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Impact of PCSK9 Gene Polymorphism on Atorvastatin Efficacy in a sample of Iraqi Hyperlipidemic Patients

A Thesis

Submitted to the Council of College of Pharmacy/University of Kerbala as a Partial Fulfillment Requirements for the Master Degree of Science in Pharmacology and Toxicology

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بسم الله الرحمن الرحيم

"يَرْفَعِ اللَّــهُ الَّذِينَ آمَنُوا مِنْكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ "

صدق الله العلي العظيم

سورة المجادلة ايه 11

Supervisor Certification

We certify that this thesis, which is titled (Impact of PCSK9 gene polymorphism on Atorvastatin efficacy in a sample of Iraqi hyperlipidemic patients) was prepared by (Khalid Qasim Mohammed) under our supervision at the College of Pharmacy / University of Kerbala as a partial requirement for the degree of Master of Science in Pharmacology and Toxicology.

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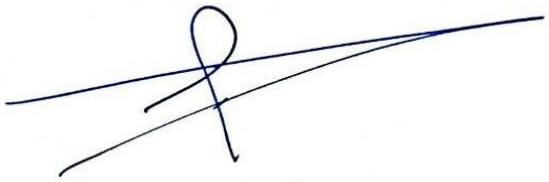

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Dedication

In loving memory of my dear mother,

who left this world but never my heart

Your love, guidance, and sacrifices shaped the person I am today.

Though you are no longer here to witness this milestone,

I feel your presence in every step I take.

This work is attribute to your unwavering belief in me

and the values you instilled in my heart.

May your soul rest in peace,

and may your legacy continue to inspire me forever.

With all my love,

Khalid

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With deep appreciation…

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Abbreviations	Full text
ADH	Autosomal dominant hypercholesterolemia
AIP	Atherogenic Index of Plasma
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
ATP	Adult treatment panel
BMI	Body mass index
CAD	Coronary artery disease
CHD	Coronary heart disease
CHOL-index	Cholesterol Index
COPD	Chronic obstructive pulmonary disease
CRI-I	Castelli's risk indexes I
CRI-II	Castelli's risk indexes II
CVA	Cerebrovascular accident
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
DNA	Deoxyribonucleic Acid
DRx	Duration of treatment
EDTA	Ethylene-diamine-tetra-acetic Acid
GOF	Gain of function
GTP	Guanosine Triphosphate
HDL	High density lipoprotein cholesterol
HMGCO-A reductase inhibitor	3-Hydroxy-3-methylglutaryl coenzyme A reductase inhibitors
IHD	Ischemic heart disease
LDL	Low density lipoprotein
LDLR	Low density lipoprotein receptor
LOF	Loss of function
Lp(a)	Lipoprotein a
mAbs	Mono clonal antibody
NOD	New onset diabetic

OxLDL	Oxidize LDL
PCR	Polymerase chain reaction
PCSK9	Proprotein Convertase Subtilisin/Kexin Type 9
RBS	Random blood sugar
ROS	Reactive oxygen species
SBP	Systolic blood pressure
SNPs	Single nucleotide polymorphisms
TB	Total bilirubin
TC	Total cholesterol
TG	Triglyceride
VLDL	Very low density lipoprotein

Abstract

Background: Hyperlipidemia, a disease characterized by elevated lipid levels in the blood, significantly increases the risk of atherosclerosis and cardiovascular diseases, particularly coronary heart disease. Clinical observations have shown that many hyperlipidemic patients continue to experience high blood cholesterol and low-density lipoprotein levels despite treatment with lipid lowering drugs like atorvastatin, a common statin used to decrease blood cholesterol levels. Proprotein convertase subtilisin/kexin type 9 plays an important role in the regulation of low-density lipoprotein metabolism via its effect on low-density lipoprotein receptor recycling. Genetic variations within the Proprotein convertase subtilisin/kexin type 9 gene (particularly rs28942111; T>A SNP) which is one of gain of function mutation which result in increasing the effect of Proprotein convertase subtilisin/kexin type 9 that may influence the therapeutic response to atorvastatin in lowering plasma cholesterol.

Aim of study: This study aims to investigate the impact of the Proprotein convertase subtilisin/kexin type 9 rs28942111; T>A SNP on the cholesterol lowering response to atorvastatin among sample of Iraqi patients diagnosed with primary hyperlipidemia. Additionally, it evaluates lipid profile parameters and atherogenic indices, including the Atherogenic Index of Plasma and Castelli Risk Indices I and II.

Methodology: A cross-sectional study was conducted involving 149 Iraqi patients aged between 28 and 85 years, all of whom were diagnosed with primary hyperlipidemia and had been taking a daily oral dose of 40 mg atorvastatin for a minimum six months. Lipid profile, liver function tests, and atherogenic indices (Atherogenic Index of Plasma and Castelli Risk Indices I and II) were assessed. Genotyping of the Proprotein convertase subtilisin/kexin type 9 rs28942111; T>A SNP was performed using allele-specific PCR.

Results: Among the 149 patients, 85.9% were TT allele carriers (the homozygous wild type) and 14.1% were AA allele carriers (the homozygous mutant type) of the rs28942111; T>A SNP. No heterozygous mutant type was detected (TA). AA allele carriers exhibited significantly higher levels of Low-density lipoprotein, total cholesterol, and aspartate transaminase compared to TT allele carriers with a p-value of 0.001. Additionally, 28.2% of patients showed a good response to atorvastatin, 35% had a moderate response, and 39% demonstrated poor or no response. Atherogenic Index of Plasma results indicated that 85% of patients were at high risk for ischemic heart disease and atherosclerosis, while Castelli Risk Indices I and II revealed that 54% and 21% patients, respectively, were at high risk for atherosclerosis and ischemic heart disease. Significant differences in body mass index, lipid profiles, and atherogenic Index of Plasma were observed between different age and duration of treatment groups.

Conclusion: The rs28942111 single nucleotide polymorphism in the PCSK9 gene appears to have a notable influence on lipid profiles and individual variability in response to atorvastatin therapy. Despite ongoing statin treatment, a considerable number of patients remain at elevated risk for ischemic heart disease, as reflected by atherogenic indices such as Atherogenic Index of Plasma and the Castelli Risk Indices. These findings indicate the importance of incorporating genetic screening and atherogenic risk evaluation into the management of hyperlipidemia, and using more personalized and effective therapeutic strategies.

Chapter One

Introduction

1.1 Hyperlipidemia

Hyperlipidemia is a disorder has long been recognized as a human health issue (Kashif et al., 2016). In 2002, research in cardiovascular epidemiology had clearly demonstrated a strong relation between elevated plasma cholesterol levels and the development of complications such as ischemic heart disease (IHD) (Darshan, 2019).

1.1.1. Definition

Hyperlipidemia is defined as conditions in which the cholesterol or triglyceride carrying lipoproteins levels in plasma exceeds the normal limits (Nagarthna et al., 2020). These lipoproteins localized in the interstitial space of arteries arising from the aorta, restricting the blood supply to the heart. This phenomenon is defined as atherosclerosis (Linton et al., 2019). Excessive accumulation of lipoproteins can obstruct coronary blood flow, leading to myocardial infarction (MI) (Upadhyay, 2023).

Worldwide, hyperlipidemia is recognized as a major and changeble risk factor for heart disease, significantly lead to both morbidity and mortality (Alloubani et al., 2021). In many parts of the world complain of a persistent rise of hyperlipidemia due to multiple factors, including poor dietary habits, obesity, sedentary lifestyles, and reduced physical activity (Uzogara, 2017). In Iraq, cardiovascular diseases are increasingly contributing to the national disease burden, ranking among the top 20 health concerns. This trend is expected to worsen in the coming years if current lifestyle and dietary patterns persist (Abd et al., 2019).

Cardiovascular disease (CVD) remains a leading global health concern, affecting populations in both Eastern and Western regions. Based on World Health

Organization data from 2017, coronary heart disease accounted for 18.5% of all deaths in Iraq, making it the leading cause of mortality among the top twenty causes of death. In this context, Iraq was ranked 19th globally for coronary heart disease-related deaths (Abd et al., 2019).

1.1.2.Types of lipids

1.1.2.A. Cholesterol

Cholesterol is an essential structural component of cell membranes in mammalian tissues and serves as a precursor for the synthesis of steroid hormones and bile acids. It exists in both free and esterified forms within animal cells, but is not present in plant derived fats (Zio et al., 2024).

1.1.2.B. Triglycerides

Triglycerides are the most prevalent type of lipid and are primarily stored in adipose tissue. They play a central role in energy storage in both animal and plant cells. When the body consumes excess calories, sugar, or alcohol, these are converted into triglycerides and deposited in fat cells (Smelt, 2010). Chemically, triglycerides are composed of one glycerol molecule esterified with three fatty acids. According to the National Institutes of Health, triglyceride levels below 200 mg/dL are considered normal, while levels exceeding 500 mg/dL are classified as high and associated with an increased risk of cardiovascular disease (Bays et al., 2016).

1.1.2.C. Lipoproteins

These are large globular particles that contain an oily core of nonpolar lipid (cholesteryl esters of triglycerides) surrounded by a polar coat of phospholipids-free (i.e. unesterified) cholesterol and apoproteins. Six lipoprotein classes differ in size, density, and properties of triglycerides and cholesterol (Feingold, 2024).

Chylomicrons

These particles are the largest in both size and density, and their levels are closely influenced by the amount of dietary triglycerides consumed (Boren et al., 2022).

Very low-density lipoprotein (VLDL)

Very low-density lipoprotein particles are produced by the liver and are smaller than chylomicrons, containing relatively lower levels of triglycerides. These lipoproteins are responsible for transporting cholesterol and triglycerides from the liver to various tissues and organs throughout the body. They are formed through the assembly of cholesterol and triglycerides (Sundaram and Yao, 2010).

Intermediate-density lipoprotein (IDL)

Intermediate-density lipoprotein is derived from the metabolic breakdown of VLDL particles by lipoprotein lipase in the capillary networks of adipose tissue and muscle. This transformation occurs as triglycerides are hydrolyzed, resulting in lipoproteins of intermediate density (Narang and Al-Horani, 2025).

Low-density lipoproteins (LDL)

Low-density lipoproteins (LDL) play a crucial role in lipid transport, carrying cholesterol and other lipids through the bloodstream. LDL is synthesized partly in intestinal chyle and partly through the lipolysis of very-low-density lipoproteins (VLDL), contributing significantly to cholesterol homeostasis (Zak, 2024). Elevated LDL levels are directly correlated with the progression of atherosclerosis andan increased risk of coronary heart disease (CHD).(Iglesias, 2025)

According to the American Heart Association (AHA) and the National Heart, Lung, and Blood Institute (NHLBI), the recommended LDL cholesterol levels (in mg/dL) are:

- Optimal: Below 100 mg/dL
- Near optimal: 100–129 mg/dL
- Borderline high: 130–159 mg/dL
- High: 160–189 mg/dL
- Very high: 190 mg/dL and above (Sciriha Camilleri, 2024)

High density lipoprotein (HDL)

Often known as "good cholesterol," HDL is synthesized in the liver and is involved in reverse cholesterol transport. It facilitates the removal of cholesterol and other lipids from peripheral tissues, returning them to the liver for metabolism and excretion (Ridker et al., 2010). HDL is considered protective against atherosclerosis due to its antiatherogenic properties.

Lipoprotein (a) [Lp(a)]

It is cholesterol-rich lipoprotein synthesized by the liver. Elevated levels of Lp(a) are strongly associated with an increased risk of atherosclerosis and related cardiovascular conditions (Kostner and Kostner, 2022).

1.1.3. Classification of hyperlipidemia.

1.1.3.A. Based on the lipid type

Hyperlipidemia is classified based on the type of lipid that is elevated in the blood. The two primary categories are hypercholesterolemia which characterized by

elevated LDL or total cholesterol (TC) and hypertriglyceridemia which defined by elevated TG, often associated with VLDL, with some mixed forms involving both cholesterol and triglycerides (Goldstein and Brown, 2015).

1.1.3.B. Based on the causing factor

Hyperlipidemia is categorized as either primary (familial) or secondary, depending on the underlying cause (Shattat, 2015). Fredrickson's classification system identifies five distinct types of familial hyperlipidemia, based on the electrophoretic or ultracentrifugation patterns of lipoproteins (Sullivan and Lewis, 2011).

• Type I–Raised cholesterol with high triglyceride levels.

• Type II–High cholesterol with normal triglyceride levels.

• Type III–Raised cholesterol and triglycerides.

• Type IV–Raised triglycerides, atheroma and uric acid.

• Type V–Raised triglycerides.

Acquired hyperlipidemia (secondary dyslipoproteinemia) results from underlying disorders and leads to alterations in plasma lipid and lipoprotein metabolism(Cicero and Cincione, 2022). The most common causes of acquired hyperlipidemia are diabetes mellitus, alcohol consumption, use of drugs such as diuretics and estrogens, hypothyroidism, renal failure, nephrotic syndrome, and some rare endocrine disorders and metabolic disorders (Stewart et al., 2020).

1.1.4. Causes of hyperlipidemia

A diet rich in saturated fat and cholesterol increases blood cholesterol and triglyceride levels. Furthermore, other disorders such as obesity, diabetes mellitus, and hypothyroidism increase the risk of hyperlipidemia. Smoking and not exercising may lead to hyperlipidemia. Beside, excessive use of alcohol increases the risk of hyperlipidemia. Certain drugs as steroids and β–blockers may cause hyperlipidemia. As well as hereditary factors are also one of the common causes of hyperlipidemia. In some cases, hyperlipidemia occurs during pregnancy, and lipoprotein lipase mutations (Sheeba and Gandhimathi, 2021).

1.1.5. Diagnosis of hyperlipidemia

Hyperlipidemia often presents without any noticeable symptoms and can typically be identified only through a blood test. The standard method for screening is a lipid profile, which measures various lipid levels in the blood. According to the National Cholesterol Education Program (NECP), screening should begin at age 20, with subsequent tests every five years if initial results are normal (Stone et al., 2005) Normal ranges for a lipid profile are provided in Table 1.1 (Parhofer and Laufs, 2023).**Table1.1 Normal levels for a lipid profile**

Lipids	Desirable value	Borderline	High risk
Total Cholesterol	Less than 200 mg/dl	200-239 mg/dl	240 mg/dl
Triglycerides	Less than 140 mg/dl	150-199 mg/dl	200-499 mg/dl
HDL cholesterol	60 mg/dl	40-50 mg/dl	Less than 40 mg/dl
LDL cholesterol	60-130 mg/dl	130-159 mg/dl	160-189 mg/dl
Cholesterol/HDL ratio	4.0	5.0	6.0

1.1.6.Symptoms of hyperlipidemia

Hyperlipidemia usually has no noticeable symptoms and tends to be discovered during routine examination for atherosclerotic cardiovascular disease (Bhatnagar et al., 2008).

Symptoms of hyperlipidemia can include chest pain (angina), heart attack, or stroke. In cases of severely elevated cholesterol levels, deposits may form in tendons or beneath the skin, particularly around the eyes. Hyperlipidemia can also lead to organ enlargement, affecting the liver, spleen, or pancreas, and cause blockages in blood vessels, particularly in the brain and heart. Additionally, it may contribute to higher rates of obesity, glucose intolerance, and the appearance of pimple-like xanthomas (Duell, 2021).

1.1.7. Complications of hyperlipidemia

1.1.7.A. Atherosclerosis:

Atherosclerosis is a prevalent condition that occurs when fat, cholesterol, and calcium accumulate in the inner walls of arteries (Wouters et al., 2005). This buildup leads to the formation of fibrous plaques. A typical plaque is composed of three main components:

1. An atheroma, which is a fatty, soft, yellowish nodular mass at the plaque's core, containing macrophages cells involved in immune response.
2. A layer of cholesterol crystals.
3. An outer calcified layer.

Atherosclerosis remains the primary cause of cardiovascular disease (Frąk et al., 2022).

1.1.7.B. Coronary Artery Disease (CAD)

Atherosclerosis is the primary cause of CAD. It is characterized by the narrowing of the arteries that deliver blood to the myocardium, restricting blood flow and depriving the heart of sufficient oxygen to meet its demands. As this narrowing progresses, the heart muscle may experience damage due to inadequate blood supply. An elevated lipid profile is strongly associated with the development of coronary atherosclerosis (Gao et al., 2012, Zhao et al., 2021b).

