



Association Between the Hematopoietically Expressed Homeobox (HHEX) Gene (Rs1111875 AND Rs7923837) Polymorphism and Type 2 Diabetes Mellitus in Iraqi Patients

¹Israa T. Hassan , ²Basima Q. Hasan, ³Alaa T. Hassan

¹Department of biology ,college of science, Al-Ameen University .

²Institute of Genetic Engineering and Biotechnology for Postgraduate studies, University of Baghdad.

³Ministry of Health, Baghdad Health Directorate - Al-Karkh, Baghdad, Iraq.

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Abstract : This study was carried out in Baghdad Training Hospital Hereditary Research Laboratories in Baghdad, through the time from the first of March to the end of the first day of December 2022. The aim of this study is to investigate the relationship between rs1111875 and rs7923837 polymorphisms in the HHEX gene in patients with Iraqi type 2 DM. The total number of patients is 120 Iraqi individuals, including 80 patients suffering from T2DM, 40 visibly healthy samples, and the age range of these people (25-75). significant changes were noticed in high FBS level, and HbA1C level ($p < 0.0001$). There is no significant difference in BMI. The findings found that the percentage of T2DM cases was found in the second age group, which reached 55% of the total patients, while the lowest percentage was observed in the first age group with 11.25%. The occurrence of DM was associated with family history and the effects of smoking. It was revealed that the sum of biochemical parameters Cholesterol, triglycerides, VLDL, were significant in patients, while serum HDL and LDL did not differ significantly in the control group compared to the patient group. The odds ratio C/T was more than one (rs 1111875) SNP polymorphism genotype was highly associated with the risk of developing diabetes in this studied sample.

Keywords: HHEX gene, rs1111875, rs7923837, Polymorphism, Type 2 DM, Iraqi patient.

Corresponding author: (Email: israa30i@yahoo.com)

Introduction

The diabetes mellitus, it is an ongoing the status of the metabolic which defined as a hyperglycemia that does not go away, it is possible that this is a decreased insulin production, resistance to the peripheral effects of insulin, or both of these factors working together. DM patients able sustain bad damage to organ of the systems as a result of glucose metabolism, which, in conjunction with other metabolic abnormalities, can lead to the

implementation of health complications that are both disabling and life-threatening. The microvascular and macrovascular consequences stand out because they are the most significant of these adverse effects, which most probably causes the CD that is anywhere from two to four times higher than it would be otherwise (3).

According to projections made by the IDF, there were about 415 million people worldwide (20 - 79 years old), who were coping with diabetes mellitus

in the year 2015. (25). T2DM or known as polygenic diabetes, is a disease that is passed down through generations due to environmental factors in addition to the genetic combination (17, 13). It has been a difficulty to find genetic variations that explain the increased the complecation of diabetes that is linked with this disease (family history). Only three mutations out of a broad range of potential genes always related to type 2 diabetes (11, 10, 6).

Many studies has been conducted in iraq including the association and relation between the occurrence and severity of DMtype2 in iraqi population such as (24), (21), (1), (19), (4), (21).

Many GWAS done on people of many different ethnicities, and the results of these research consistently point to the Hematopoietically expressed homeobox HHEX gene as a credible candidate for the T2DM risk (9).

The HHEX gene can be found on chromosome 10q23.33; it is comprised of 4 exons; and it takes up around 5.7 kb of space in the genome (18). No phenotype, other than type 2 diabetes and maybe decreased b-cell activity, has been established to be associated with SNPs in the HHEX gene area (16, 7, 23, 20).

Numerous studies have been conducted to determine whether or not two common variations (rs1111875 and rs7923837) found close to the HHEX gene are associated with a higher risk of developing type 2 diabetes (5).

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A signaling pathway that is involved in signaling and that is essential for the early implementation for both the liver in addition to the ventral pancreas are encoded by the HHEX gene (14). Some previous research on mutations in the HHEX gene area has not proven any phenotypes, with the exception of T2DM and maybe decreased beta-cell function (23, 20).

The reduced insulin production, diminished hepatic insulin breakdown and insulin sensitivity are early processes that play an essential role in the etiology of T2DM (22). Because of this, it was thought to be a potential risk gene for T2DM. Many mutations and SNPs frequent within or bordering the HHEX gene locus on chromosome 10q23.33 have been found. The HHEX gene locus is located on chromosome 10q23.33. The aim of the present study is to investigate the relation ship between the occurance of polymorphism in the HHEX gene with incidence and severity of DMtype2

Materials and Methods

This study was done in the Baghdad Teaching Hospital Laboratories in Baghdad Teaching Hospital in Baghdad throughout the period from the first march until the end of the first of December 2022. Two study groups were investigated. The total numbers of samples are 120 Iraqi individuals, including 80 patients with T2DM patients, 40 samples from apparently healthy control and there age was ranged from (25-70) years. Patients groups were selected from Baghdad Teaching Hospital as shown in figure 1

