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EVALUATION THE INFLUENCE OF LIPOPROTEIN LIPASE GENE POLYMORPHISM ON ROSUVASTATIN TREATMENT OUTCOME IN PATIENTS WITH CORONARY ARTERY DISEASE IN AL NAJAF GOVERNORATE

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Abstract

Background: Coronary artery disease is a heterogeneous, complex metabolic disorder characterized by elevated cholesterol and triglyceride concentrations called dyslipidemia, It is classified according to lipoprotein level and other further classes.

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 an 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.

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).

Physicians and basic researchers classify dyslipidemias in two distinct ways. One way is its

presentation in the body (including the specific type of lipid that is increased)(McCance & Huether, 2018).The other way is due to the underlying cause for the condition (genetic, or secondary to another condition)(McCance & Huether, 2018). This classification can be problematic, because most conditions involve the intersection of genetics and lifestyle issues. However, there are a few well-defined genetic conditions that are usually easy to identify(Hasnat et al., n.d.). 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). Based on the Framingham Risk Scores, there are different thresholds that indicate whether treatment should be initiated(Pearson et al., 2021). 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). 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 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 8 weeks for each patient.

Materials

Kits and chemicals used in the present study are shown in table (1). Instruments and equipment used in the present study are shown in table (2.2).

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)

Table.2: Instruments and equipment used in the present study

Item	Devices	Company and country of origin
1	Balance	Sartoriius (Germany)
2	Electrophoresis	Biocom direct (Korea)
3	Freezer	Egur (Turkey)
4	Thermomixer	Eppendorf (USA)
5	Microcentrifuge	Eppendorf (USA)
6	Minispin	Eppendorf (USA)
7	Biodrop	Biochrom (UK)
8	Thermocycler (PCR)	Biometra (Germany)
9	Photo documentation system	Biometra (Germany)
10	Vortex	Cyan (Belgium)
11	ELISA system	Bio-Tech (USA)
12	Spectrophotometer	EMC lab. (Germany)

Methods

Permission of the Ethical Committee: The procedure has been explained to the patient or relative for permission beside permission from 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).

BMI was calculated before treatment at time zero and following 8 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, for extraction of the genome DNA. The other 5ml of blood were collected with gel tube left for 10 minutes in a room temperature then centrifuged for 10 min at speed of 3000 rpm to get serum. Finally, the serum aliquot into 1.5 ml Eppendorf tubes to avoid frequent frozen 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 Baiolabo (France) kit in lab.

Measurement of complete fasting cholesterol (TC) concentrations

Total cholesterol (TC) determination depend on an enzymatic procedure. All samples has been assessed in 500 nm (Artiss & Zak, 2000).

Cholesterol−ester $\xrightarrow{Cholesterol-esterase}$ Cholesterol+ FFA

Cholesterol+O2 $\xrightarrow{Cholesterol-oxidase}$ Cholesterol−4−one−3+H2O2

Phenol+2H2O2+4−Amino−Antipyrine $\xrightarrow{Peroxidase}$ 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 $H2O$ $Lipase \rightarrow FFA$+$Glycerol$

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

$H2O2+4\ Chlorophenol+PAP\ Peroxidase\rightarrow Quinoeimine+H2O$

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

Study Results

Table (3): Distribution of studied cases according to demographics characteristics after follow up

		Number Total (51)	Percentage %
Age	< 50 years	20	38%
	≥ 50 years	31	62%
Gender	Male	29	57%
	Female	22	53%
Smoking	Smoker	30	59%
	Non smoker	21	41%
Diabetic	diabetic	28	55%
	Non- diabetic	23	45%
Hypertension	Hypertension	29	57%
	Non hypertension	22	43%
Weight status	BMI <25	13	26%
	BMI >=25	38	74%

Significant difference of serum biomarker before and after Rosuvastatin treatment

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 as show in table (3-2) .

Table (3-2) : Mean difference of lipid profile , lipoprotein lipase concentration , Interleukin 6 and weight status before and after treatment with Rosuvastatin

3.3. Gene polymorphism study

3.3.1. Detection of LPL rs 320 genotyping

Highly molecular weight with good purity and integrity was obtained from (51) extracted samples in figure (3-1).

