STUDY OF STATIN EFFECTS ON PLASMA LIPID PROFILE OF PATIENTS WİTH DYSLIPIDEMIA

Master Thesis

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Biology Department

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2017

REPUBLIC OF TURKEY
BINGOL UNIVERSITY
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This master thesis on 30.01.2017 by the following jury memebers (The appropriate one will be stay, the other will be deleted) **Unanimously has been accepted.**

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LIST OF ABBREVIATIONS

TCH	: Total cholesterol
TG	: Triglyceride
HDL	: High density lipoprotein
LDL	: Low density lipoprotein
E.S.R	: Erythrocyte sedimentation rate
HMG.COA	: 3-hydroxy-3-methyglutaryl coenzyme a
LDLR	: Low density lipoprotein receptor
MI	: Myocardial infarction
CVD	: Cardio vascular disease
VLDL	: Very low density lipoprotein
OGT	: O-glc nac transferase
NAFLD	: Nonalcoholic fatty liver disease
TRLS	: Triglyceride-rich lipoprotein
ATP	: Adenosine triphosphate
ADP	: Adenosine diphosphate
ACS	: Acute coronary syndrome
ECG	: Electrocardiogram
NO	: Nitric oxide
MRI	: Magnetic resonance imaging
ASCVD	: Atherosclerosis cardiovascular disease
FH	: Familial hypercholesterolemia
FDA	: Functional data analysis
FFA	: Free fatty acid
GTPASE	: Guanosine triphosphates
CHD	: Coronary heart disease
CAD	: Coronary artery disease
L-ATP	: Lipid-assessment treatment project

LP	: Lipoprotein
HS-CRP	: High sensitivity c-reactive protein
IMT	: Intime-medial thickness
CT	: Computed tomogrephy
ABI	: Ankle/brachial blood pressure
MAG	: Monoacylglycerol
IDL	: Intermediate density lipoprotein
APO	: Apolipoprotein
LPL	: Lipoprotein lipase
NPC1L1	: Niemann-pick c1 like1
GPIHBP1	: Glycocyl-phosphatidylinositol-anchored high-density-binding protein1
TGRLP	: Triglyceride-rich lipoprotein
PCSK9	: Proprotein convertase subtilisin/kexin type 9
ASP	: Acylation-stimulating protein
LCAT	: Lecithin-cholesterol acyltransferase
CETP	: Cholesterylester-transfer-protein
PLTP	: Phospholipid transfer protein
FDB	: Familial defective apolipoprotein b-100
ER	: Endoplasmic reticulum
DHC	: Dehydrocholesterol
IPP	: Isopenteny-pyrophosphate
DMAPP	: Dymethylallyl-pp
FPPS	: Fernesyl-pp synthase
GPP	: Gernayl-pyrophosphate
NADP+	: Necotinamide adenine dinucleotide
NAD(P)H	: Necotinamide adenine dinucleotide phosphate-oxidase
SREBP	: Sterol regulatory element-binding protein
GGPP	: Geranylgeranyl pyrophosphate
FPP	: Farnesyl pyrophosphate
OR	: Odds ratio
CI	: Confidence interval
S1P	: Sphingosine-1-phosphate
CHF	: Congestive heart failure

AST	: Aspartate aminotransferase
ALT	: Alanine aminotransferase
CE	: Cholesterol esterase
POD	: Peroxidase
4-APP	: 4-aminoantipyrine
GK	: Glycerol kinase
GPO	: Glycerol phosphate oxidase
HSDA	: N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxy aniline
CHOD	: Cholesterol oxidase
PON	: Paraoxonase
OR	: Oxygen radical
POS	: Reactive oxygen species
DM	: Diabetes mellitus
ROCK	: Rho-kinases
IL	: Interleukin
PAF	: Platelets activation factor

