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Study of Lipoprotein Lipase Gene Variants in Dyslipidemic Type-2 Diabetes Mellitus

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ABSTRACT

Background: Diabetic dyslipidemia is an important factor in the development of diabetic vascular complications. It may be caused or aggravated by variants of the genes coding for enzymes and proteins involved in lipoprotein metabolism as lipoprotein lipase (LPL).

Objective: To identify the genotype distributions of LPL (rs320) and (rs1801177) and to study its associative role in the development of diabetic dyslipidemia in Egyptian patients.

Subjects & Methods: This cross-sectional case-control study was conducted on 200 subjects, 140 patients with T2DM and 60 sex- and age-matched control subjects. Lipoprotein lipase (LPL) gene variants (rs320 & rs1801177) were genotyped by real-time PCR using the allele discrimination by TaqMan assay.

Results: Diabetic patients with dyslipidemia had a higher frequency of TT (wild) genotype of LPL (rs320) in comparison to non dyslipidimic patients (p=0.034). However, there was no difference between any of studied groups regarding LPL (rs1801177) genotype distributions. Mutant variant of LPL rs1801177 gene of diabetic dyslipidemia group was associated with increased triglycerides (TGs)
, cholesterol, low density lipoprotein cholesterol (LDLc) (p= 0.001), & decreased high density lipoprotein cholesterol (HDLc) (p=0.004) .Also, mutant variant of LPL rs320 gene had higher levels of TGs, cholesterol, & LDLc levels (p= 0.011, 0.001, 0.001 respectively), and lower levels of HDLc cholesterol (p=0.001) in the same group.

Conclusions: Association was detected between LPL (rs320) & (rs1801177) gene variants and dyslipidemia in diabetic patients. The interaction of LPL (rs320) and LPL (rs1801177) possibly plays a role in diabetic dyslipidemia.

Keywords: LPL, Real time PCR, Dyslipidemia, Diabetes mellitus.

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disease of high prevalence, characterized by hyperglycaemia with disruption of protein and lipid metabolism. T2DM is mainly caused by insulin resistance among other factors. T2DM affects nearly 15.6 % of Egyptians aged 20 - 79 years, hence it's considered as a public health issue of great impact on morbidity, mortality and health care resources [1]. T2DM patients are at increased risk of vascular complications including cardiovascular disease (CVD), dyslipidemia is a key factor for such aggravated risk [2].

Alterations in the characteristics of lipoproteins (both quantitative and qualitative), genetic predispositions, lipoprotein metabolism degradation, and environmental factors are the primary causative agents of dyslipidemia in diabetes. Researchers have revealed that variants of genes coding proteins and enzymes involved in lipoprotein metabolism may play a significant role the occurrence of diabetic dyslipidemia [3].

The gene of lipoprotein lipase (LPL) codes for a LPL protein made up of four hundreds and seventy five amino acids and is made up of 10 exons and 9 introns, found in region 21.3 on the short arm of chromosome 8. (8p21.3). LPL hydrolyzes TG in circulating lipoproteins, which has an impact on serum levels of TG. This is the process that limits the removal of lipoproteins from the body, including those produced by endogenous sources like VLDL and exogenous sources like chylomicrons [4].

Deficiency or dysfunction due to genetic variations of LPL resulted in various diseases as atherosclerosis, obesity & diabetic dyslipidemia & insulin resistance. In T2DM, the increased TGs serum level and the decreased HDL level is commonly related to dysfunction or deficient LPL activity [5]. Variants of LPL found in 88 locations of DNA. Functional single nucleotide variation affect whether synthesis or function of LPL including (rs328), which is considered among the most important variations of this gene [6]. The LPL (rs320) (minor allele A/G) contributes to an improved lipid profile, it has a protective effect (low TG and LDL and high HDL). Conversely, the existence of the common allele (T) is connected to atherogenic dyslipidemia with higher TG blood levels [4].

The purpose of this research was identification of the LDL (rs320) and (rs1801177) genotype distributions and studies its associative role in the development of diabetic dyslipidemia in Egyptian patients.

SUBJECTS & METHODS

Our study was case-control one conducted over 6 months from January 2021 to August 2022 including 200

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participants; 140 patients with T2DM who were following up at Diabetes Outpatient Clinic, Kasr Alainy Hospitals and 60 age- and sex- matched healthy subjects as the control group. The T2DM patients were classified into two subgroups: **Subgroup I** included 70 diabetic patients with dyslipidemic blood profile and **subgroup II** that included 70 diabetic patients without dyslipidemia.