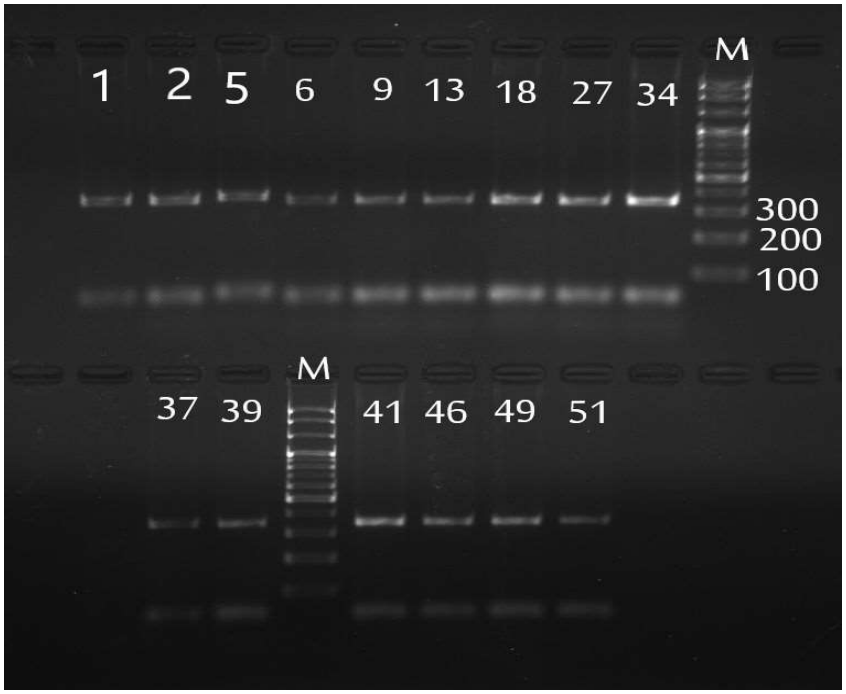

Figure (3.1) Example of DNA bands show by UV light using the BioMetra Imaging System/Germany purified on 1.5% agarose gel at 100v for 30 min. where M: ladder 1kp, (1-51): extracted genomic DNA of high molecular weight**.**

The amplified PCR products (350bp) as show in figure (3.2)

Mean of lipid profile before and after treatment with Rosuvastatin in relation to polymorphism

Mean difference of LDL level before and after treatment with polymorphism shown in Figure (3.5).

As result in Mean difference of LDL in heterogenous (28.09) , homogenous (20.72) and wild (15.78).

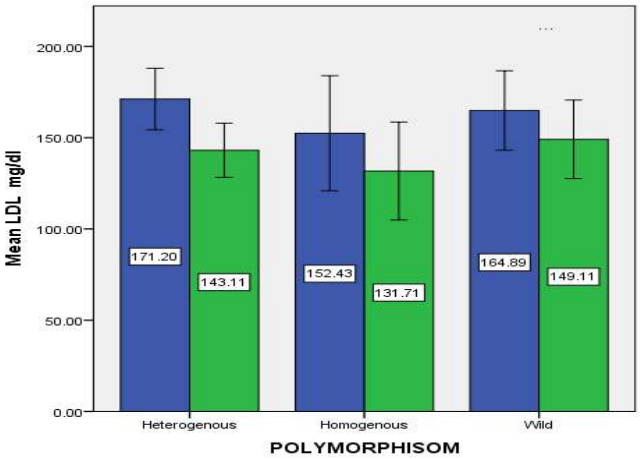

Figure (3.5): Mean difference of LDL level before and after treatment with polymorphism , blue column : before treatment , green column : after treatment

Mean difference of VLDL level before and after treatment with polymorphism shown in Figure (3.6). As result in Mean difference of VLDL in heterogenous (9.03) , homogenous (13.63) and wild (5.23).

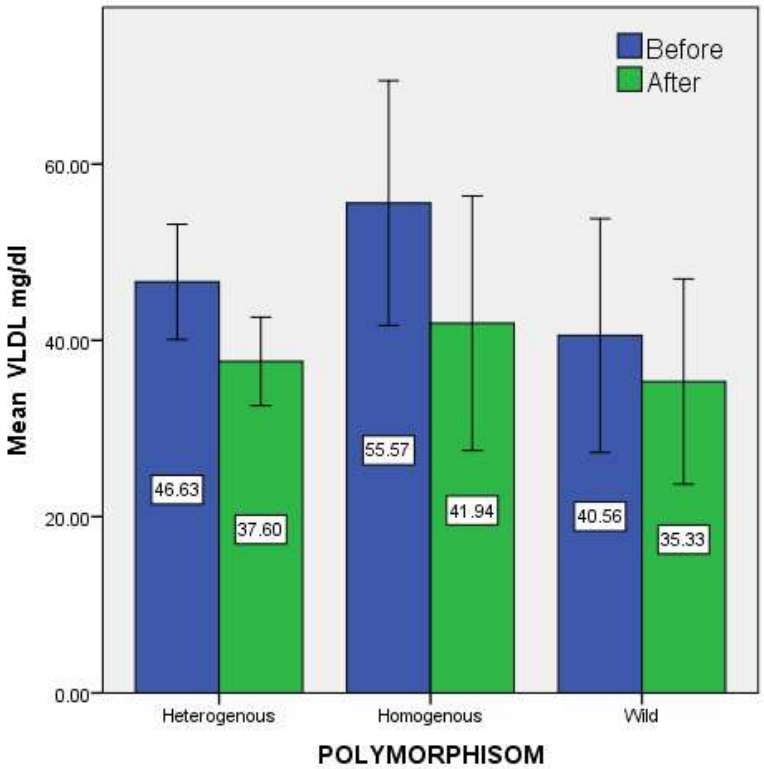

Figure (3.6): Mean difference of VLDL level before and after treatment with polymorphism

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). 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). 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).

Also with aging, CAD prevalence increases after 35 years of age in both men and women (Sanchis-Gomar et al., 2016). 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).

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).

In other study in Iran from January 2005 to December 2015, data for 90,094 patients were included in this analysis, total of 61,684 (68.5%) were men and 28,410 (31.5%) were women (Hosseini et al., 2021).

In other study, gender distribution in dyslipidemia that was affected males rather than females (52% versus 48%) respectively, because estrogen effect which has protective effect on the women against cardiovascular disease (Mansour et al., 2017).

As in our study, the distribution of coronary artery patients according to age (=>50year: 62 %) more than (<50 year: 38 %) table (3.1). That included 51 patients, 29 males (57 %) and 22 females (43 %) with a male to female ratio of 1.32:1 respectively.

4.1.2. Distribution of patients according to Weight state and smoking.

Overweight and obesity contribute to the development of cardiovascular disease (CVD) in general and coronary artery disease in particular in part by their association with traditional and nontraditional CVD risk factors (Katta et al., 2021).

Over 80% of patients with CHD are overweight or obese (Ades & Savage, 2017).

Patient classified as overweight and obese (body mass index [BMI] ≥25 kg/m2) based on National Institutes of Health recommended guidelines (Misra, 2015).

In our study, the distribution of patients according to weight state, in the BMI > 25 kg/m2:74 % more than BMI < 25 kg/m2: 24% table (3-1).

Smoking is known to increase total cholesterol (TC), triglycerides (TG), and low-density lipoprotein (LDL), while acting to decrease the cardio-protective high-density lipoprotein (HDL)(Campbell et al., 2008).

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).

In a meta-analysis study, they found an increased risk of coronary artery disease in smokers

compared with nonsmokers (Qin et al., 2013).
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).
In our study, the distribution of patients according to smoking. The smoker: 59 % more than non-smoker: 41 % table (3.1).
4.1.3. Distribution of patient according to Diabetic Mellitus and hypertension.
Diabetes is a common disease (hyperglycemia) with prevalence rates that are predicted to grow significantly over the next several decades (Hoff et al., 2003).The major cause of morbidity and mortality in diabetic patients is macrovascular atherosclerosis, most commonly in the coronary arteries (Hoff et al., 2003).
The incidence of hypertension, dyslipidemia, and current smoking status was significantly higher in diabetic patients than in non-diabetic patients (Cho et al., 2019).
Diabetic patients had significantly higher incidences of CAD (59.0% vs. 39.0%) and obstructive CAD (15.0% vs. 6.6%) than did non-diabetic patients (all $p < 0.001$)(Cho et al., 2019).
Additionally, the incidences of calcified plaque (44.5% vs. 26.9%), non-calcified plaque (25.0% vs. 16.2%), and mixed plaque (16.6% vs. 7.7%) were significantly higher in diabetic patients than in non-diabetic patients (all $p < 0.001$) (Cho et al., 2019).
In our study, the distribution of patients according to diabetic. Diabetic: 55 % more than non-diabetic: 45 % table (3.1).
Blood vessels damaged by high blood pressure can narrow and rupture the artery (Kassell et al., 1982). High blood pressure can also cause blood clots to form in the arteries leading to coronary artery disease (Heinrich et al., 1995).
In the United States alone, it is estimated that 60 million people have hypertension, the estimate varying according to whether hypertension is defined as a blood pressure of >140/90 mm Hg or, as has emerged from the Sixth Joint National Commission on Hypertension (JNC VI) consensus conference, of >130/90 mm Hg. Coronary artery disease frequently accompanies hypertension (Williams, 2005).
Hypertensive elderlies were commonly found to already have target organ damage such as impaired renal function, silent myocardial infarction, strokes, transient ischemic attacks, retinopathy, or peripheral artery disease. At least 60% of older men and 50% of elderly women with hypertension in the Framingham study had one or more of these conditions (Kannel, 2009).
Studies in USA that include of >5,000 patients with coronary artery disease, half had hypertension (Pepine, 1998).
In our study, the distribution of patients according to hypertension. hypertension: 57 % more than non-hypertension: 43 % table (3.1).
4.2. Significant difference of serum biomarker after rosuvastatin treatment
4.2.1. Mean difference of lipid profile before and after treatment with Rosuvastatin
Rosuvastatin was more effective than other statins in reducing LDL, triglyceride, and total cholesterol levels (Bullano et al., 2006). Significantly more patients taking rosuvastatin than

patients taking other statins attained their LDL goals (Bullano et al., 2006).

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).

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 (3.2).

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).

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).

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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