Experimental design

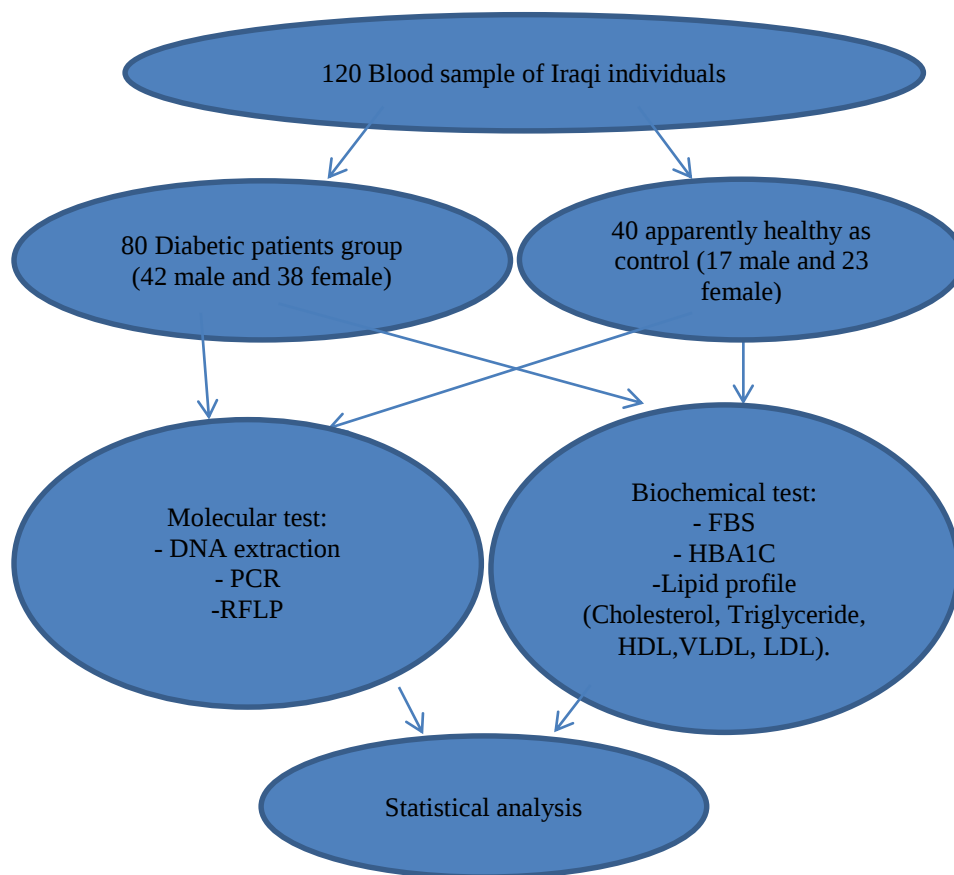


Figure (1): Study design

Methods

All participants were interviewed during their current medical workup to collect important information, including; age, family history, weight, height, duration of illness and Body mass index (BMI) and were measured Fasting blood glucose (FBG) using kits supplied by (Biotek, Spain) and Measurement of glycosylated hemoglobin (HbA1C) by (Nycocard, Norway).

The Triglycerides (TG), Total cholesterol (TC) and High-density lipoprotein cholesterol (HDL) were measured using kits supplied by (Spainreact, Spain)

The LDL concentration was measured according to as shown in Equation $LDL = Total\ cholesterol - (HDL + TG/5)$

The very low-density lipoprotein-cholesterol (VLDL) was measured according to as shown in Equation $VLDL\text{-Cholesterol (mg/dL)} = TG/5$.

Genotyping

Study Primers

The primers were using the Primer 3plus, V4, and double checked by the University Code of Student Conduct (UCSC) programs, and with their reference sequences in the NCBI database, they were synthesized and lyophilized by Alpha DNA Ltd. (Canada). Table1 displays all primer sequences utilized in this study's assays(5).

Table (1): The study's primers

Primer	Sequence (5'→3' direction)	Primer size bp	Product size bp	Ta °C
HHEX gene for rs7923837				
Forward	TGCTCACTGAACCTTGGCTA	20	222 bp	-20 °C
Reverse	TGGCTCTTGGCCTTCTTAAA	20		
HHEX gene for rs1111875				
Forward	CATCATAACTTCTCACTCCCTTCC	24	161 bp	-20 °C
Reverse	GCTGCTTATGGAACTGCATTACT	24		

Conventional PCR reaction

A partial sequence was chosen for this study to evaluate the *HHEX* gene's association with Diabetes in Iraqi Patients. To start the PCR, the reaction was tuned by testing four annealing temperatures: 56, 58, 60, and 62°C. The annealing temperature of 58 °C was the optimum for producing clear and sharp bands in agarose gel (2.0% agarose gel

stained with 10 µL of ethidium bromide hence it was used in the current study. This protocol employs 2xEasyTaq® PCR SuperMix. All PCR reactions were carried out in a 25 µL final volume and according to the manufacturer's instructions. The PCR program conditions were designed for this study as shown in Table 2 and 3.