The diagnosis of T2DM was based on fasting blood glucose (FBG) levels of ≥ 126 mg/dL and/or postprandial glucose levels of ≥ 200 mg/dL and/or hemoglobin A1c (HbA1c) levels of ≥ 6.5%. While, the presence of diabetic dyslipidemia was defined as total cholesterol > 200 mg/dl, total triglyceride > 200 mg/dL, LDL > 130 mg/dl and HDL < 40 mg/dl , patients shouldn't have other endocrinological disorders as thyroid disorders.

Sample size:

The sample size was calculated using G*Power software for sample size calculation [7]. Measuring the relationship between gene variant and the lipid profile is the primary outcome. So, it is calculated that a sample size of 140 patients and 60 control (total 200 patients) achieves 80% power to detect a medium effect size (Cohen *f*) of 0.28 assuming a numerator degree of freedom of 1 and a confidence level of 95% (alpha error of 0.05).

Ethical approval:

Ethical committee of Kasr Al-Ainy, Faculty of Medicine approved the used procedures (MS-22-2022) and written consents were obtained from patients after the protocol of work was fully explained. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

DNA Extraction and Genotyping

Three ml peripheral venous blood samples were collected into sterile (EDTA) vacutainer tubes. The genomic DNA was isolated from the blood using a QIAamp DNA blood mini kit (Qiagen, Germany) then stored at - 20°C.

- Genotyping of the (rs1801177) and (rs320) of LPL gene was done across all participants' sample sets using the TaqMan Allelic Discrimination Assay Kit (probe ID C___8804865_30 and C___1843003_10 respectively, Applied Biosystems, Foster City, CA, USA), and these SNPs were analyzed on Step One Real-Time Polymerase Chain Reaction System (Applied biosystems CA94404, Foster City, USA).

Statistical analysis:

The statistical package for the social sciences (SPSS) (IBM Corp., Armonk, NY, USA) was used. For quantitative values, standard deviation, mean, and median values were used. Frequencies and percentages were used to represent categorical variables. For comparisons of normally distributed quantitative variables, unpaired t test or ANOVA analysis was used. For the multiple comparisons post hoc test was used. When the expected frequency is less than 5, Chi square test was used to compare categorical data [8]**.** Correlations between quantitative variables were calculated by Pearson correlation coefficient**.**

RESULTS

This study consisted of 200 participants divided over three groups as the following: 70 diabetic dyslipidemic patients, 70 diabetic non-dyslipidemic patients, and 60 healthy control subjects.

HbA1c, FBG, total cholesterol, and triglyceride levels showed highly significant increase in the group with diabetic dyslipidemia than the non-dyslipidemic group ($p < 0.001$). Also, those parameters were higher in the patient groups (diabetic dyslipidemic and non-dyslipidemic) than in the healthy control ($p<0.001$). Regarding HDL level, it was significantly lower in both diabetic dyslipidemia and non-dyslipidemia than in the control group ($p=0.03$).

Among the diabetic dyslipidemic group the majority received Statins 46 (65.7%), 4 (5.7%) received Fibrates, 3 (4.3%) were on combined statin and fibrates, whereas 17 (24.3%) didn't receive any lipid lowering agents (Table 1).

Table (1): Characteristics of clinical and biochemical parameters of the study groups.

	G1 diabetic dyslipidemic patients (No.=70)	G2 diabetic non-dyslipidemic patients (No.=70)	G3 control non diabetic patients (No.=60)	P-value		
				P1	P2	P3
Sex (No., %) Male Female	 35 (50%) 35 (50%)	 35 (50%) 35 (50%)	 30 (50%) 30 (50%)	1.00 [a]	1.00 [a]	1.00 [a]
Age (Years) (mean±SD)	49±10	49.3±10.8	48.2±11	0.866 [b]	0.852 [b]	0.852 [b]
HbA1c (%) (mean±SD)	8.65±2.2	7.38±1.0	4.8±0.3	<**0.001** [c]	<**0.001** [c]	<**0.001** [c]
FBG (mg/dl) (mean±SD)	213.6±8.8	149.6±5.9	102.4±4	<**0.001** [c]	<**0.001** [c]	<**0.001** [c]
TG (mg/dl) (mean±SD)	287.54±48.6	172.7±31.5	108.9±17	<**0.001** [c]	<**0.001** [c]	<**0.001** [c]
Cholesterol (mg/dl) (mean±SD)	301.38±66.2	176.8±20.29	153.9±23.2	<**0.001** [c]	<**0.001** [c]	<**0.001** [c]
LDL (mg/dl) (mean±SD)	218.8±7.4	115.2±13.9	104.3±19	<**0.001** [c]	<**0.001** [c]	<**0.001** [c]
HDL (mg/dl) (mean±SD)	26.38±3.7	25.1±5.9	27.5±5.5	0.311 [c]	**0.033** [c]	**0.033** [c]
Lipid lowering drugs (No., %) Statins alone Fibrates alone Combined Not taken	 46 (65.7%) 4 (5.7%) 3 (4.3%) 17 (24.3%)	-	-	-	-	-