DISLIPIDEMI HASTALARININ PLAZMA LIPID PROFILI ÜZERINE STATININ ETKISININ ARAŞTIRILMASI

ÖZET

Dislipidemi gelişmiş ülkelerde önemli bir halk sağlığı problemidir ve dünya çapında daha yaygın hale gelmektedir. Ayrıca dislipidemi kardiyovasküler hastalık için önemli bir risk faktörüdür ve dünyadaki ölümlerin ilk başta gelen nedeni olarak kabul edilirken, kardiyovasküler hastalık nedeniyle Kürdistan bölgesinde daha fazla insan öldü. Bu çalışmanın amacı, dislipidemi hastalarında anti-kolesterol tedavisinin plazma lipid profili üzerine etkili olup olmadığını araştırmaktır. Yirmi dört hasta Erbil şehirdeki Cerrahi Özel Hastanesi Kardiyak Merkezinde alındı ve dislipidemi ile teşhis edildi. 20,02,2016'da başlayan ve 20,04,2016'da biten kan örnekleri koleksiyonları. Bu çalışmada dislipidemi hasta grubu olarak 12 hasta (4 erkek 8 kadın) sağlıklı grup, 30 sağlıklı hasta ve sağlıklı grup olarak dislipidemi saptanmayan 12 hasta (yaşları 30, üzeri) kullanıldı. Bu çalışmanın sonuçları, total kolesterol (TCH) ve trigliserid (TG) konsantrasyonunun, atorvastatin tedavisine başlamadan önce kontrol grubuna göre anlamlı olarak yaklaşık% 66,7 veya 1,67 kat ve% 69,5 veya 1,69 kat arttığını, Atorvastatin ile tedavi sonrasında azaldı. Düşük yoğunluklu lipoproteinin (LDL) konsantrasyonu, atorvastatin tedavisinden önce kontrol grubuna göre% 42,8 veya 1,42 kat daha yüksekti; tedavi sonrası azaldı, ancak anlamlı değildi. Bu arada, yüksek yoğunluklu lipoprotein (HDL) konsantrasyonu, atorvastatin tedavisine başlamadan önce hasta grubunda yaklaşık%23,5 veya 1,3 kat daha düşüktü; atorvastatin tedavisi sonrası arttı. CHOL / HDL konsantrasyonu, atorvastatin tedavisinden önce hasta grubunda kontrol grubuyla karşılaştırıldığında anlamlı derecede yüksek değildi, atorvastatin tedavisinden sonra anlamlı olarak azaldı. Öte yandan, atorvastatin tedavisi öncesinde hasta grubunda kreatinin konsantrasyonu kontrol grubuna göre anlamlı derecede yüksek değildi, tedavi sonrası azaldı, ancak anlamlı değildi. Üre konsantrasyonu atorvastatin tedavisine başlamadan önce hasta grubunda kontrol grubuna göre anlamlı derecede yüksekti, tedavi sonrası azaldı, ancak belirgin değildi. Glikoz konsantrasyonu, atorvastatin tedavisi öncesi ve sonrası hasta grubunda kontrol grubuna göre anlamlı derecede yüksekti; tedavi sonrası% 34,8 veya 1,34 kat azaldı, ancak anlamlı değildi. Eritrosit sedimantasyon hızının (ESR) ortalama değeri atorvastatin tedavisinden önce kontrol grubuna göre anlamlı derecede daha yüksekti, atorvastatin tedavisinden sonra azaldı, ancak anlamlı değilken, ortalama ESR seviyelerinde anlamlı farklılık vardı Atorvastatin ile tedavi öncesi ve sonrası hastalar. Gözlenen sonuçlar, atorvastatin'in lipid metabolizması ve ana klinik indeksler üzerine üre ve kreatinin gibi çok faktörlü etkisini göstermektedir. Bir araya getirildiğinde elde edilen veriler, dislipidemi hastalarının semptom verileri için statinin yumuşak pozitif etkisini ortaya koymaktadır.

Anahtar kelimeler: Dislipidemi, Statinler, Atorvastatin ve Lipit profili.

