Assessment of the impact lipoprotein lipase gene variation on the response to rosuvastatin therapy in coronary artery disease patients in Al Najaf Governorate

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Abstract

Background: One of the first line commonly used oral HMG reductase inhibitor is rosuvastatin. The variants of pharmacokinetic genes have not demonstrated a consistent and significant influence on rosuvastatin response in patients with coronary artery, in candidate gene studies. In addition, only few genome-wide signals associated with rosuvastatin pharmacological response have been detected to the present time.

Aim of the study: To study the impact of Hind iii gene single nucleotides polymorphisms (rs 320) on rosuvastatin response in coronary artery disease patients in Al-Najaf governorate.

Patients and methods: The current prospective cohort study included 51 patients with coronary artery disease. The work with the study is dated back to December 2021 to September 2022. The study was carried out at internal medicine department in Al- Sader Teaching Hospital-Najaf- Iraq. The enrolled patients with coronary artery disease have been diagnosed by a cardiologist. The age ranged from 23 to 65 years. The first blood sample was taken at time zero (time of diagnosis without treatment) and the second blood sample was taken after 8 weeks (after starting treatment) for each patient.

Results: The mean serum of triglyceride level, total cholesterol, VLDL-C, LDL-C, interleukin 6 level and mean body mass index (BMI) significantly reduced (p<0.0001). while serum of HDL-C and lipoprotein lipase level significantly increased (p< 0.0001) after treatment with rosuvastatin. The LPL rs320 genotyping was: wild allele (TT), heterozygote variant (TG) and homozygote variant (GG).

Conclusions: In our research heterozygous variant most distributed then homozygous normal and the less distributed was homozygous variant. Rosuvastatin has been shown to be effective in improvement of serum lipid profile, lipoprotein lipase level and interleukin 6 level.

Keywords: coronary artery, cholesterol, Al-Najaf governorate

Introduction

Coronary artery disease is a heterogenous, caused by plaque buildup in the wall of the arteries that supply blood to the heart (called coronary arteries). Plaque is made up of cholesterol deposits. Plaque buildup causes the inside of the arteries to narrow over time. This process is called atherosclerosis (Insull Jr, 2009). A common symptom of coronary artery disease (CAD) is angina. Angina is chest pain or discomfort that occurs if an area of your heart muscle doesn't get enough oxygen-rich blood, may feel like pressure or squeezing in your chest. You also may feel it in your shoulders, arms, neck, jaw, or back. Angina pain may even feel like indigestion with shortness of breath and fatigue. The pain tends to get worse with activity and go away with rest. Emotional stress also can trigger the pain (Kanna & Packirisamy, 2023) [12].

Statin will be choosing as 'Wonder Drugs' in CVD prevention. Whereas the pharmacogenomics research is still in the early development, these understanding will be already effectively converted from floor to bedside for other medicines such as immune-suppressants (Singer *et al*., 2016) [29]. Based on the Framingham Risk Scores, there are different thresholds that indicate whether treatment should be initiated (Pearson *et al*., 2021) [22]. Individuals with a score of >20% are considered to have a high cardiovascular risk, a score of 10-19% indicates an intermediate risk, and patients with a score less than 10% are at low risk. Statin therapy and non-pharmacological interventions are indicated in those with high cardiovascular risk. In those at intermediate risk or low risk, the use of statin therapy depends on individual patient factors such as age, cholesterol levels, and risk factors Pearson. Statins cause a 20%-22% relative reduction in major cardiovascular events (heart attack, stroke, coronary revascularization, and coronary death) for every 1 mmol/L reduction in LDL (Schubert *et al*., 2021) [28]. This study aimed to assesses the impact of Hind iii gene single nucleotides polymorphisms (rs 320) on Rosuvastatin response in coronary artery disease patients in Al-Najaf governorate.

Patients, materials, and methods

Patient

The current prospective cohort study included 51 patients with coronary artery disease, 29 males (57%) and 22 females (43%)

with a male-to-female ratio of 1.32:1. During the study time, 94 patients were included in the study; however, dropped cases were 43 patients because of poor compliance of those patients. The work with the study is dated back to December 2021 to September 2022. The study was carried out at the internal medicine department in Al- Sader Teaching Hospital-Najaf-Iraq. While Lab. Work of samples occurred in the laboratory of Al- Sader Teaching Hospital-Najaf-Iraq for biochemical tests (lipid profiles), Research Center in Faculty of Pharmacy in University of Kufa for biochemical tests (human Lipoprotein Lipase ELISA assay and human Interleukin 6 ELISA assay) and laboratory of Department of Clinical Bio-Chemistry in Faculty of Pharmacy in University of Kufa for gene analysis. The age ranged from 23 to 65 years. The first blood sample was taken at time zero, and the second blood sample was taken after eight weeks for each patient.