1.1.7.C. Myocardial Infarction (MI)

Myocardial infarction occurs when the blood and oxygen supply to the heart muscle is partially or fully obstructed, leading to damage or death of heart cells. This blockage is typically caused by the formation of a clot in one or more of the coronary arteries. Commonly referred to as a heart attack, studies have shown that approximately one-quarter of individuals who survive a myocardial infarction have hyperlipidemia (Nickolas et al., 2003, AL-Ezzy and Hameed, 2021).

1.1.7.D. Angina Pectoris

Angina is not a disease itself but a symptom of an underlying heart condition. It is typically characterized by chest pain, discomfort, or a sensation of pressure or squeezing. Angina occurs when there is a reduction or complete cessation of blood flow to part or all of the heart muscle. This diminished blood circulation is often the result of CHD, where partial or complete blockage of the coronary arteries impairs blood supply to the heart (Joshi and De Lemos, 2021).

1.1.7.E. Ischemic Stroke or Cerebrovascular Accident (CVA)

A stroke occurs when blood flow to a part of the brain is blocked or reduced. The disruption of blood supply, which carries oxygen, glucose, and other vital nutrients, leads to the death and dysfunction of brain cells. Strokes typically result from the blockage of an artery by a blood clot or a fragment of atherosclerotic plaque that detaches and travels to smaller vessels in the brain. Clinical trials have shown that reducing LDL and total cholesterol levels by 15% significantly lowers the risk of a first stroke (Amarenco and Labreuche, 2009).

1.1.8. Treatment of Hyperlipidemia

In 1987, the National Institutes of Health (NIH) established the National Cholesterol Education Program (NCEP), which is overseen by the Adult Treatment Panel (ATP). The program aims to provide guidance to healthcare professionals and the public on the testing, evaluation, monitoring, and treatment of hyperlipidemia. A key aspect of the ATP guidelines is the development of treatment goals for hyperlipidemia, tailored to the individual patient's risk of CHD [(Talwalkar et al., 2013),(Wang et al., 2024)].

ATP recommends two methods of treatment firstly by changing lifestyle and if it not enough to lowering the harmful blood lipid then use drug therapy (Wang et al., 2024).

1.1.8.A. Therapeutic lifestyle changes

Initial treatment for mild cases of hyperlipidemia should focus on lifestyle modifications, such as dietary changes, regular physical activity, smoking cessation, and weight reduction. It is important to note that while dietary cholesterol intake is reduced, the liver may compensate by increasing cholesterol production. It is

recommended that total fat intake be limited to 25%-35% of total energy, with saturated fats making up less than 7% of the total energy intake, and cholesterol intake should not exceed 200 mg per day. Additionally, the consumption of plant sterol esters and soluble fiber is beneficial. Following a healthy diet can lead to a reduction in blood cholesterol levels by 10% to 15% (Barone Gibbs et al., 2021).

1.1.8.B. Drug therapy

Since LDL is the major atherogenic lipoprotein, reduction of this lipoprotein would be expected to reduce atherosclerosis and therefore reduce cardiovascular adverse effects. In addition to high LDL, the presence of risk factors and CHD should qualify initiating drug therapy along with lifestyle changes. Monotherapy is effective in treating hyperlipidemia, but combination therapy may be required for a comprehensive approach. Currently, antihyperlipidemic drugs contain five major classes that include statins, fibric acid derivatives, bile acid binding resins, nicotinic acid derivatives and drugs that inhibit cholesterol absorption (Gaikwad and Khairnar, 2023).

1.1.9 3-Hydroxy-3-methylglutaryl coenzyme A (HMGCoA) reductase inhibitors (statins)

1.1.9.1 Statins in general

Statins are a widely used class of lipid-lowering medications that inhibit the enzyme 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase, thereby reducing endogenous cholesterol synthesis. They are the most commonly prescribed agents for both primary and secondary prevention of CVD (Zhou and Liao, 2009),(Mills et al., 2011), which remain the leading cause of death globally (Mc Namara et al., 2019). CVDs are influenced by multiple risk factors, including diabetes, elevated blood glucose levels, physical inactivity, smoking, low HDL cholesterol, and high

LDL cholesterol (Sun et al., 2015). Thus, effective management of CVDs involves controlling hypertension and dyslipidemia.

Currently, statins are considered the most effective oral therapy for reducing the risk of cardiovascular events associated with hypercholesterolemia (Ramkumar et al., 2016).

Numerous clinical trials have demonstrated that statins not only decrease LDL cholesterol and triglyceride levels but also significantly reduce the incidence and mortality of coronary heart disease, cerebrovascular events, and peripheral arterial disease (Mills et al., 2011).

1.1.9.2 Development of statins

During the 1970s, Japanese scientist Akira Endo discovered compactin (also known as mevastatin), illustrated in Figure 1.1, from *Penicillium citrinum* cultures. This compound was the first identified inhibitor of HMG-CoA reductase and demonstrated the ability to suppress cholesterol synthesis in various mammalian cells, including those from monkeys, dogs, and humans with hypercholesterolemia (Endo, 2010).

Although compactin showed promise, it was never commercialized due to its toxic effects observed in canine studies. Subsequently, lovastatin was isolated from *Aspergillus terreus* and became the first statin to receive approval for clinical use in 1987 (Goswami et al., 2013). The development of simvastatin followed in 1988 as a semi-synthetic derivative of lovastatin, created by methylation (Kant Belwal and Patel, 2019).

Later advancements led to the production of fully synthetic statins, including cerivastatin, fluvastatin, rosuvastatin, and atorvastatin, which differ structurally from their fungal-derived predecessors (Shuhaili et al., 2017). However, cerivastatin

was withdrawn from the market on August 9, 2001, after it was linked to 52 deaths caused by rhabdomyolysis-induced kidney failure (Chaudhry, 2021).

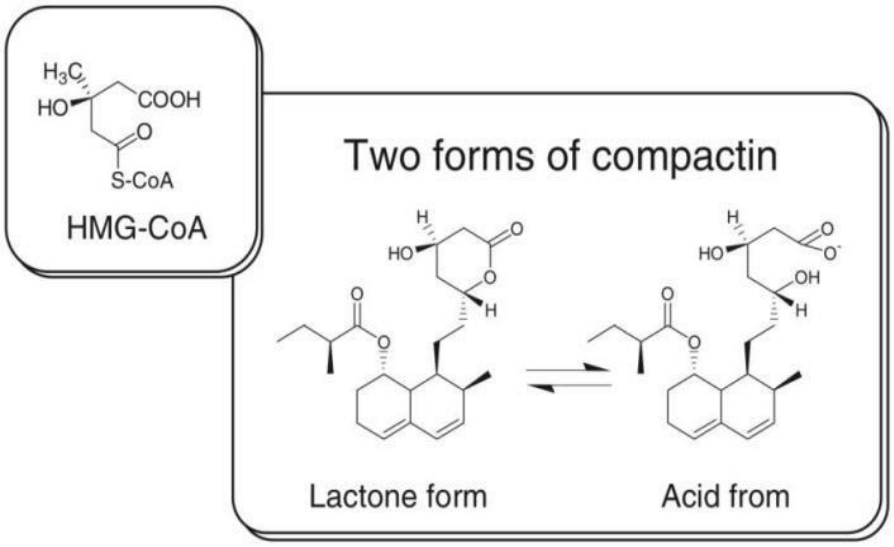


Figure 1.1: Structure of HMG-CO and Compactin (Endo, 2010).

1.1.9.3 Mechanism of action

Statins exert their effect by competitively inhibiting the enzyme 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, the rate-limiting step in the biosynthetic pathway of cholesterol. This enzyme catalyzes the conversion of HMG-CoA to mevalonate, a critical precursor in cholesterol synthesis Figure 1.2. Statins structurally resemble the natural substrate and bind to the enzyme's active site, thereby preventing HMG-CoA from accessing it and ultimately reducing intracellular cholesterol production (Hilal-Dandan et al., 2014).

The liver is the primary site of cholesterol synthesis, and hepatocytes are the main target for statin action (BERTRAM G KATZUNG, 2021). Statins lower cholesterol through two complementary mechanisms: they inhibit hepatic

cholesterol synthesis and enhance the removal of LDL cholesterol from the bloodstream by upregulating LDL receptors on liver cells (Sirtori, 2014) Figure 1.3.

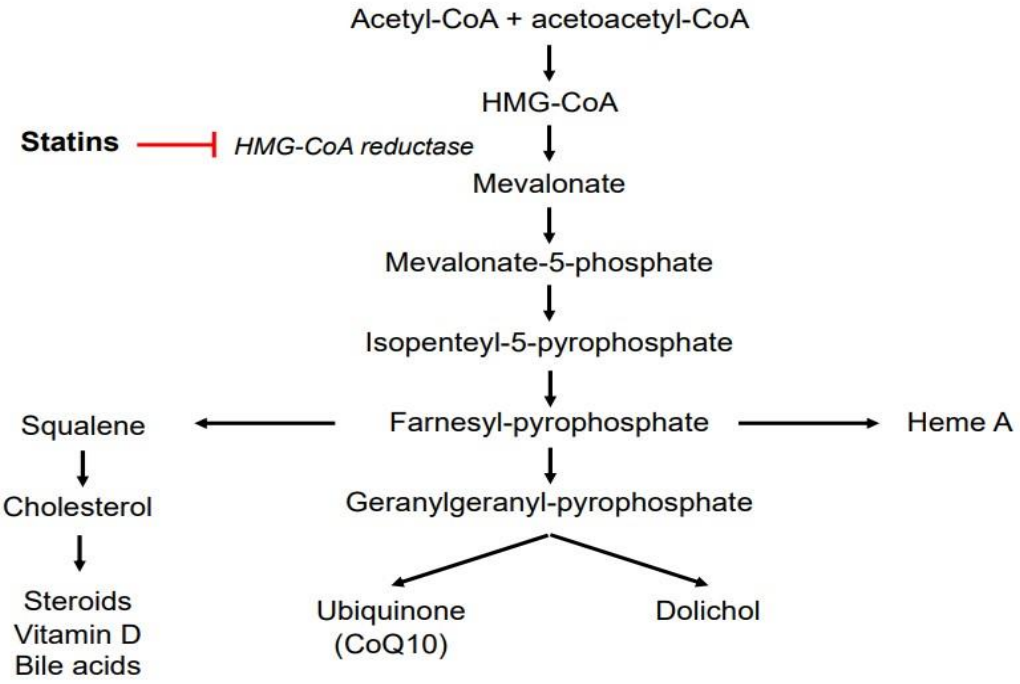


Figure 1.2: Pathway of cholesterol biosynthesis (Bouitbir et al., 2020).

Intracellular levels of cholesterol and fatty acids are controlled by a feedback mechanism involving a family of transcription factors called sterol regulatory element-binding proteins (SREBPs). These proteins are initially synthesized as inactive precursors anchored to the membranes of the endoplasmic reticulum [(Mullen et al., 2016),(Lo et al., 2018)].

When statins reduce intracellular cholesterol concentrations, they indirectly activate SREBPs. This activation involves the proteolytic cleavage of SREBPs, releasing their NH2-terminal fragments. These fragments then translocate to the nucleus, where they bind to sterol regulatory elements (SREs) in the promoter regions of specific genes. This process promotes the synthesis of isoprenoids and sterols, aiding in the restoration of cholesterol levels within the cell. Additionally,

SREBPs upregulate the expression of LDL receptor genes, thereby enhancing the clearance of LDL cholesterol from the bloodstream (Marquart et al., 2010),(Chen et al., 2022).

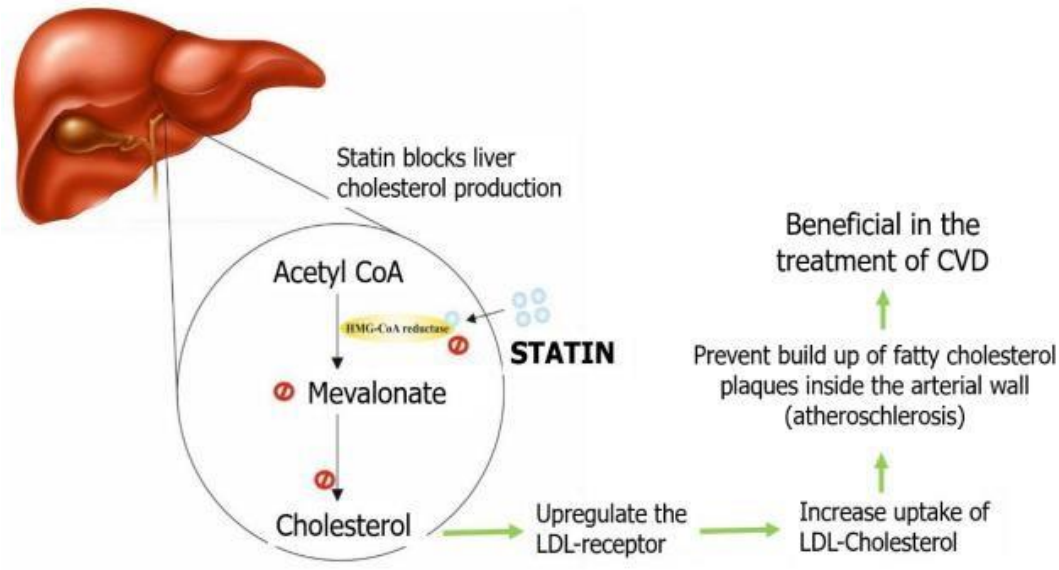


Figure 1.3 Mechanisms of action of statins (Tasik, 2019)

Furthermore, apo-lipoprotein B100 synthesis is inhibited by statins, and the liver's production of triglyceride rich lipoproteins is decreased (Althanoon et al., 2020). Powerful statin (simvastatin, rosuvastatin, atorvastatin) decreases triglycerides by up to 30% while increasing HDL (Karlson et al., 2016).

1.1.9.4 Pharmacokinetics of atorvastatin

Statins, including atorvastatin, possess amphiphilic properties. Atorvastatin, being lipophilic, can diffuse across cell membranes by integrating into the lipid bilayer and is efficiently absorbed after oral administration (Schachter, 2005). Despite this, it undergoes significant first-pass metabolism in both the

gastrointestinal tract and liver, resulting in a bioavailability of approximately 12–14% (Hirota et al., 2020).

Administered as an oral calcium salt, atorvastatin is converted into its lactone form through enzymatic processes involving acyl glucuronide intermediates as well as non-enzymatic lactonization within the intestine (Morse et al., 2019). Multiple UDP-glucuronosyltransferase enzymes are involved in this glucuronidation reaction (Filppula et al., 2021).

The liver is the primary site of atorvastatin metabolism, predominantly via cytochrome P450 enzymes. Among these, CYP3A4 accounts for roughly 85% of the metabolic activity [(Sirtori, 2014),(Hirota and Ieiri, 2015)]. Transport into hepatocytes is facilitated by organic anion transporting polypeptides (OATPs), specifically OATP1B1, OATP1B3, and OATP2B1, as well as the sodium taurocholate co-transporting polypeptide (NTCP) (König, 2011) These transport mechanisms represent the rate-limiting step in the drug's hepatic elimination (Karlgren et al., 2012). Genetic variations or defects in these transporters may impair hepatic uptake, leading to elevated systemic levels of atorvastatin and an increased risk of adverse drug reactions (Maeda et al., 2011).

1.1.9.5 Pleiotropic effects of atorvastatin

While atorvastatin's primary therapeutic benefit lies in its capacity to lower circulating levels of LDL cholesterol, it also exhibits a range of cholesterol-independent, or pleiotropic, effects that are especially advantageous to cardiovascular health (Norata et al., 2014).

One key mechanism involves the inhibition of isoprenoid synthesis, which contributes to improved endothelial function through enhanced nitric oxide bioavailability (a critical factor in vasodilation). Atorvastatin also suppresses the

activation of leukocytes and limits the proliferation of vascular smooth muscle cells (Chamani et al., 2022). Additionally, it exerts anti-inflammatory effects by curbing macrophage activity (Wang et al., 2008). Its antioxidant properties and ability to modulate immune responses are associated with reduced activity of small GTP-binding proteins (Mohammad et al., 2019). Collectively, these effects contribute to the stabilization of atherosclerotic plaques and support overall vascular and systemic health (Sirtori, 2014).

1.1.9.6 Adverse effects of atorvastatin

Statins, including atorvastatin, are widely regarded as safe and generally well-tolerated lipid-lowering agents (Toth et al., 2018). The therapeutic advantages, such as significant reductions in cholesterol levels and a range of additional cardiovascular benefits, are considered to outweigh potential adverse effects (Norata et al., 2014). Nevertheless, atorvastatin has been associated with some side effects, although most are infrequent (Thompson et al., 2016). Mild elevations in liver enzymes, indicative of hepatocellular stress, have been observed (Desai et al., 2019). However, large-scale pharmacovigilance data suggest that severe liver toxicity is extremely rare, with an estimated incidence of one case per million annually (Bays, 2006, Wang et al., 2025). Other reported side effects include decreased renal function, insomnia, upper respiratory tract infections such as nasopharyngitis, tendon ruptures, joint pain, and a reduction in testosterone levels (Banach et al., 2015). Furthermore, data from observational studies and meta-analyses of randomized controlled trials indicate a modest increase (around 10% to 12%) in the risk of new-onset diabetes (NOD), particularly with high-dose regimens and among individuals with prediabetes or metabolic syndrome (Carter et al., 2013). Despite this, the cardiovascular benefits of atorvastatin far outweigh the risk of mild

elevations in plasma glucose levels, reaffirming its role in CVD prevention (Carmena and Betteridge, 2019).

Concerns about statin-related muscle toxicity remain prominent, especially following the withdrawal of cerivastatin in 2001, which was linked to fatal cases of rhabdomyolysis (Ramkumar et al., 2016).

1.2 Lipid ratios (Atherogenic indices):

Cardiovascular diseases continue to be the primary contributors to global morbidity and mortality. Conditions such as coronary artery disease (CAD), stroke, MI, and peripheral vascular disease are commonly linked to dyslipidemia, a key modifiable risk factor. The early detection of dyslipidemia, even before the clinical manifestation of CVD, is considered a critical preventive strategy. Typically, the assessment of dyslipidemia relies on standard lipid profile components, including TC, LDL, HDL, and TG, with particular attention to LDL due to its strong association with atherogenesis (German and Shapiro, 2020). While these traditional markers are effective in clinical evaluation, reliance on single parameters such as LDL or HDL may be insufficient in patients with intermediate cardiovascular risk, especially those with coexisting risk factors like elevated lipids, hyperglycemia, hypertension, obesity, or a family history of CVD (Superko and King III, 2008). In such scenarios, alternative diagnostic tools and more nuanced biomarkers are essential for accurate risk stratification.

Over the past two decades, researchers have proposed several lipids derived ratios (commonly referred to as atherogenic indices) as more sensitive predictors of cardiovascular risk in the cases when the traditional lipid profile remain at the normal levels. Among them, the atherogenic index of plasma (AIP), Castelli’s risk index I (CRI-I) and II (CRI-II), atherogenic coefficient (AC), and the cholesterol index

(CHOLindex). Studies have demonstrated the superior advantages value of these indices compared to conventional lipid parameters in identifying and prediction of atherosclerosis and cardiovascular risk (Dobiášová et al., 2011).

In addition to enhancing predictive accuracy, these indices support more targeted and personalized treatment approaches by improving patient stratification. However, despite their clinical promise, further research is needed to validate their utility across diverse populations and disease conditions, ensuring broader applicability in preventive cardiology.

1.2.1. Atherogenic index of plasma

Atherogenic index of plasma (AIP) is an unconventional lipid ratio representing the logarithm of the molar ratio of TGs to HDL (Gómez-Álvarez et al., 2020). Accumulated evidence supports the AIP as a valuable predictive index for CVD. Numerous studies have demonstrated a significant positive correlation between elevated AIP values and increased cardiovascular risk. A large scale prospective study conducted in 2010 by *Onat et al* that evaluate 2,676 middle-aged adults in Turkey over an 8 years follow up, during which clinical and laboratory assessments revealed that AIP was a strong independent predictor of CAD in that population (Onat et al., 2010). Further supporting its clinical relevance, a study by Wang et al. 2021 in the Chinese population test the correlation between AIP and the SYNTAX score (an angiographic tool used to assess the complexity of CAD) (Wang et al., 2021). They concluded that AIP independently predicted CAD severity and could assist in CAD prevention strategies. In addition to many reports which have assessed the AIP in different populations to predict CVD in those populations (Sapunar et al., 2018) some researchers suggest a positive relation with certain other conditions such as acquired premature ejaculation and its severity (Ertas and Nas,

2021). Accordingly, evidence supports that AIP is now ready to be considered in clinical practice and for research purposes and further evaluated.

1.2.2. Castelli's risk indexes (I & II)

Castelli's risk indexes (also called cardiac risk indexes) are two lipid ratios, the CRI-I is the ratio of TC to HDL, while the CRI-II is the ratio of LDL to HDL, with notable positive associations with CVD risk (Igharo et al., 2020). They were reported by William Castelli, at the end of the past century. Later, many reports assessed and confirmed their positive correlation with CVD (Tecer et al., 2019) and some other conditions such as chronic obstructive pulmonary disease (COPD, negative correlation) (Markelić et al., 2021) and erectile dysfunction (Culha et al., 2020). CRI-I has been particularly shown to reflect coronary plaques formation and the thickness of intima media in the carotid arteries of young adults (Nair et al., 2009). Therefore, physicians should consider CRI-I and II, in clinical practice to predict or assess atherosclerosis and CVD and for further evaluation.

1.3 Proprotein convertase subtilisin/kexin type 9

It has only been 22 years since PCSK9 (proprotein convertase subtilisin/kexin type 9) was identified as an important regulator of LDL metabolism. As its name suggests, PCSK9 is the ninth member of the proprotein convertase family, a group of serine proteases that are characterized by their ability to hydrolyze peptide bonds in their cognate substrates for activation (Bell et al., 2023).

1.3.1. The discovery of PSCK9

In 2003, Seidah et al. identified the ninth member of the pro-protein convertase family, PCSK9 (Seidah et al., 2003). In the same year, the involvement of PCSK9 in regulating cholesterol metabolism became evident, with the identification of two gain-of-function (GOF) mutations in PCSK9, in two French

families with a clinical diagnosis of autosomal dominant hypercholesterolemia (ADH) which is a genetic disorder characterized by high LDL cholesterol levels, which increases the risk of cardiovascular diseases. It is inherited in an autosomal dominant pattern, meaning that a person only needs one copy of the mutated gene from either parent to be affected by the condition (Benn et al., 2012) and no detectable mutations in low-density lipoprotein receptor (LDLR) or apoB100 genes (Abifadel et al., 2003). Since this first report, several other GOF mutations have been reported (Tibolla et al., 2011), associated with mild to severe hypercholesterolemia and an increased risk of CHD (Tibolla et al., 2011). Although GOF mutations in the PCSK9 gene are uncommon and represent a minor proportion of ADH cases, significant genetic insights into PCSK9's role in LDL regulation have come from LOF variants. These LOF mutations, along with certain common polymorphisms, have been linked to reduced LDL cholesterol levels. The earliest identification of such LOF variants was reported in 2005 (Cohen et al., 2005) and the effect of lifelong reductions in LDL induced by these LOF mutations was examined in the atherosclerosis risk in communities study (Cohen et al., 2006): the LOF mutations Y142X and C679X in African-Americans were associated with a 28% reduction in LDL and an 88% reduction in the risk of coronary heart diseases, whereas the R46L mutation in Caucasians was associated with a 15% reduction in LDL and a 47% reduction in the risk of CHD (Benn et al., 2010). Numerous other LOF mutations or polymorphisms associated with decreased LDL concentrations have been identified (Tibolla et al., 2011). The link between the R46L variant and CHD risk has been thoroughly investigated across three separate studies conducted in Denmark. Meta-analytical findings revealed that individuals carrying the R46L mutation exhibited approximately a 12% decrease in LDL cholesterol levels, accompanied by a 28% lower risk of developing CHD. The fact that CHD risk reduction was considerably larger than predicted with similar LDL reductions in statin trials (Ference et al.,

2012) could be explained by the effect of long term exposure to lower LDL beginning early in life. This aligns with findings from a Mendelian randomization study, which demonstrated that prolonged exposure to reduced LDL cholesterol levels was linked to a threefold greater decrease in the risk of coronary heart disease compared to the reduction observed with statin therapy initiated later in life (Mayne et al., 2011).

1.3.1. Structure and biosynthesis of PCSK9

The PCSK9 gene in humans is situated on chromosome 1p32.3 and encodes a glycoprotein composed of 692 amino acids in its inactive form. This gene is primarily expressed in the liver, but it is also found in the intestine and kidneys (Seidah and Prat, 2012). The initial protein product, which is preproPCSK9, undergoes signal peptidase cleavage (between amino acids 1-30), followed by an autocatalytic cleavage in the endoplasmic reticulum. This process yields two products, the prodomain and the mature PCSK9, which consists of a catalytic domain and a C-terminal domain (The structure of PCSK9 is characterized by a C-terminal domain, and a catalytic domain, a prodomain, which are critical for its enzymatic activity and interaction with LDLR). The C-terminal domain facilitates the binding of PCSK9 to LDLR, promoting its internalization and subsequent degradation. The proprotein convertase domain of PCSK9 is responsible for its catalytic activity, while the prodomain serves to maintain the enzyme in an inactive form until it undergoes proteolytic cleavage (Cunningham et al., 2007). Structural studies have revealed that the interaction between PCSK9 and LDLR is essential for the modulation of cholesterol homeostasis. PCSK9 binds to the epidermal growth factor like repeat A (EGF-A) repeat of LDLR, leading to its internalization and degradation within lysosomes, reducing the receptor's availability on the hepatocyte

surface and results in lowering the number LDL particles that could uptake by LDLR (Liu et al., 2024).

Biosynthesis of PCSK9 begins in the liver, where the gene encoding PCSK9 is transcribed into mRNA, which is then translated into a precursor protein. This precursor undergoes post-translational modifications, including cleavage of the prodomain to yield the active enzyme. The secretion of PCSK9 from hepatocytes into the bloodstream is tightly regulated, and its half-life is relatively short due to rapid degradation. The regulatory mechanism of PCSK9 secretion and activity is primarily controlled by cholesterol levels within the liver. High cholesterol concentrations induce the expression of PCSK9, further decreasing the LDLR-mediated cholesterol uptake, creating a feedback loop that ensures the maintenance of cholesterol homeostasis (Maligłówka et al., 2022).

The cleavage of the prodomain is an important step for the proper maturation and secretion of PCSK9. A recently identified LOF, involving an amino acid substitution at the cleavage site, prevent this autocatalytic and activation process. This disruption hinders PCSK9 secretion and has been linked to a notable 48% reduction in LDL levels (Lambert et al., 2012). Following cleavage, the prodomain remains non-covalently attached to the mature PCSK9 via hydrogen bonds, resulting in the secretion of the protein as an inactive dimer complex (see Figure 1.4b). Recent studies suggest that this complex plays a regulatory role by restricting access of other substrates to PCSK9's catalytic domain (Seidah et al., 2013).

1.3.2. Functions of PCSK9

1.3.2.A. Role in the regulation of LDL concentration

The major function of PCSK9 is the degradation of the LDLR by complex mechanisms. PCSK9 directly interacts with the LDLR both within the cell and at the surface of the plasma membrane (Surdo et al., 2011).

However, evidence indicates that PCSK9 acts on the LDLR primarily as a secreted factor and promotes the reduction of LDLR protein concentrations, mainly in the liver. LDLR protein concentrations are increased in the liver of PCSK9 knockout mice (Rashid et al., 2005).

Secreted PCSK9 binds to the LDLR in a complex with its pro-segment and is subsequently internalized together with the LDLR. The binding of PCSK9 to LDLR induces modification of LDLR conformation, avoiding normal recycling of LDLR to the plasma membrane and enhancing the LDLR lysosomal degradation (Tavori et al., 2013)(Figure 1.4 b).

As a result, LDLR represents the main route of elimination of PCSK9 (Roubtsova et al., 2011). However, the mature secreted PCSK9 can be inactivated through cleavage by other proprotein convertases, particularly furin. The mature active form and the inactive form of PCSK9 circulate in the bloodstream (Oleaga et al., 2021).

1.3.2.B. Other functions of PCSK9

While PCSK9 is widely recognized for its central role in regulating cholesterol homeostasis by modulating LDLR levels, emerging research also implicates it in triglyceride metabolism and fat accumulation in visceral adipose tissue (Levy et al., 2013). Its specific role in the intestine remains unclear; however,

recent findings suggest PCSK9 may influence chylomicron production and contribute to cholesterol regulation within enterocytes (Farnier, 2011). Beyond lipid regulation, animal studies propose that PCSK9 may play additional roles in glucose metabolism (Mbikay et al., 2010), liver regeneration, and susceptibility to hepatitis C virus infection (Zhao et al., 2006). Although unforeseen side effects from PCSK9 inhibition cannot be ruled out, genetic studies provide some reassurance. For example, a woman identified with two PCSK9 mutations exhibited no measurable PCSK9 levels, extremely low LDL cholesterol (14 mg/dL), and maintained normal liver, kidney, and neurological function (Hedrick, 2009). Moreover, a recent mouse study found that PCSK9 deficiency may protect against melanoma liver invasion, hinting at a possible role for PCSK9 inhibitors in cancer therapy. Nevertheless, further human studies are essential, as current PCSK9-targeted treatments are primarily focused on managing hypercholesterolemia and preventing atherosclerosis (Hooper and Burnett, 2013).

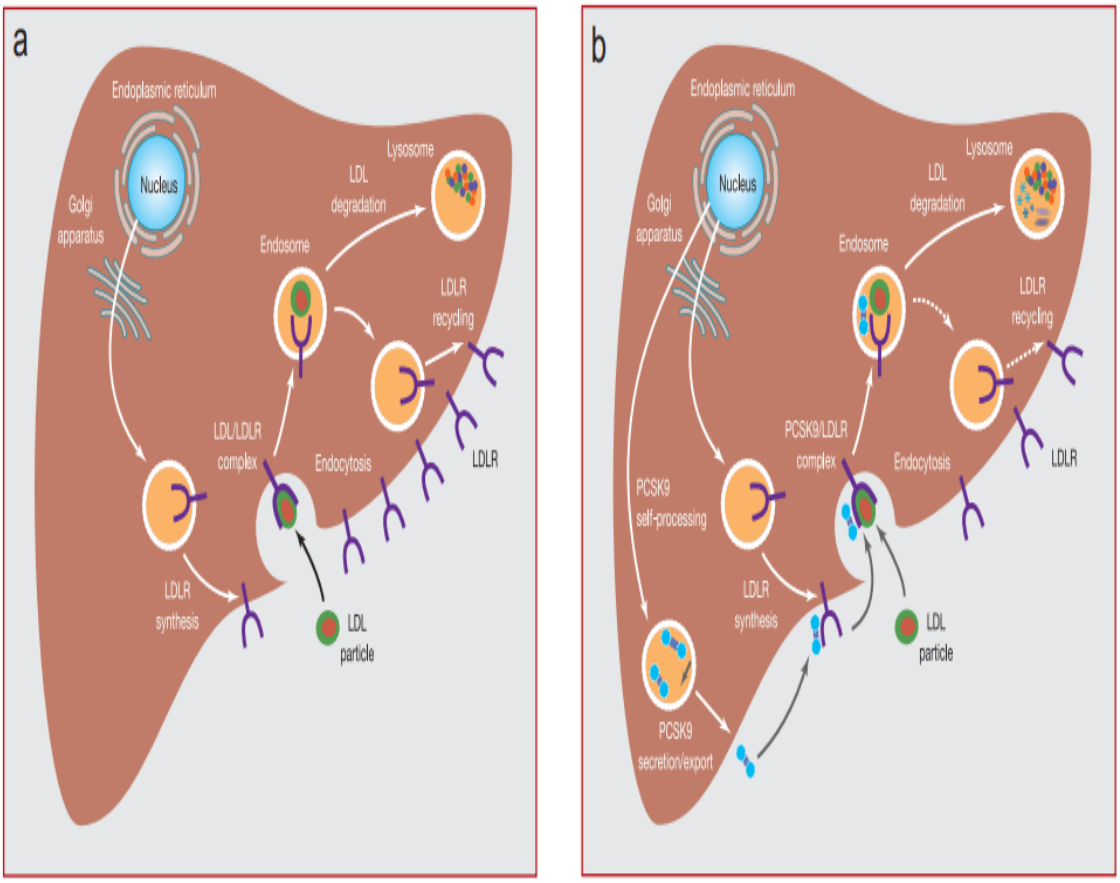


Figure 1.4 illustrates the involvement of PCSK9 in regulating LDL receptor (LDLR) activity within hepatocytes. In panel (a), the normal cycle of LDLR synthesis and recycling is depicted. Panel (b) shows how PCSK9 is synthesized and secreted, after which it binds to LDLRs. Once this PCSK9-LDLR complex is internalized, PCSK9 directs the receptor toward lysosomal degradation rather than recycling it back to the cell surface. This mechanism reduces the overall number of LDLRs available on the hepatocyte membrane, thereby decreasing LDL cholesterol clearance from the bloodstream (Farnier, 2014).

1.3.3. Strategies for PCSK9 inhibition

Multiple therapeutic strategies have been explored to inhibit PCSK9 activity (Hooper and Burnett, 2013), These include the use of gene-silencing techniques, such as antisense oligonucleotides and small interfering RNA (siRNA), to suppress PCSK9 production; monoclonal antibodies (mAbs), small peptides, and adnectins

that prevent PCSK9 from binding to LDLR; and small molecule inhibitors that disrupt the enzyme's autocatalytic processing (Rhainds et al., 2012).

Among these, monoclonal antibodies have shown promise in preclinical studies for their ability to block the interaction between PCSK9 and LDLR or inhibit PCSK9 internalization (Rhainds et al., 2012). Human data are available for three of these mAbs: alirocumab and evolocumab, two fully human mAbs developed by Sanofi/Regeneron and Amgen, respectively; and RN316/PF04950615, a humanized mAb developed by Pfizer/Rinat.

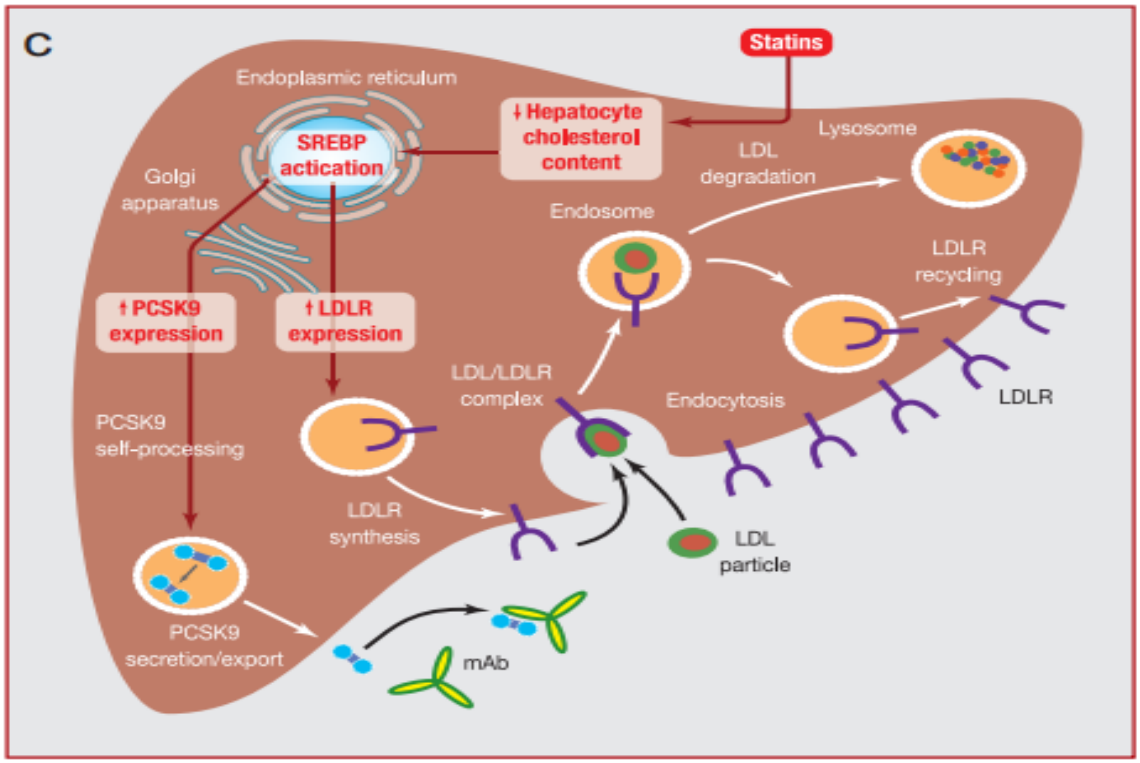


Figure 1.5: Mechanisms by which statins and PCSK9 monoclonal antibodies function. Statins work by activating SREBP2, a transcription factor that upregulates both LDL receptor (LDLR) and PCSK9 expression. While this enhances LDL clearance, the simultaneous increase in PCSK9 can reduce the number of functional LDLRs. In contrast, monoclonal antibodies against PCSK9 block its interaction with the LDLR-LDL complex, preventing receptor degradation and thereby enhancing LDLR availability on the hepatocyte surface for improved LDL cholesterol removal.(Farnier, 2014).

1.3.4. Influence of PCSK9 polymorphisms on plasma lipids and response to atorvastatin treatment

PCSK9 is a highly polymorphic gene. Gain-of-function mutations in PCSK9 cause increased enzyme activity, which results in high rate of LDLR degradation and ADH-associated increase in LDL cholesterol. Loss-of function mutations that inactivate PCSK9 result in low plasma levels of LDL cholesterol and confer protection for CAD. Previous studies have investigated the relationship of PCSK9 single nucleotide polymorphisms (SNPs), including the a common SNPs rs28942111(S127R) (Athyros et al., 2018), and changes in serum lipids showing inconsistent results. Statins are inhibitors of cholesterol biosynthesis that have high efficacy in lowering LDL cholesterol levels (Brautbar and Ballantyne, 2011) and result in clinically significant reductions in cardiovascular morbidity and mortality (Adhyaru and Jacobson, 2018). Although statin treatment has proven beneficial effects, there is large variability in clinical response to statin therapy as well as in the incidence of adverse effects. Several studies have shown that this interindividual variability is associated with SNPs in genes related to lipid homeostasis and statins metabolism (Bitto et al., 2016). Previous studies have demonstrated that carriers of LOF mutations in the PCSK9 can have a better response to statins suggesting that lipid-lowering by PCSK9 inhibitors may be synergistic to that achieved by statins treatment. GOF in PCSK9 were associated with attenuated statin-mediated reduction of LDL cholesterol (Mohammed et al., 2025).

1.4 Aims of Study

This study was designed to investigate:

1. Distribution of PCSK9 gene SNP, rs28942111; T > A in hyperlipidmic patients in Kerbala Province population.

2. The impact of rs28942111; T > A SNP on the response of atorvastatin.

3. If there is any correlation between rs28942111; T > A SNP of PCSK9 gene and efficacy of atorvastatin.

Chapter Two
Patients, Materials, and Methods

2. Patients, Materials and Methods

2.1. Patients

2.1.1. Study Population

This cross-sectional observational study was conducted at Imam Al-Hussain Medical City/ Karbala Center for Cardiac Diseases and Surgery in Karbala from November 2023 to April 2025.

2.1.1.1. Ethical Approval

The study protocol received approval from the Scientific and Ethical Committee of the College of Pharmacy, University of Kerbala. Prior to participation, all patients were informed about the study's objectives and procedures, and written informed consent was obtained from each participant.

A total of 149 individuals (both male and female) between the ages of 28 and 85 years, who had been on a daily oral regimen of 40 mg atorvastatin monotherapy for a minimum duration of six months, were enrolled in the study.

2.1.1.2. Inclusion Criteria

The study involves both male and female patients diagnosed with primary hyperlipidemia who had been receiving a daily oral dose of 40 mg atorvastatin as standalone treatment for a minimum of six months prior to the study began.

2.1.1.3. Exclusion Criteria

The exclusion criteria for the study included individuals with untreated hypo or hyperthyroidism, those of advanced age (over 85 years), and patients who had recently undergone surgery or experienced trauma. Additionally, individuals using

drugs known to interact with atorvastatin, such as fibrates and nicotinic acid, were excluded. Patients suffering from severe renal, hepatic, or malignant diseases were also not considered for inclusion in the study.

2.1.2 Patients Data Collection

The information and data was collected form medical records of patients and also through the patients themselves. And during blood sample collection the first question is if the participants were taking any other medications that could have a drug-drug interaction with atorvastatin. Collected data included age, weight, height, level of education, smoking status, duration of atorvastatin therapy, reported adverse drug reactions, and the presence of comorbid conditions.

2.1.3 Blood Sample Collection

Five ml was taken from each patient enrolled in this study and divided into two parts. Tow ml was collected in an ethylene-diamine-tetra-acetic acid (EDTA) tube for DNA extraction and genetic analysis, while the remaining 3 ml placed in a gel tube for the biochemical analysis. To ensure proper temperature and sample integrity during transport, all specimens were stored in ice filled containers until they reached to the laboratory.

2.1.4 Determination of Body Mass Index

The actual formula to determine BMI uses metric system measurements: weight in kilograms (kg) divided by height in meters, squared (m^2) BMI = Weight (kg)/Height (m^2) (Masanovic et al., 2019).

2.1.5 Measurement of Atherogenic index of plasma

The AIP is calculated according to the following formula: AIP = Log (serum triglyceride/serum HDL) (Fernández-Macías et al., 2019).

2.1.6 Determination of CRI-I and CRI-II

CRI-I is calculated using the ratio of Total Cholesterol to HDL and the CRI-II is measured by dividing the serum LDL concentration on the serum HDL concentration (Drwila et al., 2022).

2.2 Materials

The chemicals, kits, and instruments used in this study with their manufacture and origin are listed in Tables 2-1 and Table 2-2.

2.2.1 Instruments

Table 2.1 Instruments used in this study with their manufacture and origin.

Instrument	Manufacture	Origin
Cobas c 311 analyzer	Roche	UAE
Centrifuge PLC series	Gemmy Industrial	Taiwan
High-speed centrifuge	sigma 3-30K	Germany
Incubator	Binder	Germany
Nanodrop	Thermo Fisher Scientific	USA
Hot plate Stirrer	LabTech	Korea
UV-transilluminator	Major science	Taiwan
Electrophoresis apparatus	Cleaver Scientific Ltd	UK

PCR -thermal cycler veriti	Thermo Fisher Scientific	USA
Refrigerator	Denka	Japan
Electronic scale	G&G	Germany
Vortex mixer	Human twist	Germany

2.2.2 Chemicals and Kits

Table2.2 Kits and chemicals used in this study with their Manufacture and origin.

Kits and chemicals	Manufacture	Origin
Cholesterol Kit	Mindray	China
HDL kit	Mindray	China
LDL kit	BioBase	China
Triglyceride kit	Mindray	China
BILT3	Roche	Germany
ASTL	Roche	Germany
ALTL	Roche	Germany
gSYNC DNA extraction kit	Geneaid	Korea
GoTaq® G2 Green Master Mix	Promega	USA
100 bp DNA ladder	Bioneer	Korea
10x TBE buffer	MarLiJu	Korea
Agarose powder for gel	MarLiJu	Korea
Primers	Bioneer	Korea
Ethidium bromide	Promega	USA
Absolute ethanol	Honeywell	Germany
Distilled water	Pioneer	Iraq

2.3 Methods

2.3.1 Genetic Analysis

2.3.1.1 Genomic DNA Extraction

Genomic DNA was extracted from whole blood samples using the gSYNC™ blood genomic DNA extraction kit, following the protocol was adopted for DNA extraction from blood with the steps outlined below:

1. Sample Preparation: A volume of 200 µL of whole blood was transferred into a 1.5 mL micro centrifuge tube. Then, 20 µL of Proteinase K was added and mixed by pipetting. The mixture was placed in incubation at 60°C for 5 minutes.

2. Cell lysis: To lyse the cells, 200 µL of GSP Buffer was added and the mixture was vigorously shaken by vortex mixer apparatus. The tube was incubated at 60°C for an additional 5 minutes, with inversion every 2 minutes to ensure complete cell lysis.

3. DNA Binding: 200 µL of absolute ethanol was added to the sample lysate and mixed immediately by vigorous shaking for 10 seconds.

4. The entire mixture, including any precipitate, was transferred to a GS column placed in a 2 mL collection tube. The tube was centrifuged at 14,000-16,000 * g for 1 minute.

5. Get rid of the collection tube containing the flow through, and the GS column was transferred to a new 2 mL collection tube.

6. First Wash: 400 µL of W1 Buffer was added to the GS column, followed by centrifugation at 14,000-16,000 * g for 30 seconds. The flow-through was discarded.

7. Second Wash: The column was returned to the same collection tube, and 600 µL of Wash Buffer was added. After centrifuging at 14,000-16,000 * g for 30 seconds, the flow-through was again discarded.

8. Drying the Column Matrix: The GS column was placed back into the collection tube and centrifuged at 14,000–16,000 × g for 3 minutes to ensure complete drying of the column matrix.

9. DNA Elution: The dried column was then transferred to a clean 1.5 mL microcentrifuge tube. A volume of 100 µL of pre-warmed Elution Buffer was added directly to the center of the column matrix and allowed to stand for 3 minutes to facilitate absorption.

10. Final Elution Step: The tube was centrifuged at 14,000–16,000 × g for 30 seconds to elute the purified DNA.

11. Storage: The extracted genomic DNA was stored at –20°C until further analysis.

2.3.1.1.A Determination of purity and concentration of DNA

The concentration and purity of the extracted DNA were assessed using a NanoDrop™ spectrophotometer. DNA purity was evaluated based on the absorbance ratio at 260 nm and 280 nm (A260/A280). For each measurement, 1 µL of the DNA sample was applied to the device's microdetector, and the results were recorded accordingly.

2.3.1.2. Primers design

The primers were designed by Prof Dr. Hassan Mahmood Musa, as demonstrated in Table 2.3

Table 2.3 Primers Sequences to detect rs28942111; T>A SNP by allele-specific PCR

	Sequence (5'->3')	**Length**
Allele T	GCTTCCTGGTGAAGATGAGT	20
Allele A	GCTTCCTGGTGAAGATGAGA	
Revers primer	ACTAAGCACAGTCCCCAGT	19
Product length	114	

2.3.1.3 Dilution of primers

A volume of 150 µL of distilled water was added to each tube containing lyophilized primer to prepare a stock solution at a concentration of 100 pmol/µL, following the manufacturer's instructions. From each stock solution, 10 µL was transferred into a new tube labeled accordingly and mixed with 90 µL of distilled water to obtain a working solution for each primer.

2.3.1.4 Polymerase Chain Reaction PCR

Allele-specific PCR technique was used to detect the SNP rs28942111; T>A of PCSK9 gene.

2.3.1.4.A Polymerase chain reaction protocol

Polymerase chain reaction (PCR) optimization was achieved through multiple trials aimed at determining the ideal annealing temperature, as well as the appropriate concentrations of DNA and primers, and the optimal number of

amplification cycles. The components used for PCR amplification of the target fragments are summarized in Table 2.4, while the finalized thermal cycling conditions are presented in Table 2.5.

Table 2.4 Contents of PCR reaction

Components	Volume (µL)
Forward primer	1.5
Reverse primer	1.5
DNA	2
Nuclease free water	8
PCR premix	12
Total volume	25

Table 2.5 PCR Program for Detecting rs28942111; T>A SNP

Step	Temperature / °C	Time	Number of cycles
Denaturation	95	3 minutes	1
Initial denaturation	95	30 second	30
Annealing	59	50 second	
Extension	72	40 second	
Final extension	72	5 minutes	1

2.3.1.5 Agarose Gel Electrophoresis

1. A 1.5% (w/v) agarose gel was prepared. The dimensions of the gel tray were 10 cm * 15 cm * 0.5 cm, giving a total volume of 75 cm^3. Therefore, 1.125 g of agarose powder (1.5% of 75 mL) was accurately weighed and transferred into a conical flask.

2. A total of 75 mL of 1x TBE buffer was added to a conical flask, which was then placed on a heater. The mixture was heated until bubbles began to form and the initially opaque solution became transparent.

3. After approximately 2 minutes, 1.5 µL of ethidium bromide was added, and the solution was gently shaken to mix.

4. The resulting mixture was poured into the gel casting tray, which had been prepared with casting dams and a comb. The gel was left to solidify at room temperature.

5. Once the gel had solidified, the comb was carefully removed from the tray. The tray was then placed in the electrophoresis tank, which was filled with 1x TBE buffer. DNA samples were loaded into the gel as follows: 5 µL of DNA ladder was loaded into the last well, and 10 µL of each PCR product was loaded into the corresponding wells.

6. The power supply was connected and set to 100 volts, ensuring an electric field of 5 V/cm for a 20 cm distance between the cathode and anode.

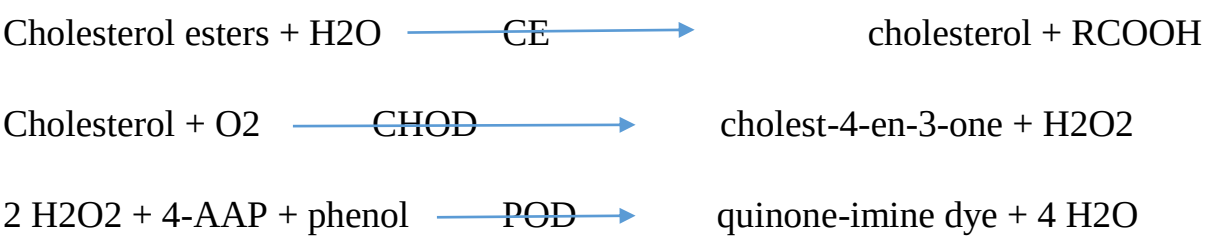
7. After 30 minutes, the gel was transferred to a UV transilluminator for visualization of the PCR products, which appeared as bands moving through the gel. The gel was then returned to the electrophoresis tray for further separation, and visualization was repeated after 1 hour, and again after 1.5 hours, until dissociation of the DNA ladder was observed (Lee et al., 2012).

2.3.2. Biochemical Parameters

2.3.2.1. Determination of Lipid Profile

2.3.2.1.A Estimation of Total Cholesterol

Total cholesterol in serum was quantified in vitro using the enzymatic colorimetric method. Cholesterol esters were hydrolyzed by cholesterol esterase (CE) to release free cholesterol and fatty acids. Subsequently, cholesterol oxidase (CHOD) catalyzed the oxidation of cholesterol to cholest-4-en-3-one and hydrogen peroxide. The hydrogen peroxide then participated in an oxidative coupling reaction with phenol and 4-aminoantipyrine (4-AAP), facilitated by peroxidase (POD), resulting in the formation of a red quinone-imine dye, the intensity of which was measured to determine the cholesterol concentration.



The content of cholesterol is exactly proportional to the color intensity of the dye produced, as assessed by an increase in absorbance at 512 nm (Allain et al., 1974).

2.3.2.1.B Estimation of Serum Triglyceride

Triglyceride levels were measured in vitro using the enzymatic quantitative colorimetric method. In this process, triglycerides are hydrolyzed by lipase, and the resulting glycerol is further processed through enzymatic reactions involving glycerol kinase and glycerol-3-phosphate oxidase, which generates hydrogen

peroxide (H_2O_2). The hydrogen peroxide then catalyzes the oxidation of 4-aminoantipyrine, leading to the formation of a colored quinoneimine dye. The increase in absorbance is directly proportional to the triglyceride concentration (TG, 1997).

triglycerides + 3 H2O $\xrightarrow{\text{lipoprotein lipase}}$ glycerol + 3 RCOOH

glycerol + ATP $\xrightarrow{\text{Glycerol Kinase}}$ glycerol-3-phosphate + ADP

glycerol-3-phosphate + O2 $\xrightarrow{\text{Glycerol-3-Phosphate Oxidase}}$ dihydroxyacetone phosphate

+ H2O2

H2O2 + 4-aminoantipyrine + 4-chlorophenol $\xrightarrow{\text{peroxidase}}$ 4-(p-benzoquinone-monoimino)-phenazone + 2 H2O + HCl

2.3.2.1.C Estimation of Serum High-Density Lipoprotein (HDL)

High-density lipoprotein levels in serum were measured directly using an enzymatic assay. The fundamental principle of the method involves the use of a blocking reagent that specifically reacts with apo-B containing lipoproteins in the sample. This reaction prevents these lipoproteins from interacting with the enzymatic cholesterol reagent, effectively excluding them from the assay. Consequently, only HDL cholesterol is quantified under these conditions. In this assay, sulfated alpha-cyclodextrin, in the presence of Mg^{2+}, forms complexes with apo-B containing lipoproteins. Additionally, polyethylene glycol-coupled cholesteryl esterase and cholesterol oxidase are used to facilitate the measurement of HDL-cholesterol.(Hafiane and Genest, 2015).

Apo-B containing lipoproteins + α-cyclodextrin + Mg+2 + dextran SO4 ⟶

soluble non-reactive complexes with Apo-B containing lipoproteins

HDL-cholesterol esters + H2O $\xrightarrow{\text{PEG-cholesterol esterase}}$ HDL-cholesterol + RCOOH

HDL-cholesterol + O2 $\xrightarrow{\text{PEG-cholesterol oxidase}}$ Δ4-cholestenone + H2O2

H2O2 + 5-aminophenazone+ N-ethyl-N-(3-methylphenyl)-N-succinyl ethylene diamine + H+ + H2O $\xrightarrow{\text{peroxidase}}$ purple blue pigment (quinoneimine) + H2O

The amount of HDL cholesterol present is proportionally correlated with the amount of blue quinoneimine dye produced

2.3.2.1.D. Estimation of Serum Low-Density Lipoprotein (LDL)

The concentration of LDL was estimated indirectly using Friedewald and colleagues' equation, which has become the more prevalent method in clinical laboratories(de Cordova and de Cordova, 2013).

LDL mmol/L = [Total-chol.] - [HDL] - [TG/5]

2.3.2.1.E. Estimation of Serum Very Low-Density Lipoprotein (VLDL)

Very Low-Density Lipoprotein (VLDL) cholesterol can be estimated using the Friedewald equation, which expresses VLDL as one-fifth of the serum triglyceride (TG) concentration: VLDL = TG/5. This estimation is widely used in clinical practice; however, it is considered reliable only when triglyceride levels are below 400 mg/dL, as higher concentrations may require direct measurement for accuracy (Islam et al., 2022).

2.3.2.2. Determination of Liver function

2.3.2.2.A. Estimation of Alanine Aminotransferase (ALT)

Alanine aminotransferase (ALT) is measured on the Cobas c311 analyzer using a kinetic UV method, following the guidelines established by the International Federation of Clinical Chemistry (IFCC). In this technique, ALT catalyzes the conversion of L-alanine and α-ketoglutarate to pyruvate and L-glutamate. Pyruvate reacts with NADH in the presence of lactate dehydrogenase, resulting in oxidation of NADH to NAD^+. The corresponding reduction in absorbance at 340 nm is directly related to ALT levels (Infusino and Mauro, 2009).

2.3.2.2.B. Estimation of Aspartate Aminotransferase (AST)

The level of AST is determined by using kinetic UV method from IFCC. In this method, AST catalyzes the reaction between L-aspartate and α-ketoglutarate that result oxaloacetate and L-glutamate. Then oxaloacetate reacts with NADH in the presence of malate dehydrogenase, resulting in the oxidation of NADH to NAD^+. The corresponding reduction in absorbance at 340 nm is directly related to AST levels (Jansen et al., 2006).

2.3.2.2.C. Estimation of Total Bilirubin (TB)

The level of total bilirubin is determined by using diazo colorimetric method on cobas c311 device analyzer. In this method, bilirubin reacts with diazotized sulfanilic acid under acidic conditions, yielding a colored azobilirubin compound. The intensity of this colored complex is measured photometrically at 546 nm, and its absorbance correlates with the bilirubin con in the sample. The Cobas c311 system enhances measurement accuracy through automated calibration and built-in quality control measures (Roche Diagnostics, 2014).

2.4 Statistical Analysis

The data from this study were entered and analyzed using the Statistical Package for the Social Sciences (IBM SPSS.26). Results are presented as frequencies and percentages, means with standard error of the mean (SEM), or mean differences, as appropriate, in tables and graphs. To examine potential associations between related variables, the Chi-square test, one-way ANOVA, and post hoc analysis were employed. The least significant difference (LSD) test was used when equal variances were assumed, while Dunnett's T3 test was applied when equal variances were not assumed, based on Levine's test for homogeneity of variances. Statistical significance was considered when the p-value was less than or equal to 0.05 ($p \leq 0.05$).

Correlation analysis was done for AIP, CRI-I, and CRI-II with same pattern.

Beside multiple linear regression was conducted to evaluate the impact of single nucleotide polymorphism on the response to atorvastatin therapy in the patients under the study.

Chapter Three
Results

3.1 Socio-demographic and Some Related Characteristics of Patients

A total of 149 Iraqi participants, age ranged from 25 to 85 years with primary hyperlipidemia taking atorvastatin 40mg for at least six months were included in this study. Their demographic details are outlined in Table 3.1. Among the participants, 28.2% of patients showed a good response to statin therapy (40 mg atorvastatin). Approximately 35% had a moderate response, while about 39% exhibited poor or no response. Table 3.1 also shows that 11.4% of patients were classified as having a low risk of ischemic heart disease (IHD), 3.4% had a moderate risk, and 85% had a high risk of IHD based on AIP results. Regarding CRI-1, about 46% of patients were classified as low risk, while 54% were considered at high risk for CVD and atherosclerosis. For CRI-2, 80% of the participants were at low risk, and approximately 20% were at high risk for CVD and atherosclerosis.

The clinical and biochemical parameters of the 149 enrolled patients are demonstrated in Table 3.2 with their maximum and minimum border.

The influence of age on the mean levels of various laboratory biomarkers, presented in Table 3.3, reveals statistically significant differences in BMI between group B (46-65 years) and group A (25-45 years) ($p = 0.014$), as well as in TC levels between group C (66-85 years) and group B ($p = 0.036$). Additionally, a significant difference in the AIP was found between group A and group C ($p = 0.048$), as well as between group B and group C ($p = 0.050$). Statistically significant differences were also observed in CRI-I between groups A and C ($p = 0.034$) and B and C ($p = 0.032$).

The effect of gender on the mean levels of laboratory biomarkers indicated no statistically significant differences between male and female patients for any of the studied biomarkers (Table 3.4).

Regarding the duration of treatment, statistically significant differences were found in TC levels between group C (25-36 months) and group B (13-24 months) ($p = 0.047$), and in AST levels between group C (25-36 months) and group A (6-12 months) ($p = 0.001$), as well as between group C and group B ($p = 0.003$). AST levels also showed significant differences between group C and group D (37-48 months) ($p = 0.003$), (Table 3.5).

The effect of smoking status on the mean levels of laboratory biomarkers, summarized in Table 3.6 revealed no statistically significant differences between smokers and non-smokers for any of the measured parameters.

Table 3.1: The demographic and atherogenic characteristics of the hyperlipidemia patients

Variable		Frequency	Percent%
Gender	Male	80	53.7
	Female	69	46.3
Age	25-45	25	16.8
	46-65	95	63.8
	66-85	29	19.5
BMI (Kg/m^2)	Under weight	1	0.7
	Normal	32	21.5
	Over weight	88	59.1
	Obese	27	18.1
Duration of treatment (months)	6-12	112	75.2
	13-24	25	16.8
	25-36	6	4.0
	37-48	6	4.0
Smoking	No smoke	100	67.1
	Smoker	49	32.9
Response to drug	<50 Good	42	28.2
	51-100 Moderate	52	34.9
	>100 None	55	36.9
AIP	<0.11	17	11.4
	0.11-0.21	5	3.4
	>0.21	127	85.2
CRI-I	<3.5	68	45.6
	>3.5	81	54.4
CRI-II	<3	118	79.2
	>3	31	20.8

BMI: body mass index, AIP: Atherogenic Index of Plasma, CRI-1: Castelli's risk indexe-1, CRI-2**:** Castelli's risk indexe-2

Table 3.2: Descriptive statistics of the clinical and biochemical data of the studied patients

Parameter	Minimum	Maximum	Mean	SEM
Age	28	85	56.38	0.977
Weight	54	107	76.07	0.864
Height	1.5	1.9	1.65	0.006
BMI (Kg/m^2)	18	41	27.69	0.310
DRx(months)	6	48	12.82	0.861
SBP(mmHg)	65	180	133.55	1.598
DBP(mmHg)	50	110	83.16	0.924
RBS(mg/dl)	86	700	144.21	6.068
LDL(mg/dl)	14.2	238	87.05	3.860
HDL(mg/dl)	18	178	46.42	1.638
VLDL(mg/dl)	5.4	85.2	27.77	1.183
TC(mg/dl)	65	298	158.23	4.209
TG(mg/dl)	27	426	138.87	5.915
ALT(U/L)	5	85	20.72	0.853
AST(U/L)	13	645	35.75	5.291
TBL(mg/dl)	0.1	3.3	0.58	0.474
AIP	-0.29	1.02	2.73	0.65
CRI-1	1.44	9.45	3.73	0.122

CRI-2	0.19	6.94	2.04	0.103

BMI stands for body mass index, SBP refers to systolic blood pressure, DBP is diastolic blood pressure, RGS indicates random blood sugar, LDL represents low-density lipoprotein, HDL denotes high-density lipoprotein, VLDL refers to very low-density lipoprotein, TC stands for total cholesterol, TG indicates triglycerides, ALT represents alanine transaminase, AST refers to aspartate transaminase, and TBL denotes total bilirubin.

Table 3.3: Description of the clinical and biochemical markers of the studied patients according to the age

Parameter	A:25-45 (25)	B:46-65 (95)	C:66-85 (29)	MC	P value
BMI(Kg/m^2)	26.25±0.67	28.32±0.41	26.86±0.47	B vs A	0.014
SBP(mmHg)	129.60±4.93	134.01±1.86	135.45±3.51	NS	0.512
DBP(mmHg)	81.36±2.25	83.72±1.08	82.90±2.50	NS	0.646
RGS(mg/dl)	149.32±23.50	139.74±5.66	154.48±15.22	NS	0.603
LDL(mg/dl)	91.40±8.49	89.78±4.92	74.34±8.79	NS	0.268
HDL(mg/dl)	43.20±3.46	46.15±1.72	50.10±5.51	NS	0.441
VLDL(mg/dl)	29.52±3.45	28.70±1.47	23.20±2.06	NS	0.160
TC(mg/dl)	160.44±10.78	163.14±5.12	140.28±9.60	C vs B	0.036
TG(mg/dl)	147.60±17.28	143.55±7.38	116.03±10.32	NS	0.160
ALT(U/L)	22.28±2.97	20.60±0.98	19.77±1.57	NS	0.669
AST(U/L)	51.00±24.81	32.38±4.79	33.66±6.61	NS	0.434
TBL(mg/dl)	0.820±0.14	0.544±0.05	0.500±0.08	B vs A C vs A	0.034 0.042
AIP	0.49±0.049	0.46±0.026	0.35±0.044	A vs C B vs C	0.048 0.050
CRI-1	4.20±0.324	3.83±0.151	3.15±0.250	A vs C B vs C	0.034 0.032
CRI-2	2.27±0.261	2.11±0.126	1.61±0.231	NS	0.105

One-way ANOVA test was used with a significant p value of less than 0.05, Results are presented as mean ± SEM.

Table 3.4: Description of the clinical and biochemical markers of the studied patients according to the gender

Parameter	Gender	Mean	SEM	P value
BMI(Kg/m^2)	Male	27.71	0.342	0.941
	Female	27.66	0.542	
SBP(mmHg)	Male	132.13	2.309	0.399
	Female	135.20	2.179	
DBP(mmHg)	Male	82.35	1.442	0.334
	Female	84.10	1.091	
RBS(mg/dl)	Male	148.36	9.744	0.464
	Female	139.41	6.671	
LDL(mg/dl)	Male	86.71	4.950	0.925
	Female	87.44	6.084	
HDL(mg/dl)	Male	45.93	2.609	0.750
	Female	46.99	1.852	
VLDL(mg/dl)	Male	26.47	1.525	0.236
	Female	29.28	1.838	
TC(mg/dl)	Male	154.99	5.501	0.408
	Female	162.00	6.489	
TG(mg/dl)	Male	132.35	7.629	0.236
	Female	146.43	9.192	
ALT(U/L)	Male	21.56	1.432	0.269
	Female	19.74	0.796	
AST(U/L)	Male	44.30	9.714	0.063
	Female	25.8	1.335	
TBL(mg/dl)	Male	0.65	0.698	0.122
	Female	0.50	0.620	
AIP	Male	0.44	0.027	0.76
	Female	0.45	0.032	

CRI-1	Male	3.75	0.162	0.873
	Female	3.71	0.187	
CRI-2	Male	2.07	0.129	0.777
	Female	2.01	0.165	

Independent t-test was used with a significant p value of less than 0.05. Results are presented as mean ± SE

Table 3.5: The description of the clinical and biochemical markers of the studied patients according to duration of treatment

Parameter	**A-6-12 months (n=112)**	**B- 13-24 months (n=25)**	**C-25-36 months (n=6)**	**D-37-48 months (n=6)**	**MC**	**P value**
BMI(Kg/m^2)	27.61±0.35	28.36±0.922	25.87±1.09	28.26±0.61	NS	0.505
SBP(mmHg)	132.01±1.85	140.16±3.72	135.00±6.70	133.33±8.79	NS	0.310
DBP(mmHg)	82.76±0.97	84.56±2.50	85.83±4.90	82.17±8.83	NS	0.826
RBS(mg/dl)	143.53±7.09	143.80±14.35	161.83±43.14	141.17±11.95	NS	0.949
LDL(mg/dl)	84.21±3.97	89.13±12.59	120.86±22.71	97.60±21.08	NS	0.280
HDL(mg/dl)	46.07±2.02	48.32±3.29	45.33±4.90	46.16±4.83	NS	0.965
VLDL(mg/dl)	28.90±1.43	24.69±2.66	25.96±3.35	21.40±1.83	NS	0.383
TC(mg/dl)	159.71±4.42	145.80±12.87	192.33±26.19	148.50±22.70	C vs B	0.047
TG(mg/dl)	144.50±7.16	123.48±13.32	129.83±16.76	107.00±9.18	NS	0.383
ALT(U/L)	20.52±0.86	19.58±1.71	35.83±10.33	14.06±1.46	NS	0.669
AST(U/L)	29.57±2.21	44.04±17.20	130.33±102.94	22.06±2.87	C vs A C vs B C vs D	0.001 0.003 0.003
TBL(mg/dl)	0.596±0.05	0.504±0.09	0.700±0.11	0.533±0.55	NS	0.851
AIP	0.47±0.025	0.37±0.04	0.45±0.77	0.37±0.06	NS	0.300
CRI-1	3.83±0.133	3.28±0.388	4.26±0.476	3.29±0.402	NS	0.258

CRI-2	2.09±0.106	1.75±0.361	2.64±0.440	1.79±0.350	NS	0.385

One-way ANOVA test was used with a significant p value of less than 0.05, Results are presented as mean ± SEM

Table 3.6: Description of the clinical and biochemical markers of the studied patients according to the smoking status (n=149)

Parameter	Status	Mean ± SEM	P value
BMI(Kg/m^2)	No	27.83±0.93	0.153
	Yes	28.32±0.49	
SBP(mmHg)	No	134.44±1.89	0.428
	Yes	131.73±2.96	
DBP(mmHg)	No	83.54±1.06	0.560
	Yes	82.39±1.79	
RBS(mg/dl)	No	142.05±5.98	0.612
	Yes	148.63±13.92	
LDL(mg/dl)	No	87.62±4.77	0.834
	Yes	85.89±6.61	
HDL(mg/dl)	No	44.42±1.44	0.156
	Yes	50.51±3.97	
VLDL(mg/dl)	No	27.22±1.41	0.508
	Yes	28.89±2.16	
TC(mg/dl)	No	156.63±5.29	0.588
	Yes	161.51±6.90	
TG(mg/dl)	No	136.12±7.05	0.508
	Yes	144.49±10.83	
ALT(U/L)	No	20.44±0.77	0.703
	Yes	21.29±2.07	
AST(U/L)	No	30.05±4.38	0.221
	Yes	47.39±13.32	
TBL(mg/dl)	No	0.581±0.05	0.979
	Yes	0.584±0.09	
AIP	No	0.45±0.02	0.804
	Yes	0.44±0.03	
CRI-1	No	3.77±0.151	0.647
	Yes	3.65±0.208	
CRI-2	No	2.06±0.127	0.731
	Yes	1.99±0.176	

3.2 Genotyping of PCSK9 rs28942111; T>A SNP

3.2.1 Results of Amplification Reaction

The primers designed to detect rs28942111; T>A SNP produced a clear band with a molecular size of 114 bps. (Figure 3.1). The size of the amplicon was estimated by comparing it to a 100-1000 bp DNA ladder.

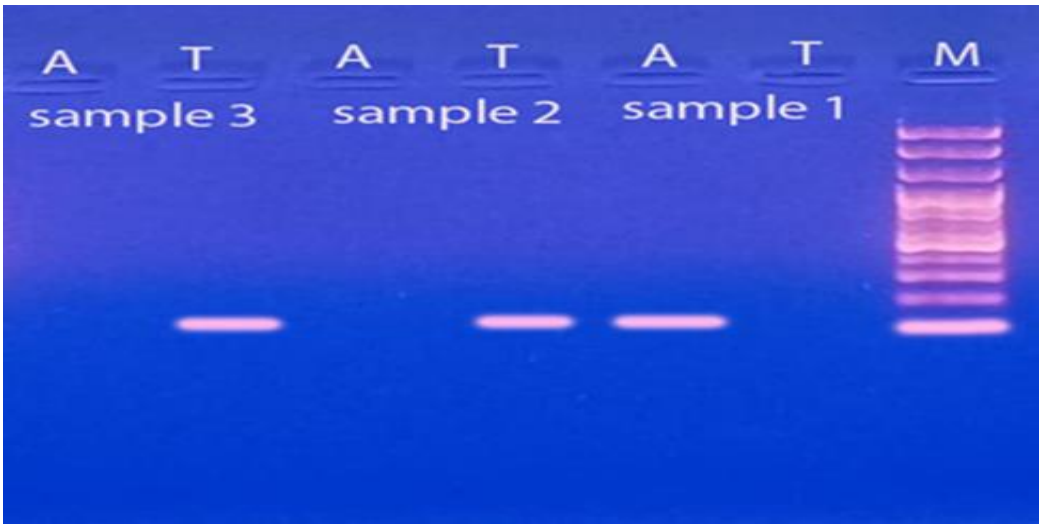


Figure 3.1: The agarose gel electrophoresis of identifying rs28942111; T>A SNP using allele specific-PCR. Sample 1 shows the mutant homozygous type (AA), samples 2 and 3 show the wild type (TT), M represents 100-1000 bp DNA ladder.

3.2.2 Distribution of Allele Frequencies of PCSK9 rs28942111; T>A SNP

For the purpose of this study, the participants were categorized in to two groups according to genotypes of rs28942111; T>A SNP:

homozygous for the T allele (TT) the wild type, and homozygous for the A allele (AA) the mutant type. There were 128 TT carriers (85.9%), and 21 AA carriers (14.1%) among 149 patients. There was no heterozygous mutant type was detected in the study subjects Table 3.7.

Table 3.7 The distribution of rs28942111; T>A SNP among hyperlipidemia patients

Variable		Frequency	Percent%
Genotype	TT wild	128	85.9
	Heterozygous mutant type (TA)	0	0.00
	Homozygous mutant type (AA)	21	14.1

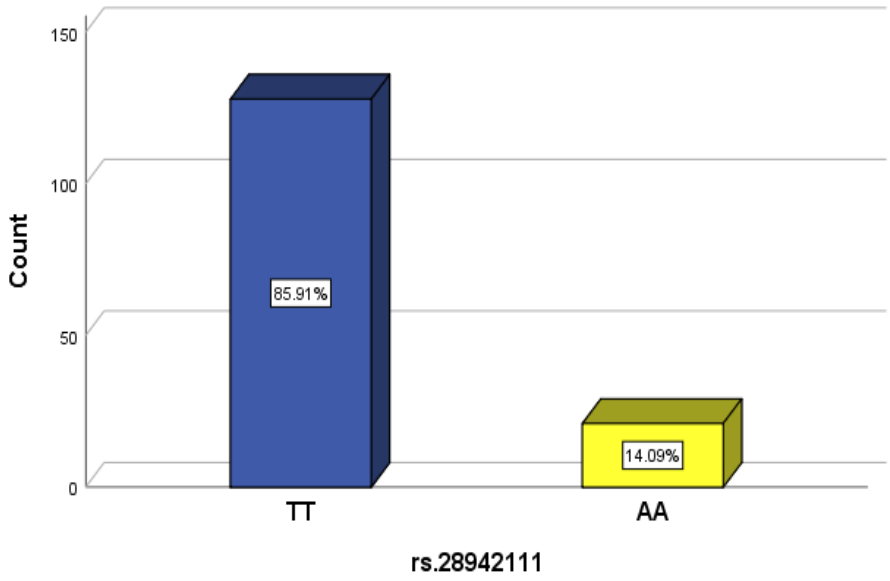


Figure 3.2 The distribution of rs28942111 SNP among hyperlipidemia patients

3.2.3 The association between rs28942111; T>A SNP and the sociodemographic and Biochemical Parameters

The levels of LDL, total cholesterol (TC), aspartate transaminase (AST), CRI-I and CRI-II differed significantly between patient's groups; those with the AA genotype exhibited higher levels of LDL, TC, and AST compared to TT carriers, with a p-value of 0.001 and higher risk to IHD according to CRI-I and CRI-II results (Table 3.8). No significant differences were found for the other laboratory biomarkers between the two groups. Although triglyceride (TG) levels were higher in the AA group compared to the TT group, but this difference was not statistically significant (p-value of 0.167) (Table 3.8).

The association between the genotype and the effect of atorvastatin based on the LDL level reduction represented in Table 3.9. There are 42 patients with TT carriers and none of AA carriers have LDL level less than 50 mg\dl and they have good response to reach the target, while 51 patients with TT carriers and only one of AA carriers have LDL level range from 51-100 mg\dl and they have moderate response to reach the target. Thirty-five patients with TT carriers and 20 of AA carriers have LDL level more than 100 mg\dl and they have poor or nonresponse to reach the target Figure 3.3.

The association of genetic variation on CRI-I is represented in Table 3.10 and show that 58.4% and 28.6% of patients that carry TT and AA respectively have a low risk to IHD and atherosclerosis while 51.6% and 71.4% of patients from our study that they carry TT and AA allele respectively have high risk to IHD and atherosclerosis.

The association of genetic variation on CRI-II is represented in Table 3.11 and show that 82% and 61.9% of patients that carry TT and AA respectively have a low risk to IHD and atherosclerosis while 18% and 38.1% of patients from our study that they carry TT and AA allele respectively have high risk to IHD and atherosclerosis.

The correlation analysis in Table 3.12 revealed a statistically significant positive association between AIP and triglyceride levels ($r = 0.745$, $p = 0.001$), indicating that higher TG levels are strongly linked with elevated atherogenic index of plasma among the study population.

The results in Table 3.13 demonstrated that CRI-I was significantly positively correlated with LDL, VLDL, and CRI-II (with p-value < 0.001), while showing a significant negative correlation with HDL ($r = -0.511$, $p < 0.001$). Similarly, CRI-II

exhibited significant positive correlations with LDL ($r = 0.793$, $p < 0.001$) and significant negative correlations with HDL ($r = -0.471$, $p < 0.001$). No significant correlation was observed between CRI-II and VLDL ($p = 0.293$).

Multiple linear regression analysis in Table 3.14 revealed a statistically significant effect of the rs28942111; T>A SNP on LDL levels ($r = 0.537$, $R^2 = 0.288$, $F = 59.494$, $p = 0.001$), indicating that this polymorphism accounts for approximately 28.8% of the variability in LDL concentrations among the study population.

Table 3.8: The association between rs28942111; T>A SNP and the clinical and biochemical markers in the hyperlipidemia patients.

Parameter	Genotype TT (n=128) AA (n=21)	Mean ±SEM	P value
BMI(Kg/m^2)	TT	27.65±0.34	0.726
	AA	27.96±0.74	
SBP(mmHg)	TT	133.35±1.75	0.760
	AA	134.76±3.85	
DBP(mmHg)	TT	83.16±0.96	0.994
	AA	83.14±2.92	
RGS(mg/dl)	TT	144.71±6.95	0.841
	AA	141.19±15.84	
LDL(mg/dl)	TT	76.84±3.54	0.001
	AA	149.28±8.33	
HDL(mg/dl)	TT	45.82±1.82	0.366
	AA	50.09±3.45	
VLDL(mg/dl)	TT	27.11±1.20	0.167
	AA	31.81±3.99	
TC(mg/dl)	TT	150.85±4.04	0.001
	AA	203.24±13.40	
TG(mg/dl)	TT	135.55±6.04	0.167
	AA	159.10±19.99	
ALT(U/L)	TT	20.20±0.73	0.393
	AA	23.85±4.11	

AST(U/L)	TT	28.93±1.73	0.001
	AA	80.63±35.16	
TBL(mg/dl)	TT	0.569±0.052	0.496
	AA	0.662±0.106	
AIP	TT	0.45±0.02	0.976
	AA	0.44±0.06	
CRI-1	TT	3.36±0.122	0.033
	AA	4.38±0.428	
CRI-2	TT	1.93±0.100	0.011
	AA	2.69±0.382	

Table 3.9 The impact of rs28942111; T>A SNP on atorvastatin response based on LDL level reduction

Variable		**<50mg\dl of LDL conc. Good response**	**51-100mg\dl of LDL conc. Moderate response**	**>100mg\dl of LDL conc. None response**	**P value**	**Eta value**
Genotype	TT	42 (32.8%)	51 (39.8%)	35 (27.3%)	0.001	0.490
	AA	0 (0.0%)	1 (4.8%)	20 (95.2%)		

Chi square test was used to detect the association.

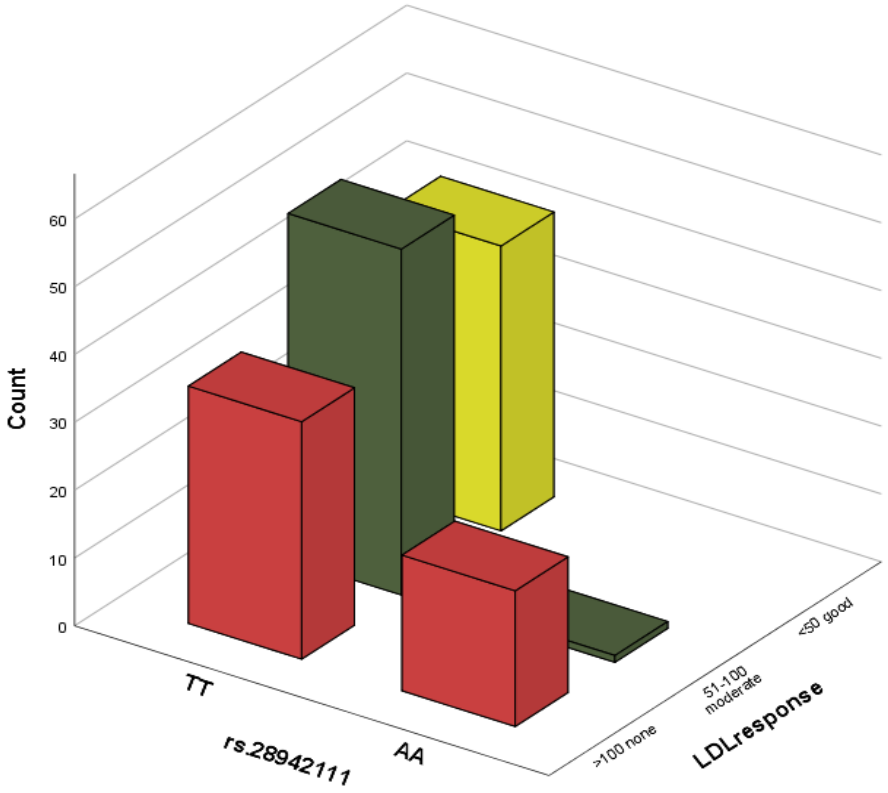


Figure 3.3 The distribution of LDL level reduction according to the genotype group of rs28942111; T>A SNP

Table 3.10: The association between rs28942111; T>A SNP and CRI-1

Variable		Low risk	High risk	P value
Genotype	TT	62(58.4%)	66(51.6%)	0.090
	AA	6(28.6%)	15(71.4%)	

Table 3.11: The association between rs28942111; T>A SNP and CRI-2

Variable		Low risk	High risk	P value	Eta value
Genotype	TT	105 (82.0%)	23 (18.0%)	0.035	0.173
	AA	13 (61.9%)	8 (38.1%)		

Table 3.12: The correlation matrix between AIP and some of the related parameters under the study.

Parameter	Status	AIP	SBP	LDL	TG
AIP	Correlation	1	0.041	0.618	0.745**
	p Value		0.618	0.153	0.001
SBP	Correlation	0.041	1	-0.106	0.057
	P value	0.618		0.200	0.492
LDL	Correlation	0.153	-0.106	1	0.134
	P value	0.062	0.200		0.103
TG	Correlation	0.745**	0.001	0.134	1
	P value	0.001	0.057	0.103	

Table 3.13: The correlation Matrix between CRI-I and CRI-II and some of the related parameters under the study.

		LDL	HDL	VLDL	CRI2
CRI1	Pearson Correlation	.706**	-.511**	.302**	.954**
	P value	.000	.000	.000	.000
CRI2	Pearson Correlation	.793**	-.471**	.087	
	P value	.000	.000	.293	

Table 3.14: Multiple linear regression for the effect of rs28942111; T>A SNP on the LDL level.

Dependent variable	Pearson correlation (r) rs28942111	R square	F value	P value
LDL	0.537	0.288	59.494	0.001**

Correlation matrix of biochemical and demographic parameters is demonstrated in Table 3.15 and showed that LDL vs. TC have strong positive correlation (r = 0.789, p = 0.001) which indicates that LDL is a major contributor to total cholesterol levels. As well as HDL vs. TC shows moderate positive correlation (r = 0.260, p = 0.001) which explained that HDL contributes positively but less strongly to total cholesterol. Besides VLDL vs. TC and TG have high correlation with both (r = 0.438, p = 0.001), and r = 1.000. TG as expected, VLDL and TG are closely linked (VLDL is calculated from TG).

Regarding liver enzymes ALT vs. LDL have weak but significant positive correlation (r = 0.209, p = 0.011). While ALT vs. TG/VLDL: (r = 0.198, p = 0.016) for both. As well as ALT vs. AST have strong correlation (r = 0.520, p = 0.001) which reflects concurrent liver involvement and consistency in liver enzyme elevation. AST vs. LDL have weak positive correlation (r = 0.188, p = 0.021)

Clinical Insights reflects that LDL and TC are closely related important for cardiovascular risk evaluation. ALT and AST correlation confirms that liver enzyme elevations usually occur together.

Table 3.15 Correlation matrix of biochemical and demographic parameters in studied participants

Parameter	Status	Age	LDL	HDL	VLDL	TC	TG	ALT	AST	TBL
Age	Correlation	1	-0.131	0.131	-0.117	-0.137	-0.117	-0.078	-0.026	-0.095
	p Value		0.112	0.110	0.154	0.096	0.154	0.347	0.750	0.249
LDL	Correlation	-0.131	1	-0.150	0.134	.789**	0.134	.209*	.188*	0.078
	P value	0.112		0.068	0.103	0.001	0.103	0.011	0.021	0.342
HDL	Correlation	0.131	-0.150	1	0.126	.260**	0.126	-0.149	-0.061	-.203*
	P value	0.110	0.068		0.127	0.001	0.127	0.070	0.458	0.013
VLDL	Correlation	-0.117	0.134	0.126	1	.438**	1.000**	.198*	0.071	0.154
	P value	0.154	0.103	0.127		0.001	0.001	0.016	0.391	0.061
TC	Correlation	-0.137	.789**	.260**	.438**	1	.438**	.185*	0.057	0.022
	P value	0.096	0.001	0.001	0.001		0.000	0.024	0.487	0.788
TG	Correlation	-0.117	0.134	0.126	1.000**	.438**	1	.198*	0.071	0.154
	P value	0.154	0.103	0.127	0.001	0.001		0.016	0.391	0.061
ALT	Correlation	-0.078	.209*	-0.149	.198*	.185*	.198*	1	.520**	0.082
	P value	0.347	0.011	0.070	0.016	0.024	0.016		0.001	0.319
AST	Correlation	-0.026	.188*	-0.061	0.071	0.057	0.071	.520**	1	0.077
	P value	0.750	0.021	0.458	0.391	0.487	0.391	0.001		0.348
TBL	Correlation	-0.095	0.078	-.203*	0.154	0.022	0.154	0.082	0.077	1
	P value	0.249	0.342	0.013	0.061	0.788	0.061	0.319	0.348	

Chapter Four
Discussion

4. Discussion

Hyperlipidemia is a well-established risk factor for CVDs (Alloubani et al., 2021). It is characterized by an increase in plasma lipids, including triglycerides, cholesterol, cholesterol esters, and phospholipids, as well as elevated levels of plasma lipoproteins such as VLDL and LDL. Conversely, HDL levels tend to decrease (Sheeba and Gandhimathi, 2021). Elevated cholesterol (hypercholesterolemia) and triglycerides (hypertriglyceridemia) contribute significantly to atherosclerosis, a condition closely linked to IHD (Shao et al., 2020).

Proprotein convertase subtilisin/kexin type 9 plays a critical role in cholesterol metabolism by regulating the degradation of LDLR (Baragetti et al., 2018). PCSK9, produced primarily in liver cells, binds to LDLR, promoting its breakdown. Each LDLR is capable of transporting thousands of LDL particles from the bloodstream into cells, where they are eventually degraded in lysosomes. By decreasing the number of available LDLRs on the hepatocyte surfaces, PCSK9 leads to increased LDL concentration in the blood (Lagace, 2014). Mutations in the PCSK9 gene can have significant impacts on cardiovascular health and they are either: LOF mutations are generally protective against CVD (Ruscica et al., 2016), whereas GOF mutations can cause familial hypercholesterolemia and elevate cardiovascular risk (Schulz and Schlüter, 2017). Furthermore, specific GOF mutations have been associated with liver damage, particularly in individuals with non-alcoholic fatty liver disease (Cohen et al., 2006).

4.1 Genetic analysis

4.1.1 The Proprotein Convertase Subtilisin/Kexin Type 9 Gene Polymorphism

The rs28942111; T>A SNP results from a serine-arginine amino acid substitution at codon 127, so it's also known as S127R, in the PCSK9 gene. This mutation was detected by using allele specific PCR technique and the results of the PCR reaction show that the carriers of the homozygous wild type TT were the most common accounting about 86 percent of the patients enrolled in this study, while the homozygous mutant type AA accounting only 14% of the studied patients. It is worth noting that none of the samples included in the study were carrier to the heterozygous TA allele. These findings indicate that the T allele is the predominant allele within this population, whereas the A allele occurs less frequently.

Other studies in the genotype distribution in other populations show similar results. For example, a study in the genotype distribution among Chinese population show that the wild type TT is the major allele while the AA allele occur less frequently Similar studies in genotype distribution have been observed in other populations (Zhao et al., 2021a). These results are consistent with additional research, particularly within east Asian populations, where the T allele has also been shown as the major allele (Mahdieh et al., 2020). Single nucleotide polymorphisms such as rs28942111; T>A have been associated with numerous physiological traits and disease susceptibility, understanding their distribution across populations is important factor in genetic epidemiology (Fernández-Rhodes et al., 2020).

4.1.2 Impact of PCSK9 Gene Polymorphism on Atorvastatin response and in the occurrence of hyperlipidemia

The Proprotein Convertase Subtilisin/Kexin Type 9 gene plays a critical role in the cholesterol hemostasis

The Proprotein Convertase Subtilisin/Kexin Type 9 gene plays an important role in lipid metabolism by regulating the degradation of LDLR, thereby influencing plasma LDL cholesterol level. Genetic variations within PCSK9 can lead to either GOF or LOF mutations, both of which have been shown to markedly affect the occurrence of hyperlipidemia and the risk of cardiovascular disease (Kamar et al., 2021).

This study focuses on the rs28942111; T>A SNP, and its association with the lipid-lowering efficacy of atorvastatin in Iraqi hyperlipidemic patients. The rs28942111; T>A SNP has been characterized as a GOF mutation within the PCSK9 gene (Gao et al., 2024).

GOF mutations enhance PCSK9 activity, accelerating LDLR degradation and result in increasing plasma LDL concentration. This mechanism is linked with an increased risk of developing familial hyperlipidemia and CVD (Abifadel et al., 2003). Several investigations have linked PCSK9 GOF mutations, including D374Y, S127R, and F216L, with elevated LDL cholesterol concentration and an increased risk of atherosclerotic and CVDs (Kotowski et al., 2006).

Significant association between the PCSK9 rs28942111; T>A polymorphism and atorvastatin response based on LDL level reduction is highlighted in Table 3.9, with a P-value of 0.001, indicating a strong statistically significance difference in the number of the drug responders between the wild type and the mutant type carriers. The Eta value (0.490) suggests a moderate to strong effect size, implying that genetic variation plays a crucial role in determining atorvastatin response.

Individuals with the TT genotype exhibited a better response to LDL lowering therapy, with 32.8% showing a good response and 39.8% showing a moderate response. A smaller proportion 27.3% of TT carriers had no significant response, suggesting that this genotype is associated with improved LDL clearance and effective cholesterol reduction.

In contrast, the AA allele carriers displayed poor response to the lipid lowering therapy, with one from the studied patients achieving a good response target which is less than 50mg/dl of LDL plasma0 concentration and only 4.8% demonstrating a moderate response which is between 50-100mg/dl. A substantial proportion (95.2%) of studied patients with the AA genotype exhibited minimal reductions in LDL levels (more than 100mg/dl), indicating a notable genetic resistance to the lipid-lowering effects of atorvastatin.

The TT allele carrier's patients, in contrast, may facilitate more effective LDL reduction due to enhanced recycling of LDL receptors and more efficient inhibition of PCSK9 activity. On the other hand, the AA genotype is likely associated with increased PCSK9 function, resulting in increasing degradation of LDL receptors and diminished to statin therapy effectiveness (Cohen et al., 2006).

These findings are consistant with existing literature indicating that PCSK9 gene polymorphisms significantly influence lipid metabolism and treatment outcomes. Prior studies have shown that GOF mutations in PCSK9 can compromise LDL receptor activity, increase plasma LDL cholesterol concentration, and reduce statins effectiveness, that may result in increasing the risk of cardiovascular disease (Maligłówka et al., 2022).

In the current study, statistically significant differences in LDL and total TC levels were observed among the genotypic groups. Patients with the AA genotype exhibited notably higher LDL and TC concentrations compared to TT carriers ($p = 0.001$), as detailed in Table 3.8. The GOF mutation (rs28942111; T>A) resulted in a decrease in the amount of cell surface LDLR and the amount of LDL internalization because PCSK9 gene with this mutation had a greater affinity for LDLR than did the wild type of PCSK9 (five-fold at pH 7.5). This could be one of the causes of the failure of atorvastatin therapy which is presented as the sustained high LDL levels among the AA carriers as shown in (Table 3.8). When rs28942111; T>A mutation is found in PCSK9 gene, the PCSK9 enzyme is characterized by poor autocatalytic cleavage, a failure to be secreted from cells, and a reduction in LDLR expression and activity compared to wild type PCSK9. Genetic analyses and studies on overexpression have shown that the rs28942111; S127R mutation is causally linked to the familial hypercholesterolemia phenotype (Huijgen et al., 2021).

Interestingly, AA genotype carriers also exhibited significantly higher AST levels, suggesting possible hepatocellular stress or damage (Table 3.8).

Previous research has demonstrated that PCSK9 significantly enhances the production of triglyceride-rich lipids in the intestine, partly through its posttranscriptional impact on LDL receptors and microsomal triglyceride transfer protein. This mechanism plays a role in the development of non-alcoholic fatty liver disease (Rashid et al., 2014). Elevated levels of PCSK9 in the liver or bloodstream are crucial for lipid storage in muscles and the liver, as well as for adipose energy storage and the regulation of fatty acid and triglyceride secretion, further contributing to the progression of non-alcoholic fatty liver disease (Rashid et al., 2014). This could explain our finding of significant high (p-value=0.001) levels of AST in the AA allele carriers compared with the wild-type (TT) carriers.

The S127R mutation in the PCSK9 gene is of particular significance due to its unique functional characteristics. Despite impairing autocatalytic cleavage and preventing secretion, this mutation still markedly reduces the expression and activity of the LDLR, suggesting that PCSK9 can exert intracellular effects on LDLR function. This is consistence with earlier findings that hepatic overexpression of PCSK9 leads to suppressed LDLR expression (Leren, 2004). Further evidence from studies in PCSK9 deficient mice demonstrated a significant reduction in hepatic lipid accumulation under both low and high cholesterol dietary conditions, indicating a protective role of PCSK9 deficiency against hepatic steatosis (Ruscica et al., 2016). Additionally, post-transcriptional mechanisms appear to influence LDLR regulation via PCSK9 (Zhang et al., 2016) reinforcing the gene’s complex role in lipid homeostasis. Aberrant regulation of LDLR can contribute to pathological lipid deposition in tissues such as vascular smooth muscle, liver, and renal cells (Zaid et al., 2008).

Furthermore, the improper regulation of the LDLR pathway has been implicated in the pathogenesis of organ damage caused by lipid disorder, including conditions such as atherosclerosis, non-alcoholic fatty liver disease (NAFLD), and renal disease (Zhang et al., 2016). Elevated plasma concentration of PCSK9 may also been associated with elevated hepatic production and secretion of triglyceride-rich lipoproteins, suggesting a potential role for PCSK9 in the progression of NAFLD (Tavori et al., 2015).

The significant variation in LDL-reduction response between individuals with the TT and AA genotypes highlights the potential clinical utility of PCSK9 genotyping in guiding personalized treatment strategies. For patients carrying the AA genotype, who may exhibit reduced responsiveness to statin therapy, alternative lipid-lowering approaches, such as PCSK9 inhibitors (e.g., evolocumab or alirocumab) or the cholesterol absorption inhibitor ezetimibe, could be more effective in achieving target LDL plasma concentration (Meyer and Merkel, 2018).

This study focus on the importance of personalized medicine in the management of hyperlipidemia. Integrating genetic screening for PCSK9 SNPs into routine clinical practice could help to take the best choice treatment, allowing clinicians to select cholesterol-lowering strategies that align with each patient's genetic profile. Such an approach has several advantages involve the potential to enhance treatment effectiveness, lower the risk of cardiovascular diseases, and improve overall patient outcomes (Ference et al., 2015).

A significant association was identified between the PCSK9 rs28942111; T>A SNP and CRI-II levels. Patients with the AA genotype were more commonly found in the high cardiovascular risk group. This result suggests that the AA genotype may contribute to an increasing risk of CVD, through its influence on lipid

metabolism. These results are consistent with previous research showing that mutations in the PCSK9 gene may effect on individual's lipid profile and cardiovascular health outcomes (Cohen et al., 2006).

According to the correlation between AIP and some of other biochemical parameters as shown in Table 3.12, a strong direct correlation was found between AIP and TG levels. When TG concentrations increase AIP levels will rise, which in turn are associated with a greater risk of IHD (Kim et al., 2021).

Moreover, both CRI-I and CRI-II showed strong direct correlations with LDL cholesterol levels, suggesting that higher LDL levels are closely associated with increased cardiovascular risk and atherosclerosis. In contrast, HDL cholesterol showed a negative relationship with both CRI-I and CRI-II, which support its protective role against CVD. These findings align with existing evidence linking lipid ratios to cardiovascular risk assessment (Edwards et al., 2017).

The multiple linear regression analysis Table 3.14, revealed a statistically significant relationship between the PCSK9 rs28942111; T>A SNP and LDL concentrations. A moderate positive correlation was observed (Pearson's $r = 0.537$), indicating that the presence of the A allele may be associated with elevated LDL cholesterol levels. The coefficient of determination ($R^2 = 0.288$) indicate that 28.8% of the variation in LDL concentrations among patients can be explained by this SNP, highlighting the effect of genetic variation on lipid hemostasis. As well as, the model's F-statistic ($F = 59.494$) indicate its overall statistical significance.

These results are compatible with recent research which highlighting the influence of PCSK9 variants on LDL receptor activity and cholesterol regulation, where functional mutations can significantly affect both lipid levels and cardiovascular risk (Fairoozy et al., 2016, Lakoski et al., 2009). The observed association with rs28942111; T>A suggests that this SNP could serve as a useful

genetic marker for hyperlipidemia susceptibility and may be considered when optimizing personalized lipid lowering strategies, particularly in genetically diverse or high risk populations.

4.2 Demographic Data and Biochemical Finding

The study included 149 Iraqi patients, both male and female, diagnosed with primary hyperlipidemia. According to the European Society of Cardiology/European Atherosclerosis Society (ESC/EAS) guidelines for treatment of hyperlipidemia (Mach et al., 2020), 28% of the patients responded well to statin therapy, achieving LDL plasma concentration less than 50 mg/dL. Around 35% showed a moderate response, with LDL plasma concentration ranging from 51 to 100 mg/dL, while about 39% exhibited poor to no response, with LDL levels more than 100 mg/dL despite at least six months of treatment.

When assessing lipid ratios, the AIP was notably elevated in about 85% of the patients, indicating an increased risk of IHD and atherosclerosis. AIP is calculated using the formula log(TG/HDL), and studies have shown an inverse relationship between TG and HDL, with the TG/HDL ratio being a strong predictor of MI (Sami Khaza, 2013). Healthcare providers often use AIP as a key indicator for assessing atherosclerosis risk. AIP values below 0.1 indicate low risk, values between 0.1 and 0.21 suggest moderate risk, and values above 0.21 are associated with a high risk of CVD (Sadeghi et al., 2021).

In this study, 54% of studied patients had a CRI-I value exceeding 3.5, which represents the upper normal limit (Jamil and Siddiq, 2012). Elevated CRI-I levels are associated with the formation of coronary plaques and an increased risk of IHD. Additionally, CRI-II was found to be above the normal limit (>3) in 21% of the studied patients (Jamil and Siddiq, 2012). The PROCAM study reported that

individuals with an LDL/HDL ratio exceeding 5 had a six fold increase in the likelihood of experiencing cardiovascular events (Bhardwaj et al., 2013).

Dyslipidemia is characterized by abnormalities in lipid metabolism, including elevated plasma concentration of LDL, triglycerides, and TC, along with decreased HDL cholesterol levels (Kopin and Lowenstein, 2017). LDL is responsible for transporting approximately 60–70% of serum cholesterol (Talbert et al., 2008), carrying cholesterol from the liver to peripheral tissues. High LDL levels contribute to the formation of atherosclerotic plaques by promoting cholesterol accumulation in the arterial vessel walls (Elshourbagy et al., 2014). Atherosclerosis progresses when the endothelial layer of blood vessels is damaged by risk factors such as hypertension, smoking, diabetes, and increased LDL concentration (Frąk et al., 2022, Salekeen et al., 2022).

The structural integrity of the arterial wall is compromised due to this damage, increasing its permeability and enabling LDL particles to infiltrate the subendothelial space (Khatana et al., 2020). When these particles goes inside, these LDL particles become trapped by proteoglycans within the extracellular matrix and undergo oxidative modifications due to reactive oxygen species (ROS) and enzymes such as lipoxygenase, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, and myeloperoxidase (Gianazza et al., 2021). The oxidation process transforms LDL into a highly reactive and cytotoxic form, triggering an immune response. The immune system recognizes oxidized LDL (oxLDL) as a harmful substances, prompting endothelial cells to release adhesion molecules, including vascular cell adhesion molecule 1 and intercellular adhesion molecule 1,which act in recruiting white blood cells (mainly monocytes) to the affected site (Razeghian-Jahromi et al., 2022).

Upon entering the arterial wall, monocytes differentiate into macrophages that engulf oxLDL through scavenger receptors such as SR-A and CD36 (Lee and Choi, 2020). Unlike typical LDL uptake, this process is unregulated, resulting in excessive lipid accumulation within macrophages. Over time, these lipid-laden macrophages transform into foam cells, which are a hallmark of early atherosclerotic plaque formation.

Foam cells intensify inflammation by secreting cytokines like tumor necrosis factor-alpha, interleukin-1 beta, and monocyte chemoattractant protein-1 (Chistiakov et al., 2017). This inflammatory response attracts additional immune cells and promotes further LDL retention, accelerating plaque progression. As the plaque enlarges, smooth muscle cells (stimulated by growth factors like platelet-derived growth factor) migrate to the site and produce extracellular matrix proteins such as collagen, forming a fibrous cap over the lipid core to enhance stability. However, ongoing inflammation and mechanical stress can weaken the fibrous cap, increasing the risk of rupture (Jansen et al., 2024). If the cap ruptures, the exposed thrombogenic material interacts with circulating blood, leading to platelet activation and thrombus (blood clot) formation. This can obstruct blood flow and result in life threatening cardiovascular events such as heart attacks or strokes (Alkarithi et al., 2021).

According to the age that describe in the Table 3.3 middle-aged group B (age 46-65 years) adults tend to gain more weight than younger individuals group A (age 25-45 years), largely due to many factors such as decreasing in the metabolic rate, also decreasing levels of physical activity, as well as dietary shifts. Decreasing in growth and sex hormone production and hormonal changes may play a role in fat accumulation and muscle loss (Jensen, 2008). Studies indicate that middle-aged

individuals are more prone to sedentary behavior due to work and family commitments, further promoting weight gain (Flegal et al., 2016).

Additionally, patients in group C (ages 66–85 years) show significantly lower TC levels compared to group B and a decreased risk IHD and atherosclerosis, as indicated by the AIP and CRI-I results, compared to younger age groups (groups A and B) (Table 3.3). This could be due many factors such as physiological, pharmacological, and behavioral factors. An additional important factor is liver metabolism to atorvastatin. As people age, liver metabolism slows, leading to higher circulating drug levels, which may enhance its cholesterol-lowering effectiveness (Hirota et al., 2020).

Additionally, lifestyle habits play a significant role in cholesterol regulation. Older adults tend to adhere more closely to medication schedules and recommended lifestyle changes, such as maintaining a healthier diet, which helps in better cholesterol management. Routine medical check-ups also improve health outcomes by ensuring continuous monitoring and timely treatment adjustments. On the other hand, younger individuals with high cholesterol levels may be less consistent in making lifestyle changes, such as improving their diet or increasing physical activity, often assuming they have more time to deal with their health concerns.

Also, as shown in Table 3.3 the bilirubin levels tend to decrease with age, with group B and C (age 46-65 and 66-85 years respectively) showing lower levels compared to younger patients (age 25-45). A reduction in bilirubin levels with age can be due to reduction of hemolysis or changes in liver enzyme activity because bilirubin is a byproduct of hemoglobin breakdown (Capková, 2023). Some researchers suggest that bilirubin has antioxidant properties, and lower levels in older adults might indicate shifts in oxidative stress management over time (Lundvig et al., 2012).

With regard to treatment duration, the data in Table 3.5 indicate that participants in group C, who have been on treatment for 25-36 months, exhibit significantly higher TC levels compared to those in group B (13-24 months). This can be due to several factors, involving inconsistent adherence to treatment, lifestyle choices, disease progression, or suboptimal dosing.

Additionally, group C demonstrates significantly elevated AST levels compared to the other groups. This can be linked to the mechanism of action of atorvastatin, which works by inhibiting HMG-CoA reductase in the liver to reduce cholesterol synthesis (Jiang et al., 2018). Over time, this inhibition may place mild stress on liver cells, potentially leading to slight and chronic elevations in liver enzyme levels for some patients. Prolong exposure to the medication could also contribute to minor liver cell injury or increased cell turnover. When hepatocytes damage, the disruption of both plasma and mitochondrial membranes results in the release of intracellular enzymes such as AST into bloodstream (Contreras-Zentella and Hernández-Muñoz, 2016). This process is influenced by structural damage, oxidative stress, inflammation, and compromised energy production, all of which affect the cells ability to maintain its integrity, resulting in an increasing of enzyme release (Herrick et al., 2016).

Additionally, prolong use of statins has been linked with mild muscle injury in certain patients, a condition known as statin induced myopathy. Since AST is found in muscle tissue as well as the liver, muscle damage may also play a role in the observed increase in AST levels (Taha et al., 2014).

4.3 Conclusion:

This study examines one of genetic polymorphism in PCSK9 gene that can influence on the effectiveness of atorvastatin therapy in sample of Iraqi hyperlipidimic patients. We can summarize this study in the following points:

1. The rs28942111; T>A SNP in the PCSK9 gene may have a significant impact on lipid hemostasis. As shown in the study results that the AA allele carriers exhibiting higher levels of LDL and total cholesterol.
2. This mutation within the PCSK9 gene appears to affect atorvastatin efficacy, by increasing the gene activity. As shown in the study results that the AA allele carriers have a poorer response to the medication compared to TT allele carriers.
3. Form the results of this study, we can conclude that the genetic variation plays a crucial role in the treatment outcomes, which can explain why some patients suffer from high cholesterol levels despite their adherence to the treatment.
4. This research focuses on how difficult it is to treat hyperlipidemia, so we must take into account both genetic predispositions and individual responses to medication in order to improve therapeutic outcomes.

4.4 Recommendations and Future Work

1. Long-term follow-up studies are crucial for assessing how atorvastatin therapy affects lipid levels and cardiovascular health over time. These investigations could provide deeper insights into the role genetic factors play in influencing treatment effectiveness as patients age.

2. A larger number of participant is essential for generating more precise data on the influence of genetic polymorphisms, particularly in the PCSK9 gene, and other relevant genetic markers on atorvastatin efficacy. Increasing the study population would strengthen the statistical reliability of the findings, enabling more definitive conclusions about how genetic variations affect statin response and cardiovascular risk.

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Appendices

جامعة كربلاء

الموافقة على ان تكون مشاركا في البحث

عنوان الدراسة: تأثير تعدد أشكال جين PCSK9 في فعالية عقار أتورفاستاتين في عينة من مرضى ارتفاع شحميات الدم العراقيين

اسم الباحث: خالد قاسم محمد هادي

هذه دراسة بحثية وليس عليك المشاركة فيها. سيشرح لك الباحث والدكتور احمد الحيدري هذه الدراسة. إذا كانت لديك أي أسئلة يمكنك ان تسألني او تسأل الطبيب.

يُطلب منك المشاركة في هذه الدراسة لأنك كنت تتناول جرعه فموية يومية من عقار الاتورفاستاتين 40 ملغم كعلاج وحيد لفرط شحميات الدم، يقوم الباحث بجمع عينات دم منك لمعرفة المزيد حول تأثير تعدد الاشكال الجيني لجين PCSK9 على فعالية عقار الاتورفاستاتين.

إذا وافقت على المشاركة في هذه الدراسة، فستذهب إلى المختبر وتُعطي عينة دم لمرة واحدة فقط. سيتم أخذ الدم عن طريق إدخال إبرة في الذراع، ويُجمع الدم في أنبوب دم صغير. تستغرق هذه العملية حوالي خمس دقائق.

المخاطر:قد تؤلم الإبرة قليلاً، وهناك خطر ضئيل للإصابة بكدمة أو بالإغماء، كما أن خطر الإصابة بعدوى يُعد نادراً.

سنبذل قصارى جهدنا لحماية المعلومات التي نجمعها منك ومن سجلك الطبي. ستبقى المعلومات التي يتم تحديد هويتك امنة ومقيدة. إذا تم نشر معلومات من هذا البحث أو تقديمها في اجتماعات علمية، فلن يتم استخدام هويتك أو الكشف عنها. لن يتم استخدام هويتك الشخصية، وسيتم استبدال اسمك ومعلوماتك الأخرى بمعرّف رمزي.
عند اكتمال هذا البحث، سيتم تزويدك بنسخة من المعلومات التي تحدد هويتك، بالإضافة إلى نسخة من نموذج الموافقة هذا للاحتفاظ بها.

الموافقة:
إذا كنت ترغب في أن تكون جزءًا من هذه الدراسة، يُرجى التوقيع في الأسفل.

اسم المشارك:
التاريخ:
توقيع المشارك:

Questionnaire for Patients taking statin therapy

Demographic characterization

الاسم: **رقم الهاتف:**

الوزن : **الطول :**

Parameters	variable	Note
Age		
Gender		
Highs		
Weight		
Smoking	Yes: No:	
Education	Primary: Secondary: Collage:	
Duration of treatment of statin		
Other diseases		
Other medication		

الخلاصة

المقدمة:

فرط شحميات الدم هو اضطراب يتميز بارتفاع مستويات الدهون في الدم، مما يزيد بشكل كبير من خطر الإصابة بتصلب الشرايين والأمراض القلبية الوعائية، وخاصة مرض الشريان التاجي. أظهرت الملاحظات السريرية أن العديد من المرضى المصابين بفرط شحميات الدم لا يزالون يعانون من مستويات مرتفعة من الكوليسترول والبروتين الدهني منخفض الكثافة (LDL)على الرغم من العلاج باستخدام عقار الأتورفاستاتين، وهو أحد الأدوية الشائعة المستخدمة لخفض الكوليسترول. إن الإنزيم المحول للبروتين كيو 9 (PCSK9) يعد من العوامل المنظمة الرئيسية لعملية استقلاب LDL، وقد تؤثر التغيرات في الجين الخاص بهذا الإنزيم، وخاصة تعدد الشكال الوراثي rs28942111; T>A، في فعالية عقار الأتورفاستاتين.

هدف الدراسة:

هدفت الدراسة الى التحقق من تأثير التغير الوراثي في rs28942111; T>A في جين PCSK9 على فعالية عقار الأتورفاستاتين لدى المرضى العراقيين المصابين بفرط شحميات الدم، بالإضافة إلى تقييم نسبة الدهون في الدم وعوامل الخطر المرتبطة بالتصلب مثل مؤشر (AIP) ومؤشرات (CRI-I & CRI-II).

المنهجية:

أجريت دراسة مقطعية شملت 149 مريضًا عراقيًا (أعمارهم بين 28 و85 عامًا) شخصوا بفرط شحميات الدم الأولي وعولجوا باستخدام أتورفاستاتين 40 ملغ لمدة لا تقل عن ستة أشهر. كذلك قيمت نسبة الدهون في دم، اختبارات وظائف الكبد، وعوامل AIP وCRI-I و CRI-II واجري التحليل الجيني لتعدد الأشكال الوراثي rs28942111 في جين PCSK9 باستخدام تقنية allele specific PCR.

النتائج:

أظهرت النتائج انه من بين 149 مريضًا، كان 128 (85.9%) حاملين للنمط الجيني TT و21 (14.1%) كانوا حاملين للنمط الجيني AA في تعدد الأشكال الوراثي rs28942111. ولم يكتشف أي نوع متحول هجينيTA. أظهر حاملو النمط الجيني AA مستويات مرتفعة وبشكل ملحوظ من LDL، TC،AST مقارنة بحاملي النمط الجيني TT. بالإضافة إلى ذلك، أظهر 42 مريضًا (28.2%) استجابة جيدة للعلاج بالأتورفاستاتين، بينما كان 35% لديهم استجابة متوسطة و39% أظهروا استجابة ضعيفة أو لم يظهروا أي استجابة. أظهرت نتائج AIP أن 85% من المرضى كانوا في خطر مرتفع للإصابة بأمراض القلب الإقفارية (IHD)، في حين أظهرت نتائج CRI-I و CRI-II أن 54% و 21% من المرضى كانوا في خطر مرتفع على التوالي للإصابة بتصلب الشرايين و IHD. و لوحظت فروق كبيرة في نسب الدهون في الدم، مؤشر كتلة الجسم (BMI، و AIP) بين الفئات العمرية المختلفة ومدة العلاج.

الاستنتاجات:

يلعب تعدد الأشكال الوراثي rs28942111 في جين PCSK9 دورًا هامًا في التأثير على مستويات الدهون واستجابة المرضى للعلاج بالأتورفاستاتين. على الرغم من العلاج باستخدام الستاتين، ما زال جزء كبير من المرضى في خطر مرتفع للإصابة بأمراض القلب الإقفارية، كما أظهرت نتائج AIP و CRI. تؤكد هذه النتائج على ضرورة تبني طرق مخصصة في إدارة فرط شحميات الدم، مع مراعاة العوامل الوراثية وعوامل الخطر المرتبطة بالتصلب من أجل تحسين نتائج العلاج.

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة كربلاء
كلية الصيدلة
فرع الادوية والسموم

تأثير تعدد أشكال جين PCSK9 في

فعالية عقار الأتورفاستاتين في عينة من مرضى ارتفاع شحميات الدم العراقيين

رسالة مقدمة الى مجلس كلية الصيدلة / جامعة كربلاء
كجزء من متطلبات نيل درجة الماجستير في علم الادوية والسموم
بواسطة

خالد قاسم محمد هادي

بكالوريوس صيدلة (جامعة كربلاء-2015)

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