Table(2): The reaction components of PCR of *HHX* gene (rs1111875 and rs7923837)

Component	Volume 25 µL
2xEasyTaq® PCR SuperMix	12.5 µL
Forward primer	1 µL
Reverse primer	1 µL
DNA	4 µL
Nuclease free water	6.5 µL

Table (3): The PCR program of *HHX* gene (rs1111875 and rs7923837)

Step	Temperature (°C)	Time	cycle
Denaturation	94	5 min	1
Denaturation	94	30 sec	35
annealing	58	30 sec	
Extension	72	30 sec	
Extension	72	5 min	1

PCR Restriction Fragment Length Polymorphisms (PCR-RFLP) for Genotyping *HHEX* Genes

Principle of RFLP

The basic technique for the detection of RFLP involves fragmenting a sample

of DNA by restriction enzymes. For this research, PCR was used to amplify specific flanked areas of single-nucleotide polymorphisms (SNPs) namely, rs1111875 and rs7932837 of *HHEX* gene followed by digestion by

two restriction enzymes, Xba I and HPY166II, in order to prepare the DNA for RFLP.

Restriction enzymes

1. Xba I (rs1111875) SNPs of HHXE gene

E. coli that harbors the recombinant XbaI gene is used to produce the *FlyCut® XbaI* enzyme, which is then purified. The molecule has a weight of 24.7 kDa, and the TCTAGA recognition site may be found in it. The reaction is carried out at 37 degrees Celsius for five to fifteen minutes, and then it is inactivated by heat for twenty minutes at 65 degrees Celsius. This enzyme is susceptible to methylation of dam but not dcm or mammalian CpG. However, it is not sensitive to methylation of dcm.

2. HPY166II (rs7932837) SNPs of *HHEX* gene

Thermo Scientific HPY166II restriction enzyme recognizes GTN↓NAC sites and cuts best at 37°C in buffer. The HPY166II is an enzyme isolated from an *Escherichia coli* strain that carries the cloned Hpy166II gene from *Helicobacter pylori* J166.

Recognition site

5' G T N↓N A C 3'
3' C A N↑N T G 5'

Statistical Analysis

Table (4): Comparison between patients and control groups in FBS and HbA1c

Group	Mean ± SE	
	FBS	HbA1c (%)
Patients	169.21 ± 9.08	8.20 ± 0.15
Control	80.67 ± 1.12	4.45 ± 0.06
T-test	25.384 **	0.449 **
P-value	0.0001	0.0001
** (P≤0.01).		

Distribution of T2DM Patients According to Age

The highest percentage of the T2DM cases was found in the second age group which reached to 55% of total

The software Statistical Analysis System- SAS (2018) was used for the purpose of determining the impact of various variables on the parameters of the research. To make a statistically significant comparison between means, we employed both the T-test and the Least significant difference –LSD test (Analysis of Variation-ANOVA). The Chi-Square test was used in order to make a statistically significant comparison between percentages (0.05 and 0.01 probability). This study's estimates for the odds ratio and the confidence interval.

Results and Discussion

Comparison between Patients and Control Groups in FBS and HbA1c
Comparison between Patients and Control Groups in FBS and HbA1c

These results observed Highly Significant differences (0.0001) in the patient group (169.21 ± 9.08) and in the control (80.67 ± 1.12), The results showed in that the mean average of glycated hemoglobin was significantly increased (P > 0.0001) from (4.45 ± 0.06) in serum specimen of apparently health controls group to (8.20 ± 0.15) in serum specimen of T2DM patients group (Table 4).

patients, while the lowest percentage was observed in the first age group which was 11.25% as shown in Table 5.

Table (5): Distribution of sample study according to age in patients and control groups

Age group (year)	Patients No. (%)	Control No. (%)	P-value
25-40	9 (11.25%)	14 (35.00%)	0.297 NS
41-55	44 (55.00%)	19 (47.50%)	0.0016 **
56-70	27 (33.75%)	7 (17.50%)	0.0006 **
P-value	0.0001 **	0.0498 *	---
* (P≤0.05), ** (P≤0.01).			

Distribution of T2DM Patients According to BMI

Throughout the course of the trial, individuals with T2DM as well as controls who seemed to be healthy had

their BMIs tested. Table 6 demonstrated that there was no discernible difference in BMI between patients with type II diabetes and controls who seemed to be in good health.

Table (6): Comparison between patients and control groups in age and BMI

Group	Mean ± SE
	BMI (kg/m ²)
Patients	27.89 ± 0.28
Control	28.33 ± 0.57
T-test	1.145 NS
P-value	0.4507

Distribution of T2 DM Patients According to Sex, Smoking, Family History, Diseases and Duration in Patients Group Regarding the issue of sex, the numbers of males and females in the patients group were 42 (52.50%) and 38 (47.50%) respectively, consequently, the males percentage was higher than the females percentage as shown in Table 7, which demonstrates that sex does not have a significant contribution on the incidence of T2DM among the 80 diabetic patients who were involved in the present study (P-value: 0.655).

Regarding smoking, there were sixteen patients who were smokers (20%) and sixty-four patients who were non-smokers (80%), and there were significant differences between the smoking and non-smoking groups in this study, as shown in Table 7. According to the most recent findings, there is a connection between diabetes and the habit of smoking in adults (P-value 0.0001). (2) disagrees with the

findings of the present study, which concluded that there was no significant connection between smoking and diabetes.