STUDY OF STATIN EFFECTS ON PLASMA LIPID PROFILE OF PATIENTS WITH DYSLIPIDEMIA

ABSTRACT

Dyslipidemia is a major public health problem in developed countries, and it is becoming more common worldwide. Also dyslipidemia is a major risk factor for cardiovascular disease, and it is considered as the first leading cause of death in the world, more people died in Kurdistan region by cardiovascular disease. The aim of this study is to investigate whether anti-cholesterol treatment could effect on plasma lipid profile of patients with dyslipidemia. Twenty-four subjects were taken at Surgical Specialty Hospital Cardiac Center in Erbil city and diagnosed by dyslipidemia. Blood sample collections started in 20,02,2016 and finished in 20,04,2016. The present study included 12 patients (6 males and 6 females) who had dyslipidemia as patient group and 12 individuals (4 males and 8 females) who had no dyslipidemia as healthy group, patient and healthy groups aged >30 years old. The results of this study showed that the concentration of total cholesterol (TCH) and triglyceride (TG) were significantly higher about 66,7% or in 1,67 times and 69,5% or in 1,69 times in patient group before treatment with atorvastatin than that of control group, while they were decreased after treatment with atorvastatin. The concentration of low density lipoprotein (LDL) was significantly higher about 42,8% or in 1,42 times in patient group before treatment with atorvastatin than that of control group, it is decreased after treatment but not significantly. Meanwhile, the concentration of high density lipoprotein (HDL) was significantly lower about 23,5% or in 1,3 times in patient group before treatment with atorvastatin than that of control group, it is increased after treatment with atorvastatin. The concentration of CHOL/HDL was not significantly higher in patient group before treatment with atorvastatin as it compares with control group, it is decreased non-significantly after treatment with atorvastatin. On the other hand, the concentration of creatinine was not significantly higher in patient group before treatment with atorvastatin than that of control group, it is decreased after treatment but not significantly. The concentration of urea was significantly higher in patient group before treatment with atorvastatin than that of control group, it is decreased after treatment but not significantly. The concentration of glucose was significantly higher in patient group before and after treatment with atorvastatin than that of control group, it is decreased about 34,8% or in 1,34 times after treatment but not significantly. The mean value of erythrocyte sedimentation rate (E.S.R) was significantly higher in patient group before treatment with atorvastatin than that of control group, it is decreased after treatment with atorvastatin but not significantly, but there is significant difference in mean levels of (E.S.R) in patients before and after treatment with atorvastatin. Observed results show multifactorial effect of atorvastatin on lipid metabolism and main clinical indices, as urea and creatinine. Taking together, obtained data evidence soft positive effect of statin for symptom data of patients with dyslipidemia.

Key words: Dyslipidemia, statins, atorvastatin and lipid profile.

1. INTRODUCTION

1.1. Overview

Statins are inhibitors of 3-hydroxy-3-methyglutaryl coenzyme A (HMG-CoA) reductase (HMGCR), a rate-limiting enzyme in the mevalonate-cholesterol synthesis pathway. This pathway is crucial for regulating the synthesis of serum cholesterol, especially low-density lipoprotein (LDL)-cholesterol. Two thirds of the serum cholesterol levels come from hepatic cholesterol synthesis, and the rest is derived from dietary cholesterol intake. Statins regulate serum cholesterol through both synthesis and LDL clearance. The reduction of LDL-cholesterol as a result of statin treatment may additionally induce an increase in LDL receptor (LDLR) and LDL clearance.

Elevated cholesterol level is a major cause of cardiovascular/cerebrovascular diseases and dyslipidemias. Statins have been used as a prevention and treatment for these conditions for many years (Ling Q and Tejada-Simon MV 2016).

Dyslipidemia are the disorder of lipoprotein metabolism, including lipoprotein overproduction and deficiency, which manifest either as elevated total cholesterol, low-density lipoprotein cholesterol (LDL), and triglyceride levels or as decreased high-density lipoprotein cholesterol (HDL) level with the promotion of insulin resistance causing metabolic syndrome.

Dyslipidemia is a widely accepted risk factor for coronary heart disease, it was showed that relative risk of myocardial infarction (MI) correlates directly with increased TG and inversely with HDL-c levels (Singh AK et al. 2011).

Atherosclerosis is an inflammatory disease affecting the arterial blood vessels, the prevalence of atherosclerosis leading to CVD (Vanderlaan PA et al. 2004). Atherosclerotic plaques can clot blood vessels supplying the heart, causing myocardial infarction (MI) (Thygesen K et al. 2007).