Materials

Kits and chemicals used in the present study are shown in Table (1).

Table 1: Kits and chemicals used in the present study Item

N.	Kit and chemical	Company and country of origin
1	Agarose	Bioworld (U.S.)
2	Ethidium bromide solution	Promega (USA)
3	Kit for 2x PCR Taq master mix	Solgent (Korea)
4	Kit for DNA extraction	FavorPrep (Taiwan)
5	Kit for human lipoprotein lipase	BT LAB Bioassay Technology Laboratory (England)
6	Kit for HDL and LDL	Biolabo (France)
7	Kit for human Interleukine - 6	BT LAB Bioassay Technology Laboratory (England)
8	Kit for Triglyceride	Biolabo (France)
9	Kit for Cholesterol	Biolabo (France)
10	Ladder for DNA 100bp	Biolabs (England)
11	Nuclease free water	Promega (USA)
12	Primers	Alpha DNA (Canada)
13	Restriction enzyme Hind iii, 5,000u	Promega (USA)
14	Tris BE buffer (10X)	Promega (USA)

Methods

Permission of the ethical committee

The procedure has been explained to the patient or relative for permission besides permission from the Kufa University/ Pharmacy Faculty Ethical Committee.

Body mass index was calculated according to the following equation: BMI (kg/m2) = Weight (kg)/[Height (m)]2 (Weir & Jan, 2019) [30]. BMI was calculated before treatment at time zero and following eight weeks after treatment.

Collection and processing of blood specimens

About 10 milliliters (ml) of venous blood were drawn from all 51 patients: 5ml was transferred into a sterile EDTA tube with gentle mixing to extract the genome DNA. The other 5ml of blood was collected with a gel tube left for 10 minutes a room temperature and then centrifuged for 10 min at a speed of 3000 rpm to get the serum. Finally, the serum aliquot was placed into 1.5 ml Eppendorf tubes to avoid frequent freezing and thawing then stored at-20 °C until time for biochemical measurements, including cholesterol, triglycerides, high-density lipoprotein, human lipoprotein lipase ELISA assay and human Interleukin 6 ELISA assay.

Measurement of the lipid profiles

Serum levels of cholesterol, TG, HDL, LDL, and VLDL were estimated by the Baiolabo (France) kit in lab.

Measurement of complete fasting cholesterol (TC) concentrations

Total cholesterol (TC) determination depends on an enzymatic procedure. All samples have been assessed in 500 nm (Artiss & Zak, 2000) [3].

Cholesterol−ester $Cholesterol-esterase\rightarrow$ Cholesterol + FFA

Cholesterol+O2 $Cholesterol-oxidase\rightarrow$ Cholesterol−4−one−3+H2O2

Phenol+2H2O2+4−Amino−Antipyrine $Peroxidase\rightarrow$Quinoeimine colored complex+4H2O

Preparation of reagent

Reagent 2 (Enzymes) ingredient quickly are added to (Buffer) reagent 1 and softly blended until full dissolution (roughly 2 minutes).

Procedure

A group of test tubes is arranged according to number of samples beside blank and stander and each one will contain

Sample=1 ml of working reagent+10 µl of serum

Blank= 1 ml of working reagent + 10 µl D.W

Stander = 1 ml of working reagent + 10 µl of stander sol.

Then each tube will be incubation in water bath at 37° C for 5 minutes then read absorption at 500 nm.

Calculation

Cholesterol Concentration= (Absorbance of sample / Absorbance of stander) * Stander concentration

Standard Cholesterol concentration =200 mg/dl

Detection of fasting triglycerides (TG) levels

Estimation of TG concentration depends on an enzymatic method which is illustrated in the reaction below:

$Triglyceride+3\ H2O\ Lipase \rightarrow FFA+Glycerol$

$Glycerol+ATP \quad Glcerokinase\leftrightarrow Glycerol-3-phosphate+ADP \quad Glycerol-3-phosphate+O2 \quad Glyceroloxydase\leftrightarrow 2OH-acetone\ phosphate+H2O2$

$H2O2+4\ Chlorophenol+PAP\ Peroxidase\rightarrow$

$Quinoeimine+H2O$

Preparation of reagent
Reagent 2 (Enzymes) ingredient quickly added to (Buffer) reagent 1 and softly blended until full dissolution (roughly 2 minutes).

Procedure
A group of test tubes is arranged according to number of samples beside blank and stander and each one will contain:
Sample=1 ml of working reagent+10 µl of serum
Blank= 1 ml of working reagent + 10 µl D.W
Stander = 1 ml of working reagent + 10 µl of stander sol.
Then each tube will be incubation in water bath at 37° C for 5 minutes then read absorption at 500 nm.

Calculation
T.G concentration = (Absorbance of sample / Absorbance of stander) * Stander concentration
Standard triglyceride concentration = 200 mg / dl
Estimation of high-density lipoprotein cholesterol (HDL-C) levels.

Procedure
A group of test tubes is arranged according to number of samples beside blank and stander and each one will contain
Blank= 1 ml of working reagent + 25 µl D.W
Stander = 1 ml of working reagent + 25 µl of stander sol.
Sample=500 µl of serum sample + 50 ml of HDL-C sol. Then put in water path at 37° C for 5 minutes after that centrifugation for 15 minutes.
Take 25µl from supernatant and add it on other can tube that has same number of sample and contain 1 ml of cholesterol sol. Then each tube will be incubated in water bath at 37° C for 5 minutes then absorption is read at 500 nm.

Calculation
HDL-C concentration= (Absorbance of sample / Absorbance of stander) * Stander concentration
Standard HDL-C concentration = 100mg / dL

Determination of LDL-cholesterol
LDL-cholesterol could be measured by indirect method using Friedewald equation: LDL-cholesterol (mg/dl) = Total cholesterol – (HDL–cholesterol + VLDL cholesterol (Friedewald *et al*., 1972).

Determination of VLDL-cholesterol
VLDL-cholesterol could be measured by indirect method using Friedewald's Equation: VLDL-c = Triglycerides/5

Biochemical ELISA Tests
Blood samples of 45 out of 51 patients before treatment and 45 of 51 patients after 8 weeks treatment for evaluations of lipid levels, Human Lipoprotein Lipase ELISA assay and Human Interleukin 6 assay were obtained from patients between (9-11 A.M.), after at least 12 hours of fasting.

Human Interleukin 6 ELISA assay
From BT LAB Bioassay Technology Laboratory kit
Catalog number E0090Hu, Standard Curve Range: 2-600ng/L, Sensitivity: 1.03ng/L, Size: 96 wells.

Intended use
This sandwich Kit is for the accurate quantitative detection of Human Interleukin 6 in serum.

Assay principle
This Kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with a Human IL-6 antibody. IL-6 present in the sample is added and binds to antibodies coated on the wells. And then, a biotinylated Human IL-6 Antibody is added and binds to IL-6 in sample. Then Streptavidin-HRP is added and binds to the Biotinylated IL-6 antibody. After incubation, unbound Streptavidin-HRP has washed Away during a washing step. Substrate solution is added, and color develops proportionately to the amount of Human Il-6. The reaction is terminated by the addition of an acidic stop solution, and absorbance is measured at 450 nm.

Statistical analysis
The statistical analysis was performed by (SPSS) statistical package of social science software computer program (version 26/2019, SPSS inc., and Chicago, IL, USA). And descriptive statistics such as mean, number, percentage, and standard deviation were used to describe the data. Categorical variables were expressed as absolute numbers and percentages, and continuous variables were expressed as mean and SD in this cohort study; the ANCOVA (Analysis of covariance) test was used to test the significance level (*p* value) for all parameters between the study group. Paired T-test was used to determine if there is a significant difference between the means of two groups; also, Chi-square test was used to evaluate the association between any two categorical variables provided that less than 20 % of cells have an expected count of less than 5. P. Value <0.05 refer to statistically significant; *p* value < 0.001 refer to statistically highly significant; *p* value > 0.5 refer to non-statistically significant.

Study results
The total number of patients in risk of coronary artery disease in this study was (51) cases and the distribution of studied cases according to demographics characteristics according to number of cases was illustrated in Table (2). The age of the cases enrolled in this study was divided in to two groups (<50 years :19, >=50years:32cases), the gender of the cases enrolled in this study was divided in to two groups (male:29, female:22cases), the smoking state of the cases enrolled in this study was divided in to two groups (smoker:30, non-smoker:21 cases), the diabetic state of the cases enrolled in this study was divided in to two groups (diabetic:28, non-diabetic:23cases) and the hypertensive state of the cases enrolled in this study was divided in to two groups (hypertensive:29, non-hypertensive:22cases), the weight state of the cases enrolled in this study was divided in to two groups (BMI <25:13, BMI>=25:38cases).

Table 2: Relationship of patients demographic characteristics and clinical characteristics with polymorphism genotyping

Patient's characters			Patient's polymorphism N=51			Total	*p* value*
			Heterogeneous (n=35)	Homogenous (n=7)	Wild (n=9)		
Gender	Females	Count	13	4	5	22	0.441
		%	59.1%	18.2%	22.7%	100.0%	
	Males	Count	22	3	4	29	
		%	75.9%	10.3%	13.8%	100.0%	
Total		Count	35	7	9	51	
		%	68.6%	13.7%	17.6%	100.0%	
Age group (Year)	<50	Count	12	4	4	20	0.496
		%	60.0%	20.0%	20.0%	100.0%	
	≥ 50	Count	23	3	5	31	
		%	74.2%	9.7%	16.1%	100.0%	
Total		Count	35	7	9	51	
		%	68.6%	13.7%	17.6%	100.0%	
smoking	yes	Count	19	5	6	30	0.611
		%	63.3%	16.7%	20.0%	100.0%	
	no	Count	16	2	3	21	
		%	76.2%	9.5%	14.3%	100.0%	
Total		Count	35	7	9	51	
		%	68.6%	13.7%	17.6%	100.0%	
DM	yes	Count	21	1	6	28	0.063
		%	75.0%	3.6%	21.4%	100.0%	
	no	Count	14	6	3	23	
		%	60.9%	26.1%	13.0%	100.0%	
Total		Count	35	7	9	51	
		%	68.6%	13.7%	17.6%	100.0%	
Hypertension	yes	Count	23	3	3	29	0.157
		%	79.3%	10.3%	10.3%	100.0%	
	no	Count	12	4	6	22	
		%	54.5%	18.2%	27.3%	100.0%	
Total		Count	35	7	9	51	
		%	68.6%	13.7%	17.6%	100.0%	
BMI before treatment	< 25	Count	9	3	1	13	00.351
		%	25.7%	42.9%	11.1%	25.5%	
	≥ 25	Count	26	4	8	38	
		%	74.3%	57.1%	88.9%	74.5%	
Total		Count	35	7	9	51	
		%	100.0%	100.0%	100.0%	100.0%	

In our study, the response of patient to Rosuvastatin that effect on cholesterol, triglyceride (TG), low density lipoprotein (LDL), high density lipoprotein (HDL), very low density lipoprotein (VLDL), concentration of lipoprotein lipase (LPL) and Interleukin 6 (IL6) consider statistically highly significant (*p* value <=0.001) which mean good response to treatment *p* value > 0.05 that consider statistically nonsignificant.

Table 3: Mean difference of lipid profile, lipoprotein lipase concentration, Interleukin 6 and weight status before and after treatment with Rosuvastatin

Lipid profile		Mean	N	Std. Deviation	Std. Error Mean	*p* value
VLDL	Before treatment	46.7843	51	19.39104	2.71529	0.0001
	After treatment	37.7961	51	15.69002	2.19704	
TG	Before	233.3333	51	97.82447	13.69817	0.0001
	After	186.3922	51	76.61595	10.72838	
HDL	Before	44.0588	51	8.79639	1.23174	0.0001
	After	49.0000	51	8.34026	1.16787	
CH	Before	239.4118	51	60.90851	8.52890	0.0001
	After	184.3922	51	51.58491	7.22333	
LDL	Before	167.5098	51	46.02277	6.44448	0.0001
	After	142.6078	51	40.72202	5.70222	
LPL conc	Before	56.9247	45	43.66950	6.50986	0.0001
	After	61.9691	45	43.65966	6.50840	
IL6	Before	63.8580	45	44.41577	6.62111	0.0001
	After	59.4580	45	43.76420	6.52398	
BMI	Before	27.5490	51	3.87460	0.54255	0.0001
	After	26.5098	51	3.34887	0.46894	

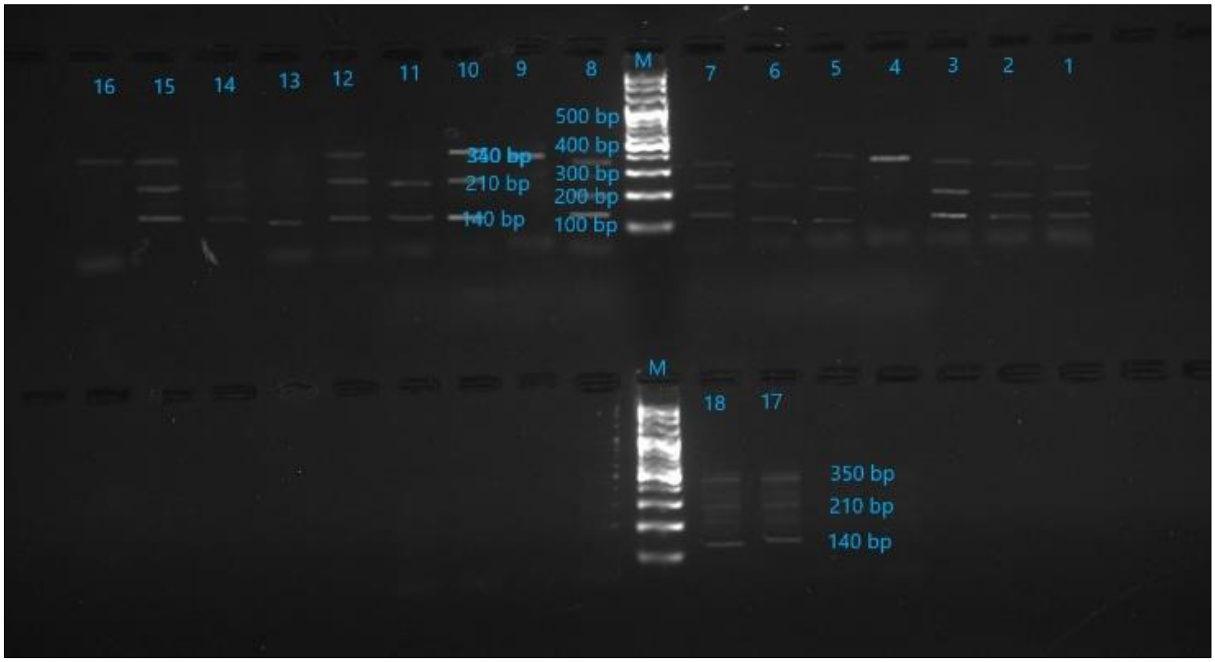

Fig 1: Example of RFLP pattern of LPL rs320 polymorphism analyzed on 1.5% agarose gel for 1 hour at 100 V show by UV light using BioMetra Imaging System/Germany. Where M: ladder. (1,2,3,5,7,8,10,12,13,14,15,17,18): sample with heterozygous variant (TG) represent (210bp+140bp) wild allele with (350bp) variant allele. (4,9,16): sample with Homozygous variant (GG) represent (350bp) variant allele. (6,11): homozygous normal (TT): (210bp+140bp) wild allele

Table 4: Restriction analysis result detected LPL rs 320 genotyping

Genotype polymorphism	Number of bands	Band size (bp)
Heterogenous variant (TG)	3	350
		210
		140
Wild type (TT)	2	210
		140
Homogenous variant (GG)	1	350

Distribution of studied cases according to LPL rs 320 genotyping and association between genotyping and treated group

The total number of cases that involved in genotyping was (51) cases. The genotypic distribution of LPL rs 320 polymorphism and allele frequency of T and G alleles was show in table (5).

Table 5: Distribution of (51) study cases after follow up by LPL gene rs 320 genotyping

LPL rs 320 SNP	N.	%	Allelic frequency	N	%
(TG)	35	68.6	T	53	52
(GG)	7	13.7	G	49	48
(TT)	9	17.7	-	-	-
Total	51	100	-	102	100

TG: Heterozygous variant. GG: Homozygous variant. TT: Homozygous normal, N: number, %: percentage

Mean difference of triglyceride level before and after treatment with polymorphism shown in Figure (2).

As result in Mean difference of triglyceride in heterogenous (48.6), homogenous (70.86) and wild (21.89).

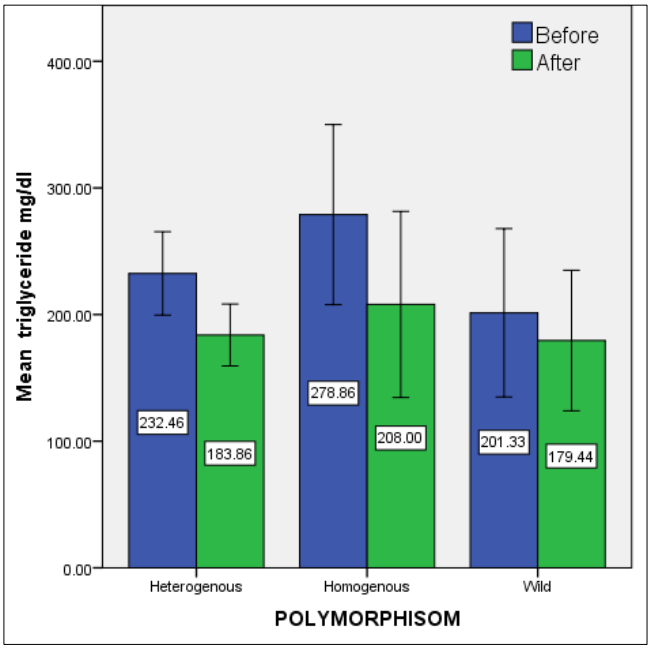

Fig 2: Mean difference of triglyceride level before and after treatment with polymorphism