Table 7 shows the distribution of patients according to their family history of disease. It found that 15 (18.75%) of 80 patients have a positive family history of asthma, while 65 (81.25%) of patients have no family history of the disease. There were highly significant differences (p 0.0001) between type 2 diabetes individuals who had a family history of the disease and those who did not have a family history of the disease.

The findings, which are presented in Table 7, demonstrated that there are statistically significant differences between the levels of diseases found in serum samples taken from patients with type 2 diabetes (P-value 0.0001). On the other side, there were 6.370.41% of T2DM patients who experienced diabetes at different times during their lives (Duration).

Table (7): Distribution of sample study according to sex, smoking, family history and diseases in patients group (Total no = 80)

Factors		No	Percentage (%)	P-value
Sex	Male	42	52.50	0.655 NS
	Female	38	47.50	
Smoking	Smoker	16	20.00	0.0001 **
	No Smoker	64	80.00	
Family history	Yes	15	18.75	0.0001 **
	No	65	81.25	
Duration (year)	Mean $\pm$ SE	80	6.37 $\pm$ 0.41	---
* (P $\leq$ 0.05), ** (P $\leq$ 0.01).				

Comparison between Patients and Control Groups in Lipid Profile Serum total cholesterol

The average cholesterol level in diabetes patients was 173.10 milligrams per deciliter, which is considerably (P = 0.0296) higher than the average cholesterol level in the control group's blood, which was 156.55 milligrams per deciliter.

Serum triglycerides (TG)

The comparison between the levels of triglycerides in diabetic patients and healthy controls revealed that the level of triglycerides in diabetic patients was significantly higher (P>0.001) than the level of triglycerides in healthy controls. The mean average of TGs in the serum specimen of T2DM patients group was (191.20 $\pm$ 8.98), whereas the level of TGs in healthy controls group was (91.62 $\pm$ 3.73), as shown in Table 8.

The findings, demonstrated that there is NOT a significant difference

(p=0.1846) in the mean serum level of HDL between patients with TIIDM (37.13 $\pm$ 1.07) and healthy controls (39.57 $\pm$ 1.44).

Serum VLDL

The findings indicated that the mean level of VLDL had dramatically increased (P 0.0001) from (37.89 $\pm$ 1.83) in T2DM to (18.35 $\pm$ 0.73) in healthy controls.

Serum LDL

The LDL level in the serum specimens taken from T2DM patients and controls was calculated, there was not a significant difference (p=0.4920) between the mean value of LDL-cholesterol in diabetes patients (113.10 $\pm$ 14.23), and the mean value of LDL-cholesterol in the control group (99.15 $\pm$ 3.91) as shown in figure 8.

Table (8): Comparison between patients and control groups in lipid profile

Group	Mean $\pm$ SE (mg/dL)				
	Cholesterol	Triglyceride	HDL	VLDL	LDL
Patients	173.10 $\pm$ 4.97	191.20 $\pm$ 8.98	37.13 $\pm$ 1.07	37.89 $\pm$ 1.83	113.10 $\pm$ 14.23
Control	156.55 $\pm$ 3.85	91.62 $\pm$ 3.73	39.57 $\pm$ 1.44	18.35 $\pm$ 0.73	99.15 $\pm$ 3.91
T-test	14.881*	25.49 **	3.614 NS	5.210 **	40.084 NS
P-value	0.0296	0.0001	0.1846	0.0001	0.4920
* (P $\leq$ 0.05), ** (P $\leq$ 0.01).					

Genetic Polymorphism Study Genomic DNA extraction

The results of the extraction demonstrated that DNA solutions extracted from blood of T2DM patients and healthy controls have a high concentration and purity as shown in

figure 2. According to the results that were obtained from the Nanodrop spectrophotometer One C, the purity of the DNA ranged between 1.8 and 1.9 while the levels of DNA ranged between 110 and 120 ng/L.

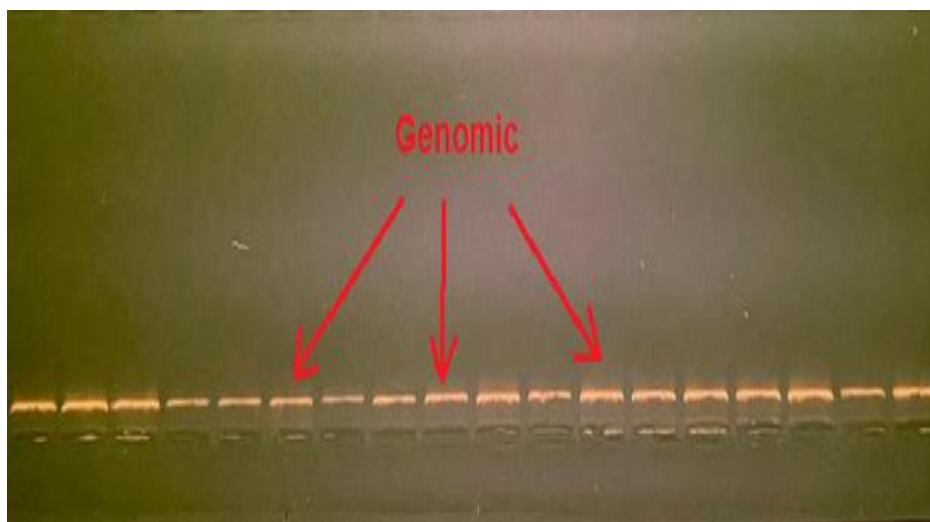


Figure (2): Gel electrophoresis shows the genomic DNA that was extracted from the blood sample.