However, apoB and apoB-I are the two major apolipoproteins involved in lipid transport and in the processes causing atherosclerosis and its complications. It is worth mentioning that apoB is the major protein in very low density (VLDL), intermediate density (IDL) and low density lipoprotein (LDL) (Walldius G. and Junger I. 2004). While, apoA-I is the major protein in high density lipoprotein (HDL) particles. The apoB number indicates the total number of atherogenic particles, the higher the number the higher is the cardiovascular risk. While apoA-I reflects the anti-atherogenic potential in HDL particles, the higher the value the better protection of cardiovascular risk. Therefore, the apoB/apoA-I ratio (apo-ratio) indicates the balance between atherogenic and anti-atherogenic particles, the higher the value, the higher is the cardiovascular risk (Walldius G. and Junger I. 2007).

2. LITERATURE REVIEW AND THEORETICAL BACKGROUND

2.1. Dyslipidemia

Dyslipidemia is an abnormal amount of lipids (e.g., cholesterol and/or fat) in the blood. In developed countries, most dyslipidemias are hyperlipidemias; that is, an elevation of lipids in the blood. This is often due to diet and lifestyle. Prolonged elevation of insulin levels can also lead to dyslipidemia. Likewise, increased levels of O-GlcNAc transferase (OGT) may cause dyslipidemia. Phenotype, or the presentation in the body, including the specific type of lipid that is increased. The classification is problematic, because most conditions involve the intersection of genetics and lifestyle issues. However, there are a few well-defined genetic conditions that are usually easy to identify LDLc levels are associated with increase of cardiovascular risk.

Nevertheless, a despite of LDLc levels at goal, a residual risk is persistent, commonly associated with persistent lipids modifications (high triglycerides and low HDLc). So, it is necessary to evaluate triglycerides and HDL to assessment cardiovascular risk. Patients with hipertriglyceridemia and low HDLc are the groups with most potential improve. In those patients with atherogenic dyslipidemia, the target for therapeutic objectives related with non-HDL-cholesterol is a priority, because non-HDL-cholesterol is considered as a more accuracy measure to assessment cardiovascular risk. The increased risk of fibrinogen on coronary atherosclerosis appeared to be enhanced by the high atherogenic lipid levels, which mediated around 24% of this effect. It has been proposed that plasma fibrinogen is associated with lipid levels and increased cardiovascular risk. However, the interrelationship has not been well-elucidated. There hypothesised that lipids may be potential mediators (Zhang Y et al. 2016). There is serious problem association dyslipidemia with liver pathology, for example, nonalcoholic fatty liver disease (NAFLD). Cardiovascular disease provides the greatest mortality risk in patients with

NAFLD (Blais P et al. 2016). NAFLD is a leading cause of chronic liver disease. The majorities of providers do not identify NAFLD as a clinically important diagnosis and do not refer to gastroenterology/hepatology providers. However, 83 % expressed a need for education on NAFLD (Wieland AC et al. 2013).

Nonalcoholic fatty liver disease (NAFLD) is associated with both dyslipidemia and increased risk for cardiovascular disease. Despite the indication to treat in patients affected by both dyslipidemia and NAFLD, an undertreatment in statin therapy due to the potential liver damage is frequently observed (Maroni L et al. 2011).

Even at low-density lipoprotein cholesterol (LDL-C) goal, patients with cardiometabolic abnormalities remain at high risk of cardiovascular events. Current evidence supports a causal association between elevated of triglyceride-rich lipoproteins (TRLs) and their remnants, low HDL-C, and cardiovascular risk. This interpretation is based on mechanistic and genetic studies for TRL and remnants, together with the epidemiological data suggestive of the association for circulating triglycerides and cardiovascular disease (Chapman MJ et al. 2011). For HDL, epidemiological, mechanistic, and clinical intervention data are consistent with the view that low HDL-C contributes to elevated cardiovascular risk; genetic evidence is unclear however, potentially reflecting the complexity of HDL metabolism. The first step should be lifestyle interventions together with consideration of compliance with pharmacotherapy and secondary causes of dyslipidemia. If inadequately corrected, adding niacin or a fibrate, or intensifying LDL-C lowering therapy may be considered (Pedersen TR. 2010).

The linear relationship between LDL-cholesterol lowering and reduction in coronary heart disease risk, as well as a lack of conclusive evidence for other mechanisms of action raise the question of whether any cholesterol-lowering agent is equally effective for reducing cardiovascular risk, but recent data from the torcetrapib clinical trial program suggest this is not the case. Future cholesterol-lowering modalities must be able to demonstrate efficacy and good tolerability in large-scale clinical trials. As far creatine provides the ADP/ATP balance and regeneration ATP pool this is critical data on creatine level in blood of patients with dyslipidemia.

