incretin levels, thereby extending the duration of postprandial insulin activity. However, interindividual difference in the response to DPP-4i was reported [43]. Hence, we enquired if genetic difference in the DPP4 gene influences GLP-1 levels and insulin secretion in individuals T2DM in Iraq.

Dipeptidyl peptidase 4 (DPP-4, also known as CD26) is a ubiquitous serine protease existing in two isoforms: a membrane-bound protein produced on the surface of several cell types and a soluble version present in most bodily fluids [44]. DPP-4 has an essential effect on homeostasis of glucose via rapidly inactivating GLP-1 and GIP, which collectively contribute to almost 50% of postprandial insulin production [45]. Consequently, the activity and expression levels of DPP-4 have been altered in conditions like T2DM [46], obesity [47], and non-alcoholic fatty liver disease [48], all analyses of DPP-4 should consider the contextual factors involved. In human, the DPP4 gene, situated on chromosome 2q24.2 and comprising twenty eight exons, encompasses nearly thirty five thousands polymorphic loci, some of DPP4 gene are linked to clinical disorders like T2DM [20], myocardial infarction [49], COVID-19 and dyslipidemia [50].

Few studies have been performed to identify whether changes in the DPP4 gene are linked to T2DM. The following section reviews some of these. In 2009, Bouchard et al. [51] examined the DPP4 gene's single nucleotide polymorphisms (SNPs), investigating their correlation with lipid levels, blood pressure, and diabetes in obese patients. However, no significant association was identified between the SNPs or cardiovascular disease risk factors and DPP4 levels in omental-derived adipose tissue.

Aghili et al. examined 875 patients with coronary artery disease (CAD) that had been angiographically documented [49] and categorized based on whether they had experienced myocardial infarction (MI) into two subgroups. Genetic loci that predispose individuals to myocardial infarction were evaluated and linked to the DPP4 gene's SNPs through a genome-wide association study. Notably, a one SNP was detected in both additive inheritance and dominant patterns, correlating with potentially elevating the risk of MI in coronary artery disease patients and reduced plasma DPP-4 levels.

A different study revealed that SNP rs6741949, a common mutation in the DPP4 gene, links with body fat and has a negative impact on secretion of insulin, glucose tolerance, and glucose-stimulated GLP-1 levels. It remains to be demonstrated if this SNP accounts for the observed interindividual variability in response to DPP-4i, particularly in individuals with elevated fat content in the body. Additionally, it showed that an association impact of SNP rs6741949 with the increase in GLP-1 induced by oral glucose (p-value

0.0021). Noticeably, there was no association impact of SNP rs6471949 on elevation of the GLP-1 was observed when body mass index was utilized rather than percent of body fat (p-value 0.8) [52].

Our findings were thought to be in line with a prior study [52] that demonstrated the DPP4 rs6741949 polymorphism interacts with body adiposity and insulin secretion which presented with a significant effect of the rs6741949 SNP on cholesterol and insulin levels but varies with respect to glucose-stimulated GLP-1 levels. Also in our results show no significant effect between rs6741949 SNP and serum GLP-1 level (p value 0.6) among different genotypes (GG, GA, and AA) for the whole participants.

Recently, a study conducted to investigate the haplotype interaction formed by two SNPs, rs6741949 & rs2268894, of the DPP4 gene (non-coding regions) with type 2 diabetes and fasting glycemic-related parameters in a specimen of older individuals in Brazil, accounting for DPP-4 serum levels and activity.

In the analysis of an entire group using a recessive model, and when each genotype was analyzed independently, T2DM was less prevalent among homozygotes for the alleles of G of rs6741949 and T of rs2268894. Indicating that glycemic parameters are significantly unevenly distributed between the two SNPs in individuals with T2DM, being less common in individuals with the GG of rs6741949 and TT of rs2268894 genotypes carriers, while overall glycemic levels were elevated in the another genotypes carriers. The findings demonstrated that the context-sensitivity of the haplotypes of DPP4 affected on glycemic levels [53].

In our study, the sitagliptin response was not found to be associated with the genotypes of rs6741949 of the DPP4 gene between the responder and non-responder groups. in whole participants, patients with T2DM were less frequent among homozygotes mutant (AA) of rs6741949, with only three patients (6.7%), despite this small number, we noticed the highest values of insulin and cholesterol concentrations in the homozygotes mutant (AA), and there are significant differences (Table IX), but not with GLP-1. The rs6741949 (G>A) SNP had a non-significant impact on serum level of GLP-1. The serum GLP-1 level was not statistically significant between responders and non-responders. ROC analysis indicates that GLP-1 levels alone are not a strong predictor of treatment response to Sitagliptin. The low AUC (0.52) and non-significant p-value (0.71) indicate that GLP-1 is not effective in distinguishing responders from non-responders in T2DM patients on Sitagliptin.

Conclusions

In conclusion, regarding the results of genetic polymorphism of DPP4, this study's findings demonstrated that rs6741949 (G>A) of gene DPP4 was present in studied groups. The frequencies of this SNP included wild and hetero genotypes and allele's carriage did not differ significantly between the groups. This indicates that these genotypes did not possess a risk on the response to sitagliptin treatment. In the corresponding pharmacogenetic studies, this gene variation was found to have no effect on clinical decisions involving the use of sitagliptin in combination with metformin therapy for T2DM. Inevitably, no published research has looked into how this mutation affects the response of sitagliptin in T2DM patients. Additionally, this SNP showed no impact on glucose-stimulated GLP-1 level but had significant effect with the serum insulin and cholesterol levels, which highlights its potential as a genetic indicator for impaired insulin sensitivity and dyslipidemia in T2DM patients. This finding underscores the importance of incorporating genetic profiling into the evaluation of diabetic patients, as it may provide insights into patient-specific risks and therapeutic strategies.

Among the many limitations of this study, it should be noted that capacity restrictions prevented the study from providing enough time for follow-up between the diabetic patients groups before and after treatment. Moreover, a main limitation was the limited sample size. The restricted participant pool may diminish the generalizability of the results, complicating the application of the findings to the entire Iraqi population.

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