Mean difference of total cholesterol level before and after treatment with polymorphism shown in Figure (3). As result in Mean difference of total cholesterol in heterogenous (60.91), homogenous (20.72) and wild (58.77).

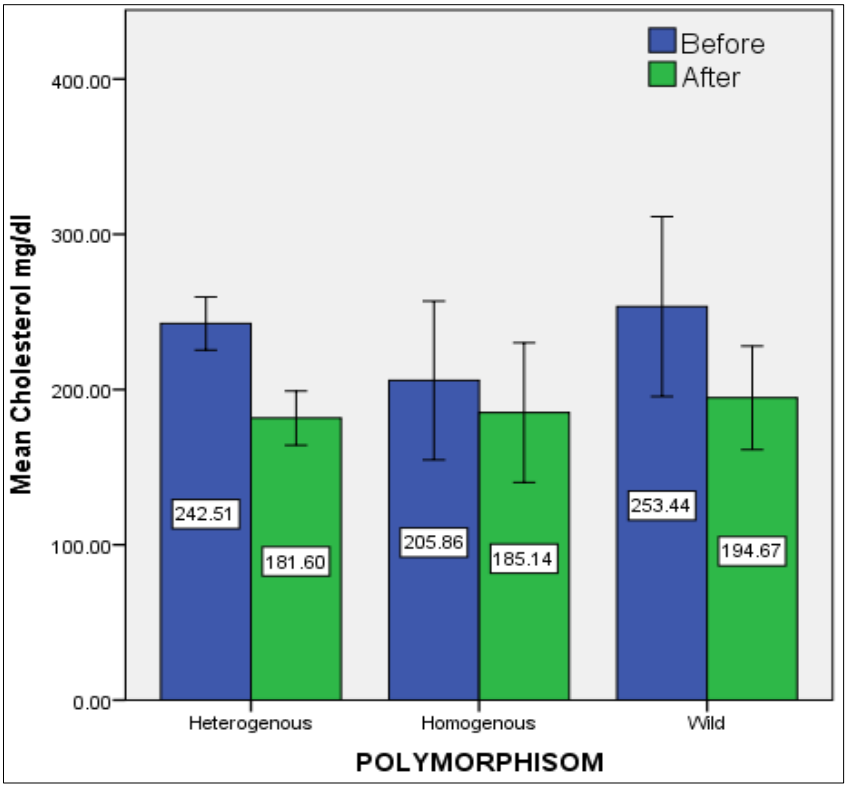

Fig 3: Mean difference of total cholesterol level before and after treatment with polymorphism Mean difference of HDL level before and after treatment with polymorphism shown in Figure (3). As result in Mean difference of HDL in heterogenous (-6.54), homogenous (0.14) and wild (-2.66).

Mean of serum lipids after treatment by polymorphism show in table (3.6). There is no significant between heterogenous, homogenous and wild (*p>0.05)*. Except HDL level it's significant (*p<0.024)*. Make multiple Comparisons of mean HDL after use rosuvastatin to identify which polymorphism affected show in table (3.7).

Found wild (TT) polymorphism significant (*p<0.006)* when compare with heterogenous (T/G).

Table 6: Mean of serum lipid after treatment in relation to LPL SNP (rs 320) polymorphism