a: chi-square test. b:independent samples t-test. c: Mann-whitney test. p1= between diabetic dyslipidemic& diabetic non-dyslipidemic. p2= between diabetic dyslipidemic & control. p3= between diabetic non-dyslipidemic & control

Higher frequency of the TT genotype of LPL (rs320) was found in patients with diabetic dyslipidemia than in the diabetic non dyslipidemia group (p=0.034). However, there was no significant difference between any of studied groups regarding LPL (rs1801177) genotype distributions as illustrated in table (2).

Table (2): Genotype distributions and allele frequencies of LPL rs1801177, and LPL rs320 gene variants in the study groups.

Gene/SNP	Genotype/ haplotype	cases		Control subjects (No., %)	P-value *		
		Patients with diabetic and dyslipidemia (No., %)	Patients with diabetic not dyslipidemia (No., %)		P1	P2	P3
LPL rs1801177	Wild (GG)	56 (80%)	62 (88.6%)	46 (76.7%)	0.206	0.837	0.142
	Hetero (AG)	7 (10%)	6 (8.6%)	8 (13.3%)			
	Mutant (AA)	7 (10%)	2 (2.9%)	6 (10%)			
LPL rs320	Wild (TT)	40 (57.1%)	26 (37.1%)	32 (53.3%)	**0.034**	0.796	0.161
	Hetero (AT)	23 (32.9%)	38 (54.3%)	23 (38.3%)			
	Mutant (AA)	7 (10%)	6 (8.6%)	5 (8.3%)			

*: chi-square test.p1= between diabetic dyslipidemic & diabetic non-dyslipidemic. p2= between diabetic dyslipidemic& control. p3= between diabetic non-dyslipidemic & control.

Regarding the association of LPL gene variants and lipid profile parameters, mutant variant of *LPL* rs1801177 gene of diabetic dyslipidemia group had higher levels of TGs, cholesterol, LDL-c levels (p= 0.001), and lower levels of HDL-c (p=0.004). Also, mutant variant of LPL Rs320 gene of diabetic dyslipidemia group had higher levels of TGs, cholesterol, LDL-c levels (p= 0.011, 0.001, 0.001 respectively), and lower levels of HDL-c (p=0.001). While, no significant association was found between the studied gene variants & lipid-lowering agents in the diabetic dyslipidemia group. These results are demonstrated in tables (3) & (4).

Table (3): Relation between LPL Rs1801177 gene polymorphism and demographic and biochemical parameters among all study groups.

variable	group	*LPL* rs1801177			p-value
		wild	mutant	hetero	
Sex Male Female	G1	27 (48.2%) 29 (51.8%)	2 (28.6%) 5 (71.4%)	6 (85.7%) 1 (14.3%)	0.085 [a]
	G2	30 (48.4%) 32 (51.6%)	2 (100%) 0 (0%)	3 (50%) 3 (50%)	0.356 [a]
	G3	23 (50%) 23 (50%)	3 (50%) 3 (50%)	4 (50%) 4 (50%)	1.00 [a]
Age (mean±SD)	G1	49±10.09	47.85±14.1	50.28±5.6	0.906 [c]
	G2	50.1±12	46.5±6.36	42±8.76	0.263[c]
	G3	47.39±10.48	47.3±12.5	49.37±9.27	0.844 [c]
TG (mean±SD)	G1	276.25±41.26	362.85±51.15	302.57±29.65	**0.001** [b]
	G2	173.7±31.35	165±35.35	165.16±37.56	0.385 [b]
	G3	110.3±17.8	98.5±1.22	108.3±17.3	0.083 [b]
Cholesterol (mean±SD)	G1	294.63±62.7	394.4±13.58	262.35±43.4	**<0.001** [b]
	G2	176.95±18.7	193.5±3.53	169.6±35.08	0.315 [b]
	G3	151.67±22.16	155.3±6.26	150.6±29.9	0.752 [b]
LDL (mean±SD)	G1	213.98±6.25	305.42±10.76	171.21±5.58	**<0.001** [b]
	G2	115.35±13	127±1.4	109.83±22.8	0.193 [b]
	G3	102±17.7	113.6±8.8	102.8±25.1	0.22 [b]
HDL (mean±SD)	G1	27.18±4.09	15.85±3.8	30.42±5.3	**0.004** [b]
	G2	25.25±5.8	18.5±3.19	26.16±3.24	0.446 [b]
	G3	27.3±3.5	31.3±2.3	26±6.2	0.134 [b]
Lipid lowering drugs (No., %) Statins alone Fibrates alone Combined Not taken	G1	 37 (66.1%) 3 (5.4%) 3 (5.4%) 13 (23.2%)	 7 (100%) 0 (0%) 0 (0%) 0 (0%)	 2 (28.6%) 1 (14.3%) 3 (5.4%) 4 (57.1%)	0.141 [a]