Detection of 161 C/T polymorphism (rs1111875) and 222 G/A polymorphism (rs 7932837) using RFLP

Polymerase chain reaction (PCR), as shown in Figure 3, the amplified regions, that have molecular weight of (161pb) which represent the region of *HHEX* gene of (rs1111875 SNP), and also shown in Figure 4, the amplified regions, that have molecular weight of (222pb) which represent the region of *HHEX* gene of Polymerase chain

reaction (PCR) as shown in Figure 3,4 the amplified regions, that have mole (rs7932837 SNP). Within the scope of this method, particular primers are utilized to demonstrate the *HHEX* gene segment (5).

This result was also obtained by (5) who photographed the gel using a digital camera. The PCR product size was determined by using a DNA ladder with increments ranging from 100 to 1500, and the ladder was photographed using a digital camera (2020).

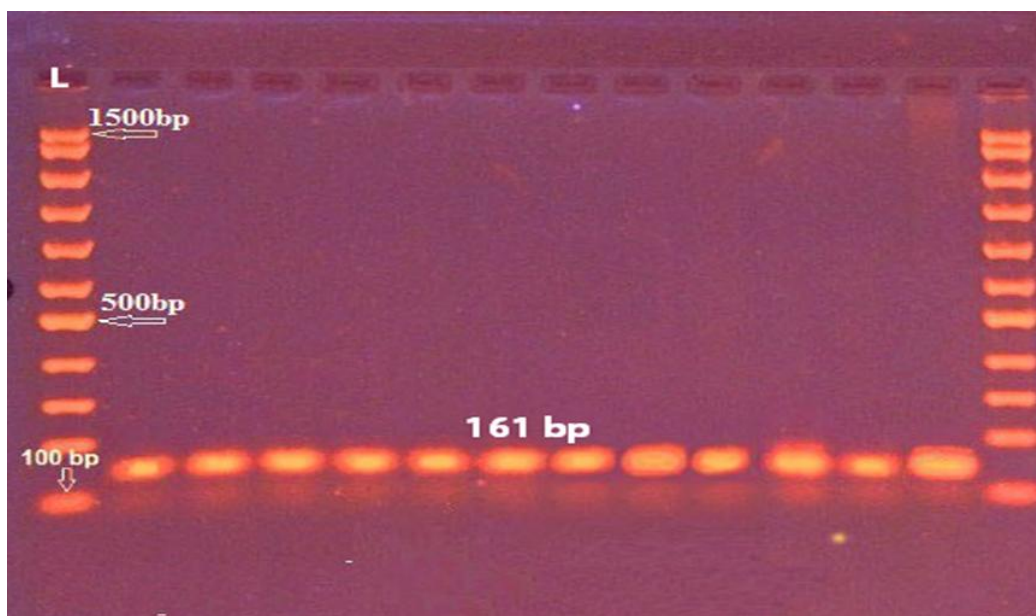


Figure (3): Electrophoresis pattern of PCR product for HHEX gene, on 2.0% agarose at 7v/cm² for 1:30 min showing bands of 161bp molecular size (Lane L: 100 bp DNA ladder, Lanes 1-12 sample)

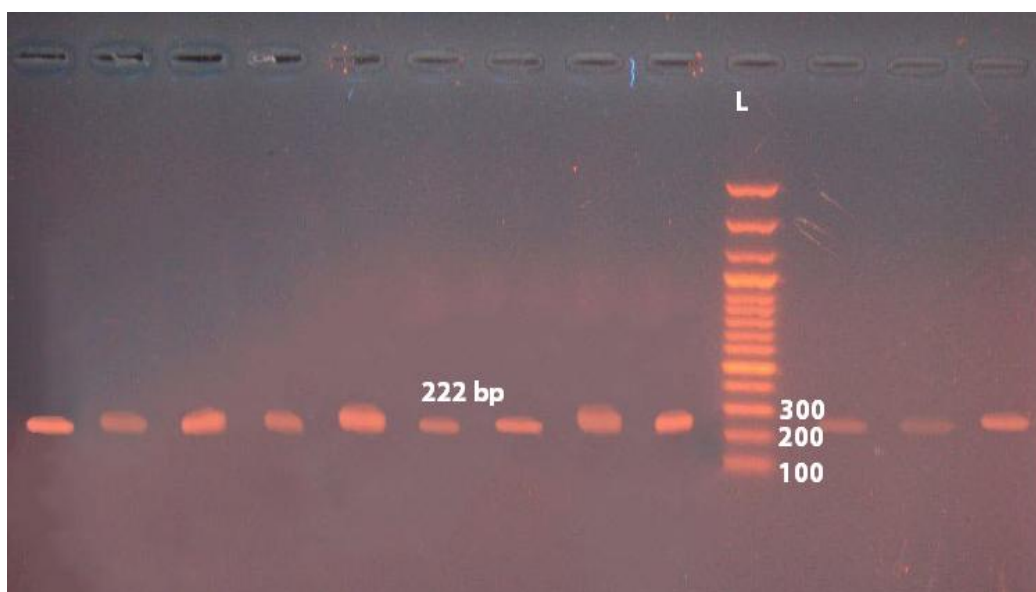


Figure (4): Electrophoresis pattern of PCR product for HHEX gene, on 2.0% agarose at 7v/cm² for 1:30 min showing bands of 222 bp molecular size (Lane L: 100 bp DNA ladder, other Lanes sample).

Genotyping and Allele Frequency of Genes

Association of HHEX Gene Polymorphism C/T (rs1111875) SNP with Diabetes

The rs1111875 polymorphism was genotyped in this study on a total of 80 Type 2 Diabetes patients and 40 healthy controls (Table 9, Figure 5). The frequencies of the CC, CT, and TT genotypes were found to be 28.75%,

60.00%, and 11.25% respectively in TIIDM patients, whereas in controls, these frequencies were found to be 62.50%, 32.50%, and 5.00% respectively in patients with TIIDM.

In patients with type 2 diabetes, the frequencies of the C and T alleles were found to be 0.59 and 0.41, while in healthy persons they were 0.79 and 0.21.

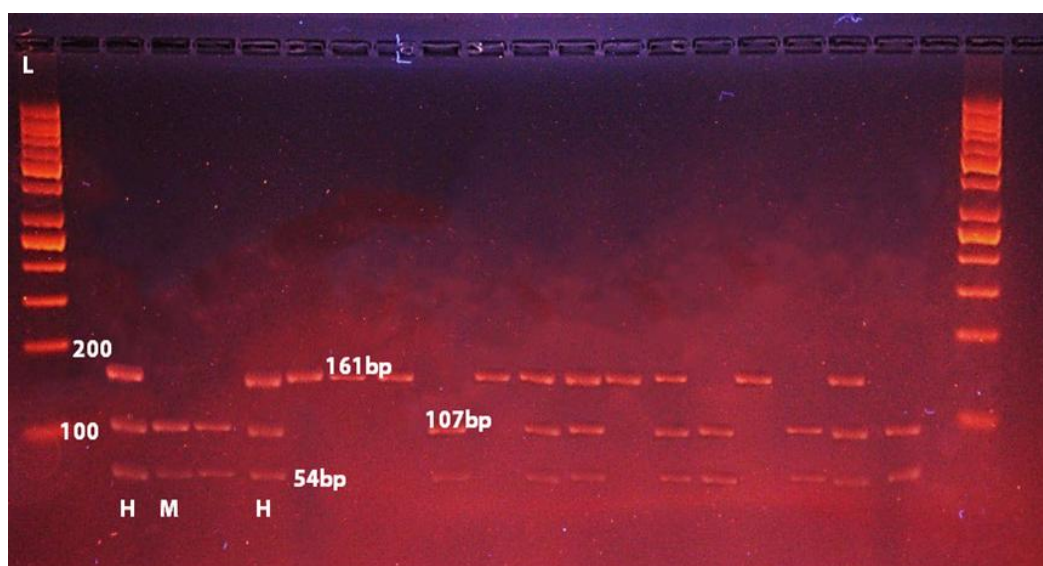


Figure (5): Electrophoresis pattern of PCR product for Gene , on 2.0% agarose at 7v/cm² for 2:00 hr showing bands of 161, 107, 54 bp molecular size (Lane L: 100 bp DNA ladder; Lanes 1-12 sample. Genotyping wild type one band 161 bp Mutant two band 107 and 54, hetero three band 161,107,54.

Table (9): Genotype distribution and allele frequency of rs1111875 C/T SNP in patients and control groups

Genotype// rs1111875 C/T SNP	Patients No. (%)	Control No. (%)	Chi-Square (χ^2)	P- value	OR (CI)
CC	23 (28.75%)	25 (62.50%)	0.083 NS	0.772	Ref.=1
CT	48 (60.00%)	13 (32.50%)	20.082 **	0.0001	1.82 (0.94-3.51)
TT	9 (11.25%)	2 (5.00%)	4.454 *	0.0348	1.17 (0.6-2.07)
Total	80 (100%)	40 (100%)	---		
P-value	0.0001 **	00001 **	---		
Allele	Frequency				
C	0.59	0.79	---		
T	0.41	0.21			
* (P≤0.05), ** (P≤0.01), NS: Non-Significant.					

Within this Table 9, in the sick group, the frequency of the CC genotype was 28.75%, whereas it was 62.50% in the control group ($p=0.772$). This difference did not reach statistical significance. While the High significance of CT Polymorphism genotype in the control group and patients group was significantly greater than that in the patients group ($p=0.0001$) (60.00% versus 32.50%, respectively), this difference was not

statistically significant. In the group of patients, the frequency of the TT polymorphism genotype was significantly different from that of the control group ($p=0.0348$). The patients' group had 11.25 percent, whereas the control group had only 5 percent.

The odd ratio was greater than one of the C/T ratio (rs1111875). In the sample that was used for this study, the SNP polymorphism genotype was found to

have a highly significant association with the chance of getting diabetes.

Association of HHEX gene polymorphism G/A (rs7932837) SNP with diabetes

The rs7932837 polymorphism was genotyped in this study on a total of 80 TIIDM and 40 healthy controls (Table 10, Figure 6). In cases of TIIDM, the frequency of the GG genotype was found to be 41.25%, while the frequency of the GA genotype was

found to be 47.50%, and the frequency of the AA genotype was found to be 11.25%. On the other hand, the frequency of the GG genotype was found to be 52.50%, 30.00%, and 17.50% in controls (Table 10, Figure 6).

In TIIDM individuals, the frequencies of the G and A alleles were found to be 0.65 and 0.35, while in healthy people they were 0.67 and 0.325.

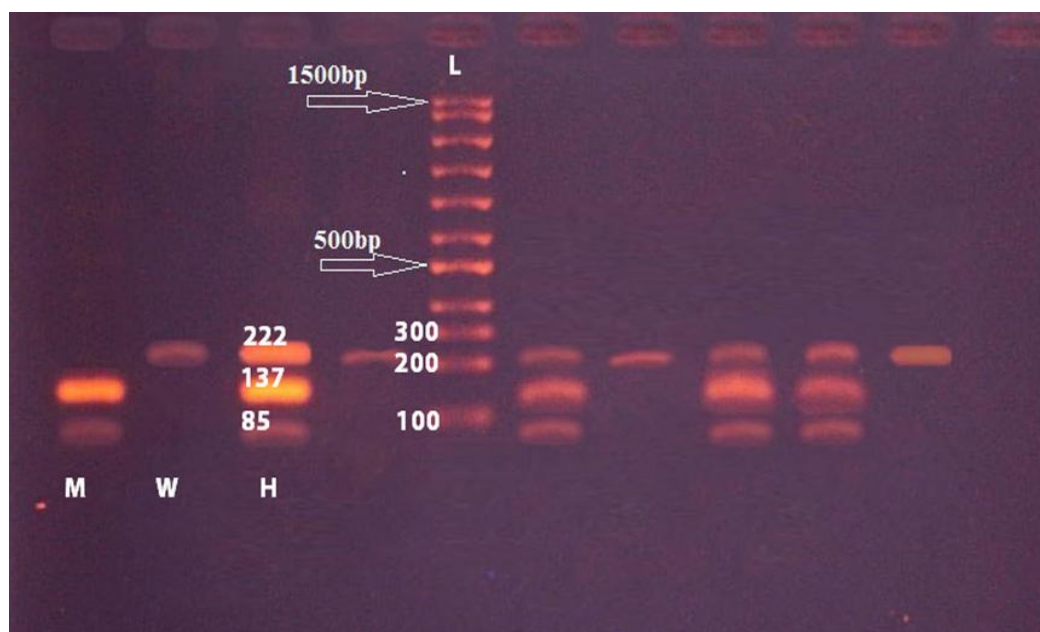


Figure (6) : Electrophoresis pattern of PCR product for Gene HHEX, on 2.0% agarose at 7v/cm² for 2:00 hr showing bands of 222, 137, 85 bp molecular size (Lane L: 100 bp DNA ladder. Genotyping wild type one band 222 bp Mutant two band 137 and 85, hetero three band 222, 137, 85).

Table (10): Genotype distribution and allele frequency of rs7932837 G/A SNP in patients and control groups

Genotype// rs7932837 G/A SNP	Patients No. (%)	Control No. (%)	Chi-Square (χ^2)	P-value	OR (CI)
GG	33 (41.25%)	21 (52.50%)	2.667 NS	0.102	Ref.=1
GA	38 (47.50%)	12 (30.00%)	13.52 **	0.0002	1.96 (1.13-3.88)
AA	9 (11.25%)	7 (17.50%)	0.250 NS	0.617	0.554 (0.24-0.91)
Total	80 (100%)	40 (100%)	---		
P-value	0.0001 **	0.0220 *	---		
Allele	Frequency				
G	0.65	0.675	---		
A	0.35	0.325			
* (P<0.05), ** (P<0.01), NS: Non-Significant.					

When compared to the control group, the frequency of the GG polymorphism genotype in the patients group did not exhibit a significant ($p = 0.102$) difference when compared to the frequency of the genotype in the control group (41.25% versus 52.50%, respectively).

The high significance of the GA Polymorphism genotype was significantly higher in the control group and patients group ($p = 0.0002$) than it was in the patients group (47.50% versus 30.00%, respectively). The frequency of the AA genotype in the patient group is 11.25 percent, which is not significantly lower than the frequency in the control group, which is 17.50 percent.

The odd ratio was more than one for both G and A. (rs 7932837) In this particular study's cohort, the SNP polymorphism genotype was not shown to be significantly related with an increased risk of acquiring diabetes (5).

Correlation between the Genotypes and Clinical Parameters in the T2DM Patients

Relationship between rs1111875 C/T SNP of gene and parameters study of patients groups

The current research demonstrated that the HHEX, SNP (rs1111875) contained a C/T dimorphism and was substantially related with the trait. Due to the fact that their wild alleles are C and their risk variant alleles are (T). Their genotypes were as follows: wild homozygous (CC), heterozygous risk variant (CT), and homozygous risk variant (TT). In terms of the connection between SNP (rs1111875) of HHEX and T2DM, the findings demonstrated that the frequency distributions of genotype of SNP (rs 1111875) were statistically significant between patients and healthy control, as shown in Table 11.

In addition, the current study found that certain parameters (cholesterol, triglyceride, HDL, and VLDL) were statistically significant at ($P = 0.0498$), ($P = 0.0361$), ($P = 0.0392$), and ($P = 0.044$) respectively between genotype (CC) and (TT) and (CT) in T2DM patients, whereas other parameters (FBS, HbA1c, and LDL) were not statistically significant between genotype.

Table (11): Relationship between rs1111875 C/T SNP of gene and parameters study of patients groups

Parameters	Genotype of rs1111875 C/T			LSD value (P-value)
	CC	CT	TT	
FBS	164.65 $\pm$ 1.44	173.15 $\pm$ 12.74	160.33 $\pm$ 15.98	54.38 NS (0.694)
HbA1c (%)	8.05 $\pm$ 0.26	8.23 $\pm$ 0.20	8.45 $\pm$ 0.64	0.977 NS (0.779)
Cholesterol (mg/dL)	151.65 $\pm$ 7.38 b	185.42 $\pm$ 6.48 a	163.56 $\pm$ 14.48 ab	28.55* (0.0498)
Triglyceride (mg/dL)	143.95 $\pm$ 10.42 b	218.19 $\pm$ 12.54 a	171.00 $\pm$ 13.25 ab	49.98* (0.0361)
HDL (mg/dL)	36.74 $\pm$ 1.73 b	35.74 $\pm$ 0.99 b	45.44 $\pm$ 6.14 a	6.11* (0.0392)
VLDL (mg/dL)	29.22 $\pm$ 2.14 b	42.87 $\pm$ 2.61 a	34.11 $\pm$ 2.66 ab	10.41* (0.044)
LDL (mg/dL)	84.73 $\pm$ 7.08	132.55 $\pm$ 23.31	84.00 $\pm$ 8.06	85.53 NS (0.106)
Means having with the different letters in same row differed significantly. * ($P \leq 0.05$).				

Relationship between rs7932837 G/A SNP of HHEX gene and parameters study of patients groups

The current research demonstrated that the HHEX, SNP (rs 7932837) exhibited G/A dimorphism and was substantially related with the trait in question. Due to the fact that their wild alleles are (G), and their risk variant alleles are (A). Their genotypes were as follows: wild homozygous (GG), heterozygous risk variant (GA), and homozygous risk variant (AA). In terms of the connection between SNP (rs 7932837) of HHEX and T2DM, the

findings demonstrated that the frequency distributions of genotypes of SNP (rs 7932837) were statistically significant between patients and healthy control, as shown in Table 12.

In addition, the current study found that parameters (FBS, Cholesterol, and HDL) showed statistically high significance at (P0.0373), (p0.0452), and (p0.0396) respectively between genotypes (GG), (AA), and (GA) in T2DM patients, whereas total HbA1c, Triglyceride, VLDL, and LDL did not show statistically high significance at (P 0.866), (P0.107).

Table (12): Relationship between rs7932837 G/A SNP of gene and parameters study of patients groups

Parameters	Genotype of rs7932837 G/A			LSD value (P-value)
	GG	GA	AA	
FBS	164.51 ±11.72 b	159.05 ±13.27 b	228.22 ±35.82 a	53.22 * (0.0373)
HbA1c (%)	8.20 ±0.27	8.23 ±0.22	8.09 ±0.32	0.956 NS (0.866)
Cholesterol (mg/dL)	170.48 ±7.80 b	169.00 ±7.57 b	199.56 ±7.73 a	27.94 * (0.0452)
Triglyceride (mg/dL)	178.24 ±16.67	203.51 ±11.80	188.11 ±9.78	48.91 NS (0.107)
HDL (mg/dL)	37.78 ±1.74 ab	35.16 ±1.06 b	42.89 ±5.19 a	5.98 * (0.0396)
VLDL (mg/dL)	35.24 ±3.37	40.29 ±2.43	37.78 ±1.96	10.19 NS (0.074)
LDL (mg/dL)	136.72 ±32.98	90.64 ±6.67	118.78 ±6.72	83.70 NS (0.238)
Means having with the different letters in same row differed significantly. * (P≤0.05).				

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