	ff	N	Mean	Std. Deviation	95% Confidence Interval for Mean		*p* value
					Lower Bound	Upper Bound	
VLDL	Heterogenous	35	37.6000	14.85459	32.4973	42.7027	0.707
	Homogenous	7	41.9429	19.10557	24.2732	59.6126	
	Wild	9	35.3333	17.46425	21.9091	48.7575	
	Total	51	37.7961	15.69002	33.3832	42.2090	
TG	Heterogenous	35	183.8571	72.23619	159.0431	208.6711	0.724
	Homogenous	7	208.0000	97.23511	118.0725	297.9275	
	Wild	9	179.4444	83.23928	115.4611	243.4278	
	Total	51	186.3922	76.61595	164.8436	207.9407	
HDL	Heterogenous	35	50.7143	7.60639	48.1014	53.3272	0.024
	Homogenous	7	49.0000	9.81495	39.9227	58.0773	
	Wild	9	42.3333	7.33144	36.6979	47.9688	
	Total	51	49.0000	8.34026	46.6543	51.3457	
CH	Heterogenous	35	181.6000	51.62147	163.8674	199.3326	0.801
	Homogenous	7	185.1429	59.45987	130.1516	240.1341	
	Wild	9	194.6667	50.01000	156.2256	233.1078	
	Total	51	184.3922	51.58491	169.8837	198.9006	
LDL	Heterogenous	35	143.1143	43.99116	128.0028	158.2258	0.701
	Homogenous	7	131.7143	35.49983	98.8824	164.5462	
	Wild	9	149.1111	32.27787	124.3001	173.9221	
	Total	51	142.6078	40.72202	131.1546	154.0611	

Table 7: Multiple Comparisons of mean HDL after use of rosuvaststin

Dependent Variable	Polymorphisom	Polymorphisom	Mean Difference (I-J)	Std. Error	*p* value	95% Confidence Interval	
						Lower Bound	Upper Bound
HDL2	Heterogenous	Homogenous	1.71429	3.25968	0.601	-4.8397	8.2683
		Wild	8.38095*	2.94242	0.006	2.4648	14.2971
	Homogenous	Heterogenous	-1.71429	3.25968	0.601	-8.2683	4.8397
		Wild	6.66667	3.96756	0.099	-1.3106	14.6440
	Wild	Heterogenous	-8.38095*	2.94242	0.006	-14.2971	-2.4648
		Homogenous	-6.66667	3.96756	0.099	-14.6440	1.3106

According to table (6), there was no significant difference in mean LDL ($p = 0.701$); therefore, we the chose the cutoff value of < 130 mg/dl for LDL to split the group of patient into two groups, patients with LDL < 130mg/dl were considered as controlled group ($n = 26$), and the group with LDL of ≥ 130mg/dl were considered uncontrolled group ($n = 25$). We found also no significant association between LPL SNP (rs 320) polymorphism and control according to a LDL level cutoff of < 130mg/dl ($p = 0.317$), as shown in table (7).

Discussions

Age plays a vital role in the deterioration of cardiovascular functionality, resulting in an increased risk of cardiovascular disease (CVD) in older adults (Rodgers *et al*., 2019) [26]. The prevalence of CVD has also been shown to increase with age, in both men and women, including the prevalence of atherosclerosis and myocardial infarction (Rodgers *et al*., 2019) [26]. The American Heart Association (AHA) reports that the incidence of CVD in men and women is ~40% from 40–59 years, ~75% from 60–79 years, and ~86% in those above the age of 80 (McGarry & Shenvi, 2021) [20]. Also with aging, CAD prevalence increases after 35 years of age in both men and women (Sanchis-Gomar *et al*., 2016) [27]. The lifetime risk of developing CAD in men and women after 40 years of age is 49% and 32%, respectively (Sanchis-Gomar *et al*., 2016) [27]. At 50 years of age, lifetime risks were 61.7% (95% CI, 59.1 to 64.3) for men and 39.2% (95% CI, 37.0 to 41.4) for women (Lloyd-Jones *et al*., 2006) [17].

In patients with atherosclerotic coronary artery disease, cigarette smoking increases myocardial oxygen demand that cause an inappropriate decrease in coronary blood flow and vasoconstriction (Winniford *et al*., 1986) [32]. In a meta-analysis study, they found an increased risk of coronary artery disease in smokers compared with nonsmokers (Qin *et al*., 2013) [24]. A 2015 meta-analysis revealed that smoking resulted in a 51% increased risk (21 studies, RR 1.51, 95% CI 1.41.1-62) of coronary heart disease in patients with diabetes (Brown *et al*., 2020) [4]. In our study, the distribution of rosuvastatin was more effective than other statins in reducing LDL, triglyceride, and total cholesterol levels (Bullano *et al*., 2006) [5]. Significantly more patients taking rosuvastatin than patients taking other statins attained their LDL goals (Bullano *et al*., 2006) [5].

For 17 802 patients in the JUPITER trial, rosuvastatin 20 mg per day reduced the incidence of the primary endpoint of first non-fatal myocardial infarction or stroke, hospitalization for unstable angina, arterial revascularization, or cardiovascular death by 44% (p<0·0001) (Ridker *et al*., 2012) [25].

In our study, the mean of lipid profile (LDL, VLDL, TG, total CH and HDL) before and after treatment it is significant (*P* value <0.0001) table (2).

In other randomized trial in the cardiovascular unit of a tertiary care hospital in Pakistan from January till December 2018, use rosuvastatin 40mg for four weeks, they found mean change in lipid profile: LDL ($p < 0.0001$), VLDL ($p< 0.0001$), Total CH ($p < 0.0001$), TG ($p<0.04$) and HDL ($p <0.09$) (Kumar *et al*., 2019) [16].

In other study, one-hundred and eight trials (18 placebo-controlled and 90 before-and-after) evaluated the dose-related efficacy of rosuvastatin in 19,596 participants. Rosuvastatin 10 to 40 mg/day caused LDL-cholesterol decreases of 46% to 55%, when all the trials were combined using the generic inverse variance method (Adams *et al*., 2014) [1].

Lipoprotein lipase (LPL) hydrolyze triglyceride (TG) in circulating chylomicrons and very low-density lipoprotein (VLDL) on the surface of endothelial cells (Nilsson-Ehle *et al*., 1980). It has been demonstrated that a reduced concentration of plasma LPL mass is associated with an increased risk of coronary artery disease (Hitsumoto *et al*., 2002). Study in Japan, reported that a pre-heparin serum LPL mass (pre-LPL mass) reflected the level of functioning LPL activity in the whole body and could be deeply involved in the progression of coronary atherosclerosis of stable organic angina pectoris (Hitsumoto *et al*., 2002).

Multiple regression analysis revealed that a low pre-LPL mass and hypertriglyceridemia were independent risk factors for AMI (t value=2.1, 2.4). The result indicates that a low pre-LPL mass may be an important risk factor for AMI and stable organic angina pectoris (Hitsumoto *et al*., 2002).

Mild decreases in LPL activity are thought to contribute to the hypertriglyceridemic atherogenic phenotype in Type 2 diabetes and the metabolic syndrome (Meksawan, 2004).

In our study, found significant effect of rosuvastain treatment will increase mean level of lipoprotein lipase ($p<0.0001$) table (3.2).

Mean difference of interleukin 6 level before and after treatment with Rosuvastatin

IL-6 increased C-reactive protein (CRP) secretion by liver show in figure (3). Rosuvastatin reduced CRP expression by 46% (Mayer *et al*., 2007). Rosuvastatin reduced CRP mRNA by 73% compared to IL-6-stimulated cells. IL-6 activated the transcription factors STAT3 up to 2.6-fold. Rosuvastatin attenuated the activation of STAT3 by 48% (Mayer *et al*., 2007).

Study in Korea included thirty (30) patients who had been diagnosed by coronary artery disease, after doubling the dose of statin, The interleukin-6 concentration also significantly decreased from 8.55 pg/dL to 4.81 pg/dL ($p<0.001$) (Kim *et al*., 2012). In our study, found significant effect of rosuvastain treatment will decrease mean level of Interleukin 6 ($p<0.0001$) table (2).

Statin use is associated with increased calorie intake and

consequent weight gain. It is speculated that statin-dependent improvements in lipid profile may undermine the perceived need to follow lipid-lowering and other dietary recommendations leading consequently to increased calorie intake (Ward *et al*., 2019). However, increases in calorie intake in statin users may also be related to statin-dependent decreases in satiety factors such as leptin, an adipocyte-derived adipokine (Barra *et al*., 2020). In our study, found different expected BMI value after use rosuvastatin table (2).

Mean of BMI decreased from 27.5490 kg/m2 to 26.5098 kg/m2.

Conclusions

In accordance with the results and findings reached in this work, the following conclusions were obtained:

1. The coronary artery disease was most distributed in age more than 50 year, in male gender than female, hypertensive, diabetic, obese and smoker patient.
2. There were good responses to treatment in serum biomarker that include lipid profile, lipoprotein lipase level and interleukin 6 level.
3. Heterozygous variant was the most distributed one then homozygous normal and the less distributed was homozygous variant.
4. There is no association among LPL SNP (rs 320) polymorphism with demographic and some clinical characteristics.

Recommendations

Based on the conclusions obtained here, it is recommended that:

1. Performing study with control group to determine the dominant gene in healthy person.
2. Performing study with different study design to overcome the problem of losing patients through follow up.
3. Study other SNP polymorphism in relation to disease.
4. Search other center in study.
5. Determine lipoprotein lipase and interleukin 6 activity in relation to another treatment.

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