a: Chi-square test. b: Kruskal-wallis test. c: One way ANOVA test.G1=among diabetic dyslipidemic group. G2=among diabetic non-dyslipidemic group. G3=among control group.

Table (4): Relation between LPL Rs320 gene polymorphism and demographic and biochemical parameters among all study groups.

variable	group	*LPL* rs320			p-value
		wild	mutant	hetero	
Sex Male Female	G1	18 (45%) 22 (55%)	4 (57.1%) 3 (42.9%)	13 (56.5%) 10 (43.5%)	0.627 [a]
	G2	13 (50%) 13 (50%)	1 (16.7%) 5 (83.3%)	21 (55.3%) 17 (44.7%)	0.214 [a]
	G3	18 (56.3%) 14 (43.7%)	2 (40%) 3 (60%)	10 (43.5%) 13 (56.5%)	0.579 [a]
Age (mean±SD)	G1	48.25±9.3	50.5±15.4	49.8±9.8	0.761 [c]
	G2	48.88±11.11	50.66±12.42	49.42±12.53	0.945 [c]
	G3	50.3±11.7	48.2±9.1	47.2±10.6	0.593 [c]
TG (mean±SD)	G1	280.6±47.1	348±53.5	281.1±37.4	**0.011** [b]
	G2	178.8±24.66	187.66±11.92	166.2±36.37	0.273 [b]
	G3	109.2±12.2	121.6±4.1	105.6±13.3	0.683 [b]
Cholesterol (mean±SD)	G1	290.58±6.58	388.28±15.8	293.7±56.18	**0.001** [b]
	G2	176.19±13.67	187.16±14.9	175.57±24.34	0.180 [b]
	G3	154±21.5	173.2±5.5	149.6±26.1	0.133 [b]
LDL (mean±SD)	G1	206.56±8.03	304.1±10.1	214.26±58.3	<**0.001** [b]
	G2	115.96±10.03	121.16±10.14	113.75±16.49	0.385 [b]
	G3	105.1±18.6	112.8±8.8	101.3±20.9	0.499 [b]
HDL (mean±SD)	G1	28.7±5.68	15.8±3.3	25.5±3.1	**0.001** [b]
	G2	24.88±5.9	27.66±4.45	24.92±3.24	0.616 [b]
	G3	27±4.9	25.8±1.09	27.08±6.2	0.534 [b]
Lipid lowering drugs (No., %) Statins alone Fibrates alone Combined Not taken	G1	 25 (62.5%) 2 (5%) 1 (2.5%) 12 (30%)	 5 (71.4%) 0 (0%) 1 (14.3%) 1 (14.3%)	 16 (69.6%) 2 (8.7%) 1 (4.3%) 4 (17.4%)	0.656 [a]

a: Chi-square test. b: Kruskal-wallis test. c: One way ANOVA test. p1=among diabetic dyslipidemic group. p2=among diabetic non-dyslipidemic group. p3=among control group.

DISCUSSION

The Occurrence or prevention of any disease in human is related to the gene /environment interplay. Similarly, scientists have proposed a link between lipid serum levels and certain interactions between genetic variations and environmental considerations [9]. Results of studies that targeted *LPL* gene variants and their association with dyslipidemia and so predisposition to cardiac diseases and thrombotic events are conflicting, with no general agreements on the role of certain variants in the development of dyslipidemia [10]

One of the most prevalent *LPL* gene variations is the *LPL* (rs320) variant at which thymine transition to guanine base occurred at position +495 in intron [4] According to NCBI *LPL* (rs320) variant has the major allele (T) and minor allele that is either (G) or (A), with worldwide frequencies T=79.785%, A=0.001% and G= 20.21%[12] .

What was intriguing about our study was that while no (G) allele was found in any of the groups, we discovered allele (A) in our study group with frequencies of 37% in diabetic dyslipidemic, 5 % in diabetic without dyslipidemia and 33 % in control group. This allele was not analyzed in Egyptians or even similar ethnic groups as Jewish and Mediterraneans. Hence, there is shortage of data regarding its frequency that needs further studies with larger sample size. According to our knowledge, the association between *LPL* (rs1801177) and (rs320) gene variants and diabetic dyslipidemia were not previously studied in Egyptian patients. Our study is the first one to examine the possibility of such association.

Regarding *LPL* (rs320) TT, AT, AA genotypes, the distribution was 57.1%, 32.9%, and 10% in dyslipidemic group, 37.1%, 54.3%, and 8.6% in non-dyslipidemia group, and 53.3%, 38.3%, and 8.3% in the control subjects respectively. Genotype TT was significantly higher in the dyslipidemic compared to non-dyslipidemic group **(p=0.034).** Our results are similar to **Bogari *et al.*[13]** and **Abu-amero *et al***. [14] where both studies examined *LPL* (rs320) in Saudi Patients with CAD and the genotype frequencies were (TT 47.0%, GT 42.9% & GG 10.2%) and (TT 53.7%, TG 39.2% & GG, 7.1%), respectively. In contrast to our results, **Vardarlı *et al.*** [15] detected high prevalence of the *LPL (rs320)* mutant genotype in Turkish diabetic dyslipidemic patients as their distribution was (TT 10.1%, TG 38%, & GG 51.9%). While, the distribution of genotype in control subjects was close to our results (TT 69.8%, GT 25.5%, & GG 4.7%).

In the current study *LPL* (rs1801177) genotype distribution was GG 80%, AG 10%, and AA 10% in diabetic dyslipidemic patients, GG 88.6%, AG 8.6%, and AA 2.9% in diabetic non-dyslipidemic patients, and GG 76.7%, AG 13.3%, and AA 10% in the control subjects. Close to ours were **Malek *et al.*** [16] analyzed the above mentioned genotype in Kuwaiti cohort, the frequency was (GG 97%, AG 3% and AA 0%). Contradictory to our results, **Daoud [17]** study included patients from Saudi Arabia with CAD and the genotype distribution was (GG 5.14%, AG 23.72% and AA 71.14%) in CAD patients and (GG 1.93%, AG 16.43% and AA 81.64%) in healthy control subjects. We believe that due to population-dependent penetrance, age, and sample size, discrepancy in some studies can be attributed to heritability variations.

We analyzed the association of *LPL* gene variants and lipid profile parameters, mutant variant of *LPL* Rs1801177 gene of diabetic dyslipidemia group had higher levels of TGs, cholesterol, LDL-c levels (p= 0.001), and lower levels of HDL-c (p=0.004). Also, mutant variant of *LPL* Rs320 gene of diabetic dyslipidemia group had higher levels of TGs, cholesterol, *LDL* levels (p= 0.011, 0.001, 0.001 respectively), and lower levels of HDL cholesterol (p=0.001).

On the other hand, **I *et al.* [18]** found that the AT and TT genotypes in (rs320) variant is associated with high HDL-c concentrations and low TG and LDL-c concentrations. **Similar to our results, Moghadasi *et al.*** [19] study clarified that the interaction of *LPL* rs1801177 and rs320 variants were associated with different level of HDL-c. Subjects having no mutant genotype of either variant had high levels of HDL-c. The minor allele of rs1801177 variant was found to be related to low HDL-c levels and high LDL-c levels in Iranian adolescents [19]. **Corsetti *et al.*[20]** found that increased CVD risk can be attributed to rs1801177 variant that affects serum lipids. Another case–control study, on the other hand failed to detect significant correlation between rs1801177 genotype and dyslipidemia [21].

CONCLUSION

Association detected between *LPL* (rs320) (rs1801177) gene variants and dyslipidemia in diabetic patients. The interaction of *LPL* (rs320) and *LPL* (rs1801177) possibly plays a role in diabetic dyslipidemia.

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