2.2. Atherosclerosis

The term atherosclerosis is derived from the Greek “athero” (gruel or porridge) and “sclerosis” (hardening). It is a chronic inflammatory disease of the arteries in which macrophages and oxidized lipids are the primary agents, resulting in intimal fibro-fatty plaque formation and progression (Mcnair ED. 2006).

Some elevation of LDL seemingly is required for atherogenesis and hence ASCVD. LDL accounts for more than 75% of atherogenic lipoproteins, the others being cholesterol-enriched remnants of triglyceride-rich lipoproteins. The latter play a larger role when triglycerides are elevated. When LDL infiltrates into the arterial wall, it initiates and promotes atherosclerosis; indeed an elevated LDL acting alone can cause ASCVD. The role of LDL is best exemplified by familial hypercholesterolemia (FH). Persons with FH commonly develop premature atherosclerosis and clinical ASCVD even in the absence of other risk factors. No other risk factor can do the same. In populations with low levels of LDL, the presence of other risk factors—cigarette smoking, hypertension, low HDL, or diabetes don’t lead to premature ASCVD. These other risk factors appear to accelerate atherogenesis when LDL is high enough to initiate atherosclerosis. For this reason, the prime focus of prevention of ASCVD must be on lowering LDL and keeping it low throughout life. LDL promotes atherosclerosis in several ways. After entering the arterial wall, LDL is trapped and modified in a variety of ways; this leads to its uptake by macrophages. Lipid-engorged macrophages are called foam cells. Expansion of regions of foam cells creates a fatty streak. The latter initiates smooth muscle proliferation, and this response forms a fibrous cap (fibrous plaque). But continued LDL infiltration creates superficial lipid-rich areas in fibrous plaques. These areas are prone to breaking though the surface of the plaque; this breakage is called plaque rupture. When rupture occurs, plaque contents exude and precipitate a thrombosis. Plaque rupture and thrombosis in coronary arteries are responsible for acute coronary syndromes (ACS). Ruptures of carotid artery plaques produce strokes. All of these steps occur in patients with FH and demonstrate how elevated LDL alone can cause clinical ASCVD.

Since LDL is the predominant cholesterol-carrying lipoprotein, it has received the most attention in the atherosclerosis field. Yet very low density lipoproteins (VLDL) also are cholesterol enriched and have atherogenic potential. The most atherogenic form of VLDL consists of partially degraded VLDL, called remnants. The atherogenic component of VLDL is its cholesterol, not its triglyceride. VLDL remnants are particularly enriched in cholesterol. The importance of VLDL as an atherogenic lipoprotein is greatest in persons with hypertriglyceridemia (Jappesen J et al. 1998).

2.3. Coronary Heart Disease (CHD)

Coronary heart disease (CHD), the result of coronary atherosclerosis, is the largest single cause of death in the United States and is the leading cause of morbidity and mortality worldwide. Central to the pathogenesis of atherosclerosis is the deposition and retention of cholesterol in arterial walls, making lipid modification critical to CHD prevention. Lowering cholesterol levels has been shown unequivocally to reduce cardiovascular events and prevent the development of atherosclerosis (Rossana M et al. 2010).

2.4. Cardiovascular Disease (CVD)

Cardiovascular disease (CVD) is a major cause of morbidity and mortality in the United States and dyslipidemia is a major risk factor for CVD (Roger VL et al. 2012). Low-density lipoprotein (LDL) cholesterol has been and should continue to be the primary target of efforts to reduce the risk for CVD. Success in achieving goal LDL cholesterol concentrations was poor in the past and has improved in recent years. In the Lipid Assessment Treatment Project (L-TAP), a study of 4888 Americans with or at risk for CVD conducted in the late 1990s, the goal LDL cholesterol concentration was achieved in 38% of patients receiving lipid-lowering therapy (Pearson TA et al. 2000). In a subset of patients at very high risk for cardiovascular events because of established coronary heart disease (CHD), the success rate in achieving the LDL cholesterol goal was only 18%. In L-TAP 2, a follow-up survey of patients with dyslipidemia conducted in 2006 and 2007, the overall rate of success in achieving the goal LDL cholesterol concentration was 73% in 9955 patients who were receiving stable lipid-lowering therapy (statins for

most patients) (Waters DD et al. 2009). Participants lived in one of several countries, including the United States. The percentage of patients at low, moderate, high, and very high risk for CVD who achieved their goal LDL cholesterol concentration (<160 mg/dL, <130 mg/dL, <100 mg/dL, and <70 mg/dL, respectively) was 86%, 74%, 67%, and 30%, respectively. Although the success rates in achieving goal LDL cholesterol concentrations in patients treated for dyslipidemia have improved in recent years (Grundy SM et al. 2004).

2.5. Dyslipidemia As A Risk Factor For CVD

Dyslipidemia has been identified as one of the main risk factors for CVD. Our local NCVD – ACS Registry showed that dyslipidemia was present in 55% of our patients. Specific lipid abnormalities implicated are.

2.5.1. Elevated LDL-C levels

LDL-C has been shown to be atherogenic in epidemiological studies. There is a direct relationship between levels of LDL-C (or TC) and the rate of new onset CHD in men and women who were initially free from CHD. In people with established CHD, elevated LDL-C correlates with recurrent cardiac events. There is a near absence of clinical CHD in populations with very low levels of serum cholesterol throughout their life (TC<3.9mmol/L or LDL-C< 2.6 mmol/L).The risk for CHD appears to increase progressively above these levels. At levels of LDL-C above 3.4 mmol/L, atherogenesis proceeds at a significant rate particularly in the presence of other major risk factors. Randomized controlled trials have repeatedly shown that lowering of LDL-C reduces CVD events in both primary and secondary prevention. Studies have also shown that LDL-C particle concentration and size are important predictors of CVD. However measurements of these are not widely available and are not standardized.

2.5.2. Low HDL-C levels

There is substantial data linking a low HDL-C level (< 1.0 mmol/L) with increased risk of CHD. A 1% decrease in HDL-C, in epidemiological studies, has been associated with 2-3% increase in CHD risk. Clinical trials using pharmacotherapy to increase HDL-C levels have, however, showed mixed results.

2.5.3. Elevated TG levels

Data suggest that a raised TG level indicates a modest but highly significant association with CHD. This suggests that some TG-rich lipoproteins are atherogenic. Weight reduction and drug therapies (fibrates, nicotinic acid and statins) reduce remnant lipoproteins and are accompanied by a reduced risk for CHD.

2.5.4. Elevated Non-HDL-C levels

Non HDL-C reflects the concentration of cholesterol within all lipoprotein particles considered atherogenic. Many studies have demonstrated that non HDL-C is a better predictor of CV risk than is LDL-C and may be especially true in statin-treated patients.

2.5.5. Atherogenic Dyslipidemia

This consists of low HDL-C, raised TG and small dense LDL particles. The LDL-C levels are usually normal but there is a higher proportion of small dense LDL particles which are more atherogenic.

2.5.6. Lipoprotein Lp(a)

Elevated levels of Lp(a) have been shown to be related to cardiovascular risk in some but not all studies.

2.6. Other Risk Factors for CVD

More than 90% of CVD can be explained by 9 to 10 modifiable risk factors – dyslipidemia, hypertension, smoking, diabetes, abdominal obesity, psychosocial stress, low intake of fruits and vegetables, alcohol intake and physical inactivity.

2.6.1. Age

The incidence of CVD increases with age. This is due to the combined effects of age related changes in the vascular system as well the duration of exposure to adverse risk factors.

2.6.2. Gender

The incidence of CVD is about 3-4 times higher in men than women in the middle decades of life and approximately twice as high in the elderly.

2.6.3. Hypertension

Both elevated systolic and diastolic blood pressures are linked with increased CVD risk. From epidemiological data, elevated systolic blood pressure appears to be more important when compared to diastolic blood pressure as a risk factor especially in middle aged and elderly individuals. The presence of left ventricular hypertrophy is associated with increased CV risk.

2.6.4. Smoking

This is an important CV risk factor and cause of mortality in both men and women. The incidence and mortality of CVD is 2-3 times as high in cigarette smokers compared to non-smokers. The risk of developing CVD is directly related to the number of cigarettes smoked. The relative risk of CV events is greater in younger than in older patients although the absolute excess mortality related to smoking increases with age. Smoking

interacts in a multiplicative manner with other risk factors i.e. the risk is higher than that would have resulted from simply adding together the independent risks. In individuals who discontinue smoking, the risk decreases within a year or two of stopping and the curve flattens out within 4 years although the relative risk remains slightly higher compared to never-smokers.

2.6.5. Family History of Premature CVD

Familial and genetic factors may play an important role in the determination of some major risk factors, especially hypertension, lipid abnormalities and glucose intolerance. In addition there appears to be a familial predisposition to CVD. The presence of premature CVD in first degree male relatives below the age of 55 years or female relatives below the age of 65 years is a recognized independent risk factor for CVD. This risk is greater when more family members are affected and the younger their age of onset of CVD.

2.7. Others

Other risk factors include:

2.7.1. Lifestyle Risk Factors

- Abdominal obesity:

Risk for CVD is increased although a recent analysis seems to indicate that abdominal obesity just helps to identify high risk individuals with metabolic abnormalities. In Asians, a waist circumference of > 90 cm in men and > 80 cm in women has been found to be associated with increased CV risk.

- Physical Inactivity:

Numerous studies have shown that physical inactivity is associated with increased mortality and CVD.

-Intake of fruits and vegetables: Low intake (less than 5 servings a day) increases CV risk.

2.7.2. Risk Markers

The following risk markers maybe helpful in intermediate risk patients to determine the intensity of therapeutic targets.

- Inflammatory markers (hs-CRP) –

Pathological studies strongly support a role for inflammation in the pathogenesis of the early stages of atherosclerosis, plaque progression and rupture. One such acute phase reactant is high sensitivity C-reactive protein (hs-CRP). Data show that an elevated level of hs-CRP identifies healthy individuals who are at an increased risk of an initial and recurrent cardiac event. Results of a recent trial indicated that healthy persons with LDL-C levels in the normal range but with elevated hs-CRP benefited from statins.

- Hemostatic markers –

An elevated level of fibrinogen has been associated with an increased risk for coronary events.

- Subclinical atherosclerosis -

Individuals with subclinical atherosclerotic disease are at increased risk for major coronary events. Subclinical atherosclerosis may be identified by the:

• Presence of abnormalities in the resting and / or stress ECG.

• Measurement of the ankle / brachial blood pressure (ABI) (a level of less than 0.9 is significant).

• Measurement of carotid intima-medial thickness (IMT) by ultrasound (more than 75th percentile for age and sex).

• Measurement of coronary calcium score by computed tomography (CT).

• Noninvasive imaging of the coronary arteries by CT angiography.

These tests may be used for risk stratification in individuals at intermediate risk. Patients with subclinical atherosclerotic disease may warrant a more aggressive preventive treatment strategy (Ridker PM et al. 2008).

2.8. Overview of Lipoprotein Metabolism

Numerous metabolic processes are involved in the uptake, transport and storage of lipids. After the ingestion of a meal containing fat, TG are ipolyzed in the intestinal lumen into FFA and 2-monoacylglycerols (MAG) and are taken up by the enterocytes via passive diffusion and specific transporters like CD36. Cholesterol is taken up by the enterocytes via the specific cholesterol transporter Niemann-Pick C1 Like 1 protein (NPC1L1). Once in the enterocyte, cholesterol is transformed into cholesterol-esters, whereas FFA and MAG are assembled into TG again. Finally, cholesterol-esters and TG are packed together with phospholipids and apolipoprotein (apo) B48 to form chylomicrons. After assembly, the chylomicrons are secreted into the lymphatics and finally enter the circulation via the thoracic duct. The liver synthesizes TG-rich lipoproteins called very low density lipoproteins (VLDL), which increase postprandially when food derived TG and FFA reach the liver. The assembly of VLDL is almost identical to the synthesis of chylomicrons, but apo B100 is the structural protein of VLDL (and its remnants, *i.e.*, intermediate density lipoproteins (IDL) and low density lipoproteins (LDL)). The human liver lacks the editing complex necessary to change the apo B100 molecule into the smaller apoB48, by post-transcriptional modification of one base leading to a premature stop codon.

Chylomicrons and VLDL deliver FFA to the heart, skeletal muscle and adipose tissue for energy expenditure and storage. Adequate lipolysis of TG-rich lipoproteins is necessary for FFA to be released in the circulation. This process is regulated by several enzymes and proteins acting as co-factors. Lipoprotein lipase (LPL) is the primary enzyme for TG lipolysis in the circulation and is strongly expressed in tissues that require large amounts of FFA like the heart, skeletal muscle and adipose tissue. LPL serves as the docking station for chylomicrons and VLDL for adherence to the endothelium via glycosyl-phosphatidylinositol-anchored high-density-binding protein 1 (GPIHBP1), which is present on the luminal side of the endothelium. The amount of liberated FFA from chylomicrons and VLDL depends on the activity of LPL, which is stimulated by insulin. In contrast, apo C-III is an inhibitor of LPL, but also of hepatic lipase. Plasma apo C-III concentrations correlate positively with plasma TG. In addition, chylomicrons compete with endogenous VLDL for the action of LPL. The liberated FFA are avidly taken up by adipocytes and re-synthesized into TG within the cytoplasm where the acylation-stimulating protein (ASP)/C3adesArg pathway plays an important role. The scavenger receptor CD36 is the best characterized FFA transporter and is abundant in muscle, adipose tissue and the capillary endothelium. Insulin and muscle contractions increase the CD36 expression thereby facilitating FFA uptake.

The postprandial rise in insulin is one of the most important regulatory mechanisms for fuel storage. The postprandial increase of insulin results in the effective inhibition of hormone sensitive lipase, which is the key enzyme for hydrolysis of intracellular lipids. Despite the uptake of FFA by adipocytes and myocytes, a proportion of FFA remains in the plasma compartment (“spill over”) where the FFA are bound by albumin and transported to the liver. When delivery of FFA for energy expenditure is insufficient like in the fasting state, FFA can be mobilized by adipose tissue for oxidation in energy demanding tissues like cardio myocytes. Insulin is also an important regulator of FFA mobilization from adipose tissue. Therefore, insulin resistance has a major impact on the metabolism of TG-rich lipoproteins and FFA.

Eventually, chylomicrons and VLDL shrink in diameter during the process of lipolysis to form chylomicron remnants and dense LDL, respectively. Chylomicron remnants are taken up by the liver via multiple pathways including apo E, hepatic lipase, the LDL

receptor, the LDL receptor-related protein and heparan sulphate proteoglycans. In contrast, LDL is primarily taken up by the liver via the LDL receptor. The LDL receptor is recycled and re-shuttled back to the cell surface. In the last decade, many studies have extended our knowledge concerning this recycling process of the LDL receptor, which is regulated by the proprotein convertase subtilisin/kexin type 9 (PCSK9). The LDL receptor undergoes lysosomal degradation during the shuttling process when PCSK9 is bound to the LDL receptor, but is recycled back to the surface of the hepatocytes in the absence of PCSK9. Neutralization of PCSK9 increases the total LDL binding capacity of the hepatocytes leading to reduced LDL-C concentrations.

Besides the above described TG and LDL metabolism, the intestine and liver also play an important role in the reverse cholesterol transport by the synthesis of HDL particles. HDL promotes the uptake of cholesterol from peripheral tissues, including the arterial wall, and returns cholesterol to the liver. Enterocytes and hepatocytes synthesize apo A-I which is the structural protein of HDL. Nascent HDL particles acquire free cholesterol from peripheral tissues. Subsequently, the cholesterol within HDL becomes esterified into cholesterol-esters by HDL associated lecithin-cholesterol acyltransferase (LCAT). Within the circulation, the HDL particles also become enriched with cholesterol-esters by the action of cholesterylester-ransfer-protein (CETP) and phospholipid transfer protein (PLTP). In this process HDL acquires TG from TG-rich lipoproteins in exchange for cholesterol-esters as a direct consequence of the CETP action. In the liver, hepatic lipase hydrolyses HDL-associated TG and also phospholipids inducing the formation of smaller HDL particles which can contribute again to the reverse cholesterol transport. Therefore, lipid metabolism is highly dