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CORRELATION BETWEEN SERUM LEVEL OF LOW-DENSITY LIPOPROTEIN RECEPTOR RELATED PROTEIN AND ITS SINGLE GENE POLYMORPHISMS IN TYPE 2 DIABETES MELLITUS

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Abstract
Diabetes mellitus is a metabolic disease described as the presence of hyperglycemia because of failure in insulin secretion, defective action of insulin, or both. In this study, serum levels of LRP1 and genetic polymorphism (rs1800127) of the LRP1 gene were investigated in Type 2 diabetes (T2D) patients and healthy controls to determine their disease-associated risk. Allele-specific primer technique was used to determine the polymorphisms. Accordingly, a case-control analysis was carried out on 50 patients and 50 control from October 2020 – December 2020. The patients and control were characterized for age, sex, Body Mass Index (BMI), HbA1c, and lipid profile, including cholesterol, triglyceride, low-density lipoprotein, high-density lipoprotein, and very low-density lipoprotein. The results are given in the following: The mean age of patients increased significantly compared to the control (50.22 ±9.34 vs. 44.28 ±9.28 years; $p < 0.0019$). At the same time, the mean BMI of T2D patients was insignificant compared to controls (30.39 ±2.64 vs. 30.96 ±4.57 kg/m2; $p \leq 0.452$). Furthermore, there was no significant difference in LRP1 level in patients with T2DM compared with controls (49.19±18.45 vs. 33.43 ±12.66 pf/mL; $p < 1.00$). Genetic analysis of rs1800127 SNP of the LRP1 gene revealed significant differences ($p =0.0091$) between the observed and expected genotypes. In CT Polymorphism, the odds ratio for the CT genotype was 3.16 with p=0.066 indicating that individuals who carry CT are more likely to encounter T2DM, which means that the SNP (rs1800127) has a role in the incidence of the disease. Moreover, the data detected significant variations (p=0.0347) between the observed and expected genotypes of rs7968719. In addition, In CG Polymorphism, the odds ratio for the CG genotype was 0.07 with p-value=1, indicating that there was non-significant CG genotyping of T2DM patients compared with controls. The odd ratio of genotype frequencies of allele G was 1.38, which suggests that the G allele may have a critical role in the incidence of T2DM.
Keywords: SNP, genotype, LRP1, HbA1c, BMI, lipid profile.

Introduction
Diabetes mellitus is a heterogeneous metabolic disorder described as a presence of hyperglycemia because of failure of insulin secretion or defective insulin action or both (Punthakee et al, 2018) . This disease characterized by significantly increased levels of blood glucose which overrun their normal physiological ranges (Saeedi et al, 2019). Diabetes is may be occurred because of either the pancreas cannot produce sufficient amount of insulin, or the body's cells cannot respond to the produced insulin (Shoback and Gardner, 2018). In fact, A 463 million of human had the disease worldwide as the study reported in 2019. Where type 2 diabetes (T2D) cases are constituted about

90% of total cases, the data suggest that the rates will raise. The disease cause approximately 4.3 million mortalities in 2019 (Saeedi et al, 2019). It Diabetes ranks 7th in terms of diseases that cause global mortality (World Health Organization, 2019).

Type 2 diabetes is a multifactorial and chronic disease. In the past, it was termed "non-insulin-dependent diabetes mellitus (NIDDM)'. However, this term is changes because of insulin is usually utilized to manage this type. The sufficient insulin level has to meet the metabolic order for regulating the levels of blood glucose, hence, their defect results in T2DM pathogenesis (Hussein et al., 2020) (Galicia-Garcia et al., 2020). High blood cholesterol levels are the cause of type 2 diabetes mellitus. It is a type of dyslipidemia (any defects of blood lipoprotein and lipid levels), hyperlipoproteinemia (high blood lipoprotein levels) and hyperlipidemia (high blood lipid levels). Obesity, Diet, genetic conditions (including LDL receptor mutations in familial hypercholesterolemia), or the existence of other factors, including T2DM which may contribute to increased blood levels of LDL and non-HDL cholesterol (Durrington, 2003). Diabetes treatment can be decreased by various by consuming healthy diet, exercise and pharmacologic therapy. There are a lot of chemical drugs that have proven to be able to maintain blood sugar levels but there are many side complications, which have not yet been proven to be safe for use (Al-Hamdani, 2019).

LDL receptor related protein 1 (LRP1) is receptor involved in various cellular processes, including clearance of apoptotic cells, lipid homeostasis and intracellular signaling (Chang et al., 2022). Hyperglycemia reduces LRP1 expression, and its absence in neurons impairs insulin signaling pathways and glucose absorption (Liu et al., 2015; Au et al., 2017). Even though LRP1 is present in many different cell types and tissues, it is most abundant in vascular smooth muscle cells (SMCs), hepatocytes, and neurons. LRP1 is expressed in several tissues and is a member of the LDLR family (Etique et al, 2013b; Lillis et al, 2005b).

In this context, this case-control study was aimed to investigate the differences of the serum level of LRP1 in patients with type 2 diabetes and healthy controls and their correlation with single nucleotide polymorphism (SNP).

Materials and methods

Collection of samples

The current cross-sectional, retrospective study incorporated fifty T2D-suffering Iraqi individuals and fifty healthy controls. It composed individuals with T2D whose visited the Specialist Center for deaf diseases and diabetes (Al-Russafa, Baghdad, Iraq). The T2D diagnosis in each case was recorded after obtaining the medical history. Five milliliters of venous blood were withdrawn from each subject by vein-puncture under aseptic technique by syringe. The blood samples were divided into two portions; one portion was collected in EDTA tubes (2ml) to use for genetic analysis. The blood samples were stored in frozen at -20 C until used. While the other portion collected in a gel tube to use in ELISA technique and for other laboratory analysis. Demographic characteristics, including age, gender, life style (smoking and alcoholic uptake) and body mass index, were obtained for each patient and control.

Laboratory examination using manual methods

Serum levels of HbA1c and lipid profile, including cholesterol, high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL) and very low-density lipoprotein (VLDL) were estimated for each patient and healthy control.

Immunological examination using ELISA

Enzyme-linked immunosorbent assay (ELISA) technique was utilized to determinate the serum level of LRP1 based on the Human Low Density Lipoprotein Receptor Related Protein 1 (LRP1) ELISA Kit (SunLong Biotech Co.). Three milliliters of blood from venous were obtained from each case and control in a gel tube. The blood was left to clot, then the tube was placed in centrifuge (3000 rpm for 15 min at 4oC). After that, the serum was obtained and stored at -20 0C till assayed. The serum level of LRP1 was calculated utilizing a kit of enzyme-linked immunosorbent assay (ELISA), as well as the manufacturer instructions were performed. The kit detection ranges were 5pg/ml-100pg/mL.

Molecular examination using ASP technique

Allele specific primer (ASP) technique was utilized for estimation of rs1800127 (C/T) SNP in exon 6 and rs7968719 SNP (C/G) SNP in intron 6 of LRP1 gene by the amplification using PCR. Samples of DNA were extracted using kit of Mini DNA extraction based on the instructions of manufacturer (Extraction of ReliaPrep Genomic DNA Miniprep System, Promega/USA). In ASP technique, two forward primers and one reverse primer for rs1800127 as well as two reverse primers and one forward primers for rs7968719 SNP were utilized as follow:

Table 1. Primers utilized in amplification of exon 6 and intron 6 regions of LRP1 gene

Gene	Single nucleotide polymorphisms	Primer's name	Sequence	Product size (bp)
LRP1	rs1800127	LRP1 F	CCTGAGAGGTGGTCCTAGAG	203
		LRP1 R	GGTGTGATGGTAGACACCTGGG	
		LRP1 R	GGTGTGATGGTAGACACCTGGA	
	rs7968719	LRP1a F	TGCCATACAGTGGGCTCC	165
		LRP1a F	TGCCATACAGTGGGCTCG	
		LRP1a R	GTGAGTTTAGCAGACTGGGA	

Statistical analysis

The program of the Statistical Analysis System- SAS (2018) was utilized to reveal variations in parameters of the study. Chi-square test was utilized to significant comparison between percentage (0.05 and 0.01 probability). T-test was utilized to significant comparison between means. To calculating the 95% confidence intervals (CIs) and the odds ratios (ORs), a logistic regression model was utilized (SAS, 2018).

Discussion

Demographic characteristics

Type 2 diabetes mellitus (T2DM) patients nominated for the current work were 50 in total, where the age of patients was ranged between (33-65 years) with four age groups (≤30, 30-39, 40-49, 50-59 and ≥60 years). All parameters in the study were recorded, as shown in Table 2.

Table 2. Demographic Characteristics of patients with type 2 diabetes and healthy controls.

			Diabetes type 2 patients		Health Controls		*p*-value
			No.	%	No.	%	
Age (years)		≤30	0	0.00	1	2.00	1.00 NS
		30-39	2	4.00	19	38.00	**0.0001** **
		40-49	13	26.00	13	26.00	1.00 NS
		50-59	19	38.00	15	30.00	0.492 NS
		≥60	9	18.00	2	4.00	**0.0348** *
		Mean± SD (Range)	50.22 ±9.34 (33-65)		44.28 ±9.28 (30-60)		**0.0019** **
Gender		Male	22	44.00	27	54.00	0.475 NS
		Female	28	56.00	23	46.00	0.483 NS
BMI (Kg/m^2)		Normal (18.5-24.9)	0	0.00	7	14.00	**0.0466** *
		Overweight (25-29.9)	30	60.00	15	30.00	**0.025** *
		Obese (≥30)	20	40.00	28	56.00	0.145 NS
		Mean± SD (Range)	30.39 ±2.64 (27.50-33.08)		30.96 ±4.57 (25.27-35.17)		0.452 NS
Biochemical markers		HbA1c (%)	9.69 ±1.88		5.11 ±0.54		**0.0001** **
		Cholesterol (mg/dl)	280.40 ±62.55		170.96 ±15.37		**0.0001** **
		Triglyceride (mg/dl)	253.30 ±79.06		112.50 ±26.65		**0.0001** **
		HDL (mg/dl)	48.50 ±30.20		52.36 ±5.46		**0.376 NS**
		VLDL (mg/dl)	51.76 ±16.35		22.16 ±6.04		**0.0001** **
		LDL (mg/dl)	190.45 ±61.21		94.86 ±17.72		**0.0001** **
Life style	Smoking	Yes	18	36.00	21	42.00	0.631 NS
		No	32	64.00	29	58.00	0.701 NS
	Alcohol uptake	Yes	3	6.00	4	8.00	0.706 NS
		No	47	94.00	46	92.00	0.917 NS

*** (*P*≤0.05): significant**
**** (*P*≤0.01): highly significant**
NS: non-significant

Based on the data, there are no significant differences in males and females among patients (22, 44.00% vs. 28, 56.00%) and controls (27, 54.00% vs. 23, 46.00%) as shown in table 2. The sex related-studies were often reported that it is little or no sex bias within both types I and II diabetes mellitus. Women, or their offspring, are more likely to transmit T2DM (Gale and Gillespie, 2001). It was recently established that T2DM more affected women than men (Asiimwe et al., 2020). This is attributed to those women being less muscular, with no fixed glucose uptake and high estrogen and progesterone levels, which reduces the sensitivity of the body's insulin (Machado-Alba et al., 2016). Additionally, it was investigated that women are less physical and exercise to burn excessive fat in the body's tissues. Also, they take an unhealthy diet (including various starches and fats resulting in non-communicable diseases (NCDs) such as type 2 diabetes (Bommer et al., 2018).

The mean age of T2D patients was increased significantly in comparison with controls (50.22 ±9.34 vs. 44.28 ±9.28 years; $p < 0.0019$), as shown in Table 2. A significant difference between the two means was observed ($p < 0.0019$). Rahelic reported that T2DM occurred in middle and older ages (Rahelić, 2016). Diabetes is observably raised with age (Braunwald et al., 2005). The incidence of this type of diabetes is observed in all age groups, but most of this disease is higher among children (Quinn et al., 2021). Indeed, age is highly linked to T2D, and extending life expectancy and population growth lead to most of the increase in T2D prevalence (Peters et al., 2015). Children, and teenagers (10 to 19 years), however, may be diagnosed with T2D and have grown considerably, according to research conducted in the United States (US), particularly among ethnic minority groups (Dabelea et al., 2014).

The mean BMI of T2D patients was not significant in comparison with controls (30.39 ±2.64 vs. 30.96 ±4.57 kg/m2; $p \leq 0.452$), as shown in Table (4-4). The increased BMI was observed in cases with T2DM more than in controls, which supposed that these patients tend to be obese (Li et al., 2021). High BMI levels (Overweight/obesity) are an independent and dose-dependent risk factor for T2DM (Sanada et al., 2012).

Based on the data, the results show that there are highly significant differences in the value of HbA1c among patients and controls (5.11 ± 0.54 vs. 9.69 ± 1.88), as shown in table 2. HbA1c is an effective screening tool for the detection of T2D (Bennett et al., 2007). HbA1c is a sugar-hemoglobin formation, and its presence indicates excessive sugar level, an indicator of diabetes (Miedema, 2005).

The data showed highly significant differences in values of lipids (including cholesterol, triglyceride, VLDL, and LDL) except HDL. The findings of cholesterol were (280.40 ±62.55 vs. 170.96 ±15.37, $p<0.0001$), triglycerides (253.30 ±79.06 vs. 112.50 ±26.65, $p<0.0001$), HDL (48.50 ±30.20 vs. 52.36 ±5.46, $p<0.376$), VLDL (51.76 ±16.35 vs. 22.16 ±6.04, $p<0.0001$) and LDL (190.45 ±61.21 vs. 94.86 ±17.72, $p<0.0001$) among patients and control, respectively.

The results reveal that the patient group is suffering from hyperlipidemia, more precisely, hypercholesterolemia. Our results were in agreement with Gyawali and Regmi (2011), who reported that about 54% of diabetic individuals had increased LDL, > 50% of individuals had increased triglycerides, and 73% of individuals had low HDL levels (Gyawali and Regmi, 2011).

In patients with diabetes, increased HbA1c and dyslipidemia can be considered risk parameters for cardiovascular diseases (Selvin et al., 2006).

Serum level of LRP1

The result of the serum level of LRP1 was observed, and there was no significant difference between the patients and controls, as in the following table (2).

Table 3. The serum level of LRP1 proteins in patients and controls

ELISA		Type 2 diabetes patients		Controls		P value
		No	%	No	%	
LRP1 (pg/mL)	<5	0	0.00	0	0.00	1.00 NS
	5-100	49	98.00	50	100	0.984 NS
	≥100	1	2.00	0	0.00	0.984 NS
	Mean± SD (Range)	49.19 ±18.45		33.43 ±12.66		0.094 NS
NS: Non-Significant.						

Some studies have reported the essential role of LRP1 in the normal function of β-cell and the regulation of translocation of insulin signaling and modulating GLUT2 in the liver. In addition, regulation of GLUT4 trafficking in adipocytesmplicated insulin resistance in patients with T2DM (Actis Dato and Chiabrando, 2018). It indicated that the reduced expression of LRP1 protein expression related to the progression of cardiovascular disease in familial hypercholesterolemia (Ghareeb et al., 2021). The study's results investigated that cholesterol-lowering interventions exerted regulatory effects on vascular LRP1 overexpression induced by hypercholesterolemia and that simvastatin did not influence LRP1 expression beyond its cholesterol-lowering effects (Llorente-Cortes et al., 2011).

The following table (4) shows the correlation between the serum level of LRP1 proteins with different parameters. Our results revealed no significant correlation between the serum level of LRP1 and the patients' and controls' age, gender, and BMI.

Table 4. Correlation between serum level of LRP1 and all parameters

		LRP1 (pg/mL)			
		T2D patients		Controls	
		No	Mean±SD	No	Mean±SD
Age groups	≤30	0	---	0	---
	30-39	2	40.84 ±5.03	20	33.82 ±3.93
	40-49	13	54.9 ±6.21	13	28.20 ±2.78
	50-59	19	51.06 ±5.48	15	38576 ±4.78

	≥60	9	45.33 ±5.27	2	38.70 ±3.98
	p-value		0.083 NS		0.066 NS
Gender	Male	22	45.60 ±4.98	27	32.46 ±3.76
	Female	28	52.01 ±6.37	23	35.75 ±4.85
	p-value		0.149 NS		0.497 NS
BMI	Normal (18.5-24.9)	0	---	7	35.73 ±4.72
	Overweight (25-29.9)	30	47.55 ±3.19	15	34.18 ±3.98
	Obese (=>30)	20	51.65 ±5.88	28	32.46 ±4.07
	p-value		0.371 NS		0.621 NS
* ($P \leq 0.05$), NS: Non-Significant.					

Genetic studies

DNA extraction

A total of one hundred blood samples were undergone for DNA extraction using ReliaPrep™ Blood gDNA Miniprep (Promega, USA), while the purity and the concentration of each sample were examined with Nanodrop. Besides to confirm the DNA was not hydrolyzed or degraded, some samples were taken randomly and electrophoresed with agarose gel with a concentration (1%) of agarose as shown in (figure 1), and representing the range concentration of DNA ranged from (11-279 ngμL) and the purity of DNA ranged between (1. 67-2.04).

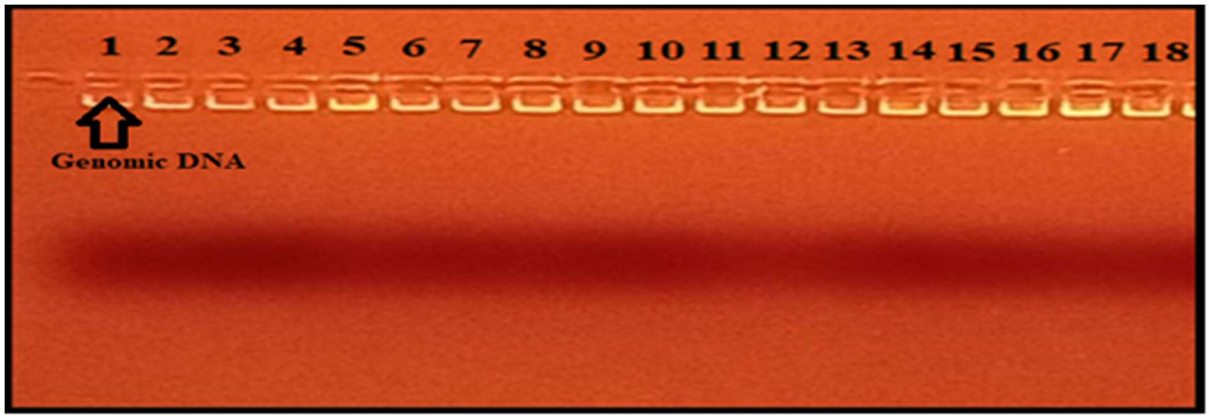

Figure 1. Genomic DNA extracted from blood samples collected from type 2 diabetes mellitus with iris pigments after electrophoresis on agarose 1% stained with ethidium bromide, carried out for 45 min at 90 volts visualized under UV transilluminator at 350 nm.

Single Nucleotide polymorphisms (SNPs) of the LRP1 gene

Genetic variations of the SNPs were investigated; rs1800127 and rs7968719. PCR genotyped them with an allele-specific primer (PCR-ASP) technique (Yang et al., 2010).

The polymerase chain reaction (PCR) was performed under optimal amplification conditions using specially designed primers for the rs1800127 SNP. The results in (figure 2) showed that the amplifying products appeared as a sharp and clear tape after electrophoresis on an agarose gel

(2%) for 85 min and 90 volts, with a molecular size of 203 bps of rs1800127 SNP of the LRP1 gene, depending on the ladder (100 – 1500) bp ladder marker. These segments represent rs1800127 SNP in the exon 6 region of the LRP1 gene, located on chromosome 12q13-14.

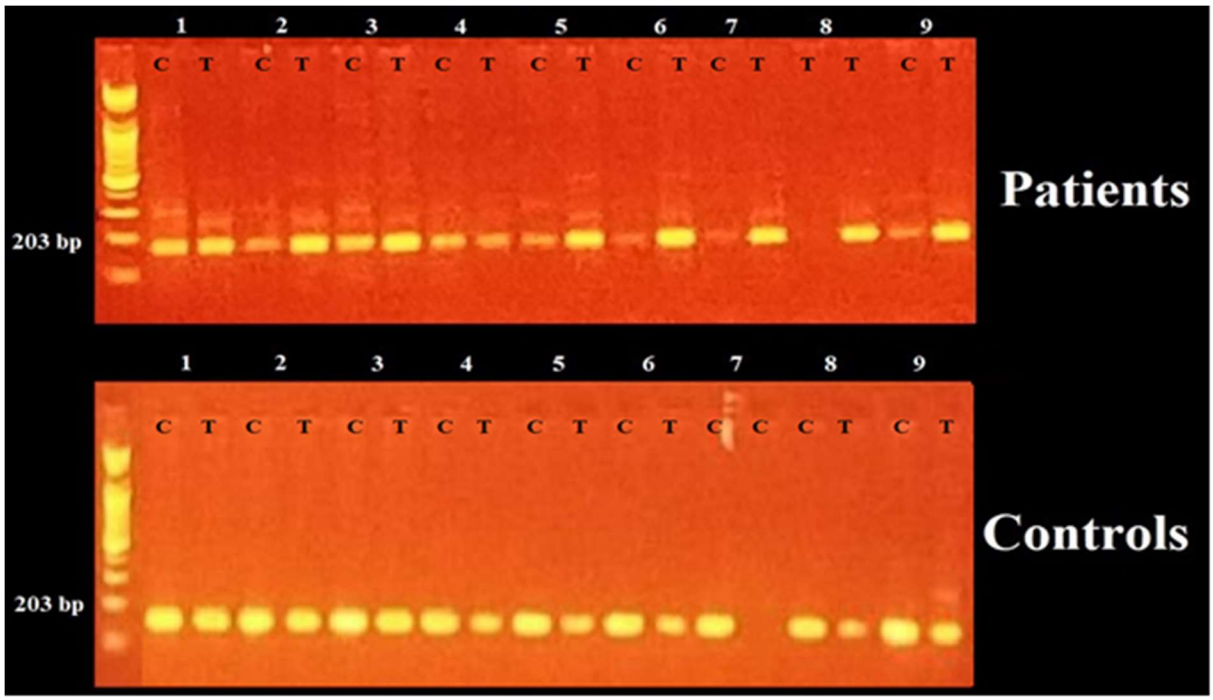

Figure 2. Electrophoresis on an agarose gel (2%) stained with ethidium bromide was carried out for 85 min at 90 volts and visualized using a UV transilluminator at 350 nm for an amplification product of the LRP1 gene that includes the rs1800127 SNP at exon 6 region.

The Hardy - Weinberg equilibrium (HWE) analysis in patients with T2DM showed that genotypes of rs1800127 SNP were compatible with equilibrium, and significant variations ($p = 0.0091$) appeared between the expected and observed genotypic frequencies. The frequencies in the control group were non-significant with p-value = 0.101, as shown in (Table 5). Inspecting genotypes of the LRP1 gene and frequencies of an allele in patients and controls detected non-significant variations between these frequencies. In CT Polymorphism, the OR for the CT genotype was 3.16 with p=0.066 suggesting that individuals who carry CT are more likely to encounter T2DM. The SNP (rs1800127) in the LRP1 gene is detected in samples from individuals with T2DM, meaning that the SNP (rs1800127) has a role in the disease. No further studies for this SNP in hypercholesterolemia, but it was reported that the presence of SNP rs1800127 within the LRP1 gene causes the creation of a target site for miR422a, which is highly expressed in Alzheimer's patients (Mallick and Ghosh, 2011).

Table 5. Number of alleles, percentage frequencies of LRP1 genotypes that include the rs1800127 SNP site and Hardy-Weinberg equilibrium (HWE) in blood samples from the control group and blood samples from type 2 diabetes mellitus patient group.

rs1800127 Genotype	Patient				Control				*p*-value	OR (95% CI)
	Expected		**Observed**		**Expected**		**Observed**			
	No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**		
CC	4.21	8.41	8	16	43.25	86.49	44	88	-	1(References)
CT	20.59	41.18	13	26	6.51	13.02	5	10	0.066	**3.16 (1.04 - 9.58)**
TT	25.21	50.41	29	58	0.25	0.49	1	2	-	-
C			29	29			93	93	-	0.03 (0.01-0.07)
T			71	71			7	7	-	**32.52(13.53 -78.17)**
HWE Analysis	*p*-value = **0.0091**				*p*-value=0.101					
	Significant				non-Significant					

p: Two-tailed Fisher's exact probability; *CI: confidence interval. *OR: odd ratio; * N: allele drop-outs.

The polymerase chain reaction (PCR) was performed under optimal amplification conditions using specially designed primers for the rs7968719 SNP. The results in Figure 3 showed that the amplifying products appeared as a sharp and clear tape after electrophoresis on an agarose gel (2%) for 85 min and 90 volts, with a molecular size of 165 bps of rs7968719 SNP of the LRP1 gene, in the presence of (100 – 1500) bp ladder marker. These segments represent rs7968719 SNP in the intron six regions of the LRP1 gene located on chromosome 12q13-14.

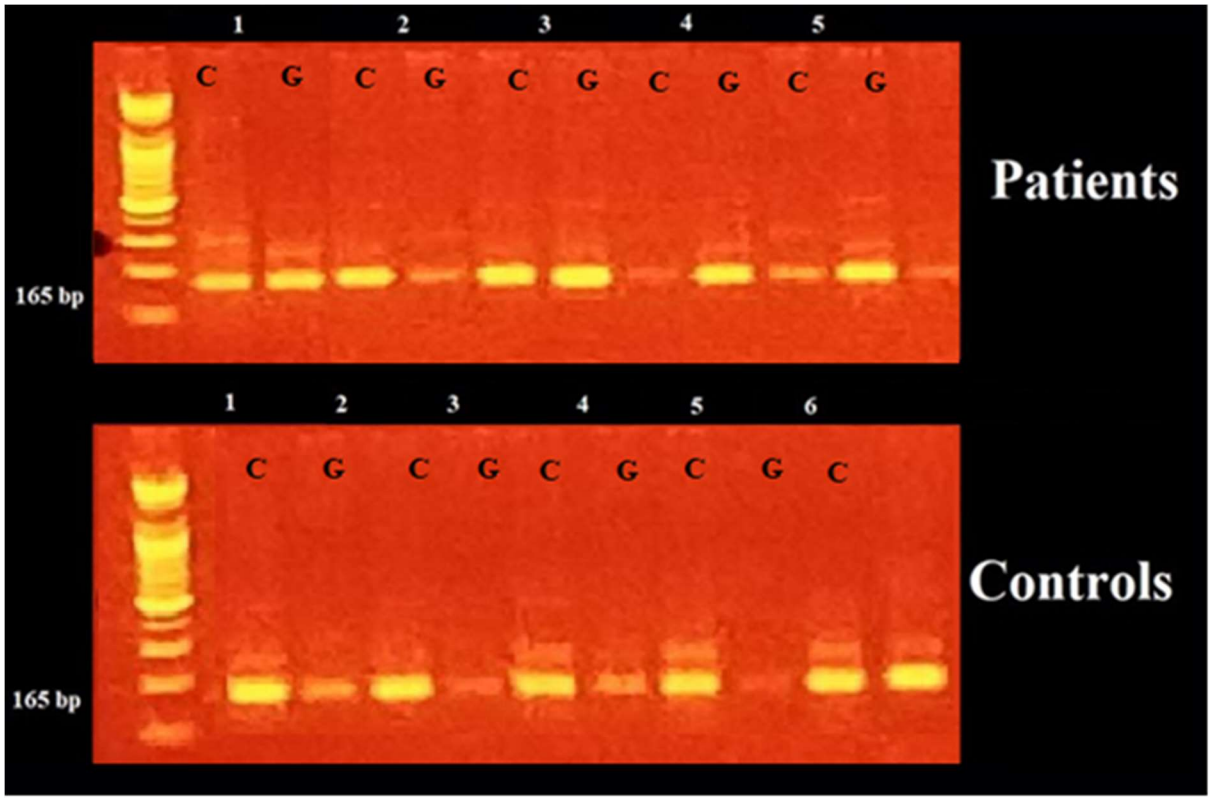

Figure 3. Electrophoresis on an agarose gel (2%) stained with ethidium bromide was carried out for 85 min at 90 volts and visualized using a UV transilluminator at 350 nm for an amplification product of the LRP1 gene that includes the rs7968719 SNP at intron 6 region.

The Hardy - Weinberg equilibrium (HWE) analysis in patients with T2DM showed that genotypes of rs7968719 SNP were compatible with equilibrium, and variations (p =0.0347) appeared between the expected and observed genotypes. The genotype frequencies were non-significant in the control group with p-value = 1, as shown in Table 6.

Inspecting genotypes of the LRP1 gene and frequencies of an allele in patients and controls revealed that there were non-significant variations between these frequencies. However, the results of the G allele revealed that this allele represented a risk factor in patients with T2D compared to the control group, Table 6.

In CG Polymorphism, the OR for the genotype of CG was 0.07 with p-value=1, indicating non-significant CG in T2DM patients. The SNP (rs7968719) in the LRP1 gene is detected in samples from individuals with T2DM, which mean that this SNP may have a role in the disease.

Table 6. Number of alleles, percentage frequencies of LRP1 genotypes that include the rs7968719 SNP site and Hardy-Weinberg equilibrium (HWE) in blood samples from the control group and blood samples from type 2 diabetes mellitus patient group.

	Patient	Control		

rs7968719 Genotype	**Expected**		**Observed**		**Expected**		**Observed**		*p*-value	OR (95% CI)
	No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**		
CC	9.68	19.36	6	12	13.52	27.04	2	4	-	1 (References)
CG	24.64	49.28	32	64	24.96	49.92	48	96	1	**0.07 (0.02-0.34)**
GG	15.68	31.36	12	24	11.52	23.04	0	-	-	-
C			44	44			52	52	0.322	0.73 (0.42-1.26)
G			56	56			48	48	0.322	**1.38** (0.79-2.40)
HWE Analysis	*p*-value = **0.0347**				*p*-value=**1**					
	Significant				non-Significant					

p: Two-tailed Fisher's exact probability; *CI: confidence interval, * N: allele drop-outs; *OR: odd ratio.

Conclusion

In this study, Age is considered a risk factor for 2 type diabetes mellites. Significant variations were observed in values of biochemical markers, including HbA1c and lipid profiling (cholesterol, triglyceride, LDL, and VLDL, except HDL) in patients with T2DM. The serum level of LRP1 protein revealed no incidence of T2DM in patients with familial hypercholesterolemia. SNP (rs1800127) of LRP1 genotypes and allele frequencies in control and patient groups demonstrated that there was a significant difference in the frequencies of the heterozygous (CT) in genotype (rs1800127), and there are significant differences between observed and expected allele frequencies of genotype for this SNP. SNP (rs7968719) of LRP1 genotypes and allele frequencies in the control and patient group demonstrated that there was a significant difference in the frequencies of the heterozygous (CG) in genotype (rs7968719) and there are significant differences

between observed and expected allele frequencies of genotype for this SNP. In SNP (rs7968719) of LRP1, the allele G is considered a risk factor for T2DM.

REFERNCES

Actis Dato, V., & Chiabrando, G. A. (2018). The role of low-density lipoprotein receptor-related protein 1 in lipid metabolism, glucose homeostasis and inflammation. International Journal of Molecular Sciences, 19(6), 1780.

Al-Hamdani, H. M. S. (2019). Effect of stevia leaves consumption on sugar and other blood characters in diabetes-induced mice. IRAQI JOURNAL OF AGRICULTURAL SCIENCES, 50(6).

Asiimwe, D., Mauti, G. O., & Kiconco, R. (2020). Prevalence and risk factors associated with type 2 diabetes in elderly patients aged 45-80 years at Kanungu District. Journal of Diabetes Research, 2020.

Au, D. T., Strickland, D. K., & Muratoglu, S. C. (2017). The LDL receptor-related protein 1: at the crossroads of lipoprotein metabolism and insulin signaling. Journal of Diabetes Research, 2017.

Bennett, C. M., Guo, M., & Dharmage, S. C. (2007). HbA1c as a screening tool for detection of type 2 diabetes: a systematic review. Diabetic Medicine, 24(4), 333–343.

Bommer, C., Sagalova, V., Heesemann, E., Manne-Goehler, J., Atun, R., Bärnighausen, T., Davies, J., & Vollmer, S. (2018). Global economic burden of diabetes in adults: projections from 2015 to 2030. Diabetes Care, 41(5), 963–970.

Braunwald, E., Fauci, A. S., Hauser, S. L., Longo, D. L., & Jameson, J. L. (2005). Harrison's principles of internal medicine. McGraw-Hill Companies, Inc.

Chang, C., Tang, X., Mosallaei, D., Chen, M., Woodley, D. T., Schönthal, A. H., & Li, W. (2022). LRP-1 receptor combines EGFR signalling and eHsp90α autocrine to support constitutive breast cancer cell motility in absence of blood supply. Scientific Reports, 12(1), 1–12.

Dabelea, D., Mayer-Davis, E. J., Saydah, S., Imperatore, G., Linder, B., Divers, J., Bell, R., Badaru, A., Talton, J. W., & Crume, T. (2014). Prevalence of type 1 and type 2 diabetes among children and adolescents from 2001 to 2009. Jama, 311(17), 1778–1786.

Durrington, P. (2003). Dyslipidaemia. The Lancet, 362(9385), 717–731.

Etique, N., Verzeaux, L., Dedieu, S., & Emonard, H. (2013). LRP-1: a checkpoint for the extracellular matrix proteolysis. BioMed Research International, 2013.

Gale, E. A. M., & Gillespie, K. M. (2001). Diabetes and gender. Diabetologia, 44(1), 3–15.

Galicia-Garcia, U., Benito-Vicente, A., Jebari, S., Larrea-Sebal, A., Siddiqi, H., Uribe, K. B., Ostolaza, H., & Martín, C. (2020). Pathophysiology of type 2 diabetes mellitus. International Journal of Molecular Sciences, 21(17), 6275.

Ghareeb, Z. A., AlKhafaji, H. A.-H., & Al-Saedi, M. K. A. (2021). The Effect of LRP-1 Gene and Mir-205 on Cardiovascular Disease in Iraqi Patients with Familial Hypercholesterolemia. Journal of Applied Sciences and Nanotechnology, 1(3), 24–31.

Gyawali, P., & Regmi, P. (2011). Association between glycaemic control and serum lipid profile in type 2 diabetic patients: Glycated haemoglobin as a dual biomarker Post chemotherapy Complications in Breast cancer View project Hemorheology and Microcirculation View project. https://www.researchgate.net/publication/215774283

Hussein, S. I., Kaluf, A. F., Ahmed, Y., Ahmed, B., & Iyad, A. (2020). Determination of inhibition activity of α-Amylase enzyme, antioxidant activity, antibacterial activity and phenolic compounds by using some medical plants. The Iraqi Journal of Agricultural Science, 51(1), 411–421.

Li, Y., Chen, S., Zhao, T., & Li, M. (2021). Serum IL-36 cytokines levels in type 2 diabetes mellitus patients and their association with obesity, insulin resistance, and inflammation. Journal of Clinical Laboratory Analysis, 35(2), e23611.

Lillis, A. P., Mikhailenko, I., & Strickland, D. K. (2005). Beyond endocytosis: LRP function in cell migration, proliferation and vascular permeability. Journal of Thrombosis and Haemostasis, 3(8), 1884–1893.

Liu, C.-C., Hu, J., Tsai, C.-W., Yue, M., Melrose, H. L., Kanekiyo, T., & Bu, G. (2015). Neuronal LRP1 regulates glucose metabolism and insulin signaling in the brain. Journal of Neuroscience, 35(14), 5851–5859.

Llorente-Cortes, V., Casani, L., Cal, R., Llenas, A., Juan-Babot, O., Camino-López, S., Sendra, J., & Badimon, L. (2011). Cholesterol-lowering strategies reduce vascular LRP1 overexpression induced by hypercholesterolaemia. European Journal of Clinical Investigation, 41(10), 1087–1097.

Machado-Alba, J. E., Medina-Morales, D. A., & Echeverri-Cataño, L. F. (2016). Evaluation of the quality of life of patients with diabetes mellitus treated with conventional or analogue insulins. Diabetes Research and Clinical Practice, 116, 237–243.

Mallick, B., & Ghosh, Z. (2011). A complex crosstalk between polymorphic microRNA target sites and AD prognosis. RNA Biology, 8(4), 665–673.

Miedema, K. (2005). Standardization of HbA1c and optimal range of monitoring. Scandinavian Journal of Clinical and Laboratory Investigation, 65(sup240), 61–72.

Peters, A. L., Buschur, E. O., Buse, J. B., Cohan, P., Diner, J. C., & Hirsch, I. B. (2015). Euglycemic diabetic ketoacidosis: a potential complication of treatment with sodium–glucose cotransporter 2 inhibition. Diabetes Care, 38(9), 1687–1693.

Punthakee, Z., Goldenberg, R., & Katz, P. (2018). Definition, classification and diagnosis of diabetes, prediabetes and metabolic syndrome. Canadian Journal of Diabetes, 42, S10–S15.

Quinn, G. Z., Abedini, A., Liu, H., Ma, Z., Cucchiara, A., Havasi, A., Hill, J., Palmer, M. B., & Susztak, K. (2021). Renal histologic analysis provides complementary information to kidney function measurement for patients with early diabetic or hypertensive disease. Journal of the American Society of Nephrology, 32(11), 2863–2876.

Rahelić, D. (2016). Of IDF diabetes atlas--call for immediate action. In Lijecnicki vjesnik (Vol. 138, Issues 1–2, pp. 57–58).

Saeedi, P., Petersohn, I., Salpea, P., Malanda, B., Karuranga, S., Unwin, N., Colagiuri, S., Guariguata, L., Motala, A. A., & Ogurtsova, K. (2019). Global and regional diabetes prevalence

estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas. Diabetes Research and Clinical Practice, 157, 107843.
Sanada, H., Yokokawa, H., Yoneda, M., Yatabe, J., Yatabe, M. S., Williams, S. M., Felder, R. A., & Jose, P. A. (2012). High body mass index is an important risk factor for the development of type 2 diabetes. Internal Medicine, 51(14), 1821–1826.
SAS. (2018). Statistical Analysis System, User's Guide. Statistical. Version 9.6th ed. SAS. Inst. Inc. Cary. N.C. USA.
Selvin, E., Wattanakit, K., Steffes, M. W., Coresh, J., & Sharrett, A. R. (2006). HbA1c and peripheral arterial disease in diabetes: the Atherosclerosis Risk in Communities study. Diabetes Care, 29(4), 877–882.
Shoback, D. M., & Gardner, D. G. (2018). Greenspan's basic & clinical endocrinology. McGraw-Hill Education.
World Health Organization. (2019). The top 10 causes of death.
Yang, Y., Qiao, J., & Li, M. (2010). Association of polymorphisms of interleukin-18 gene promoter region with polycystic ovary syndrome in chinese population. Reproductive Biology and Endocrinology, 8(1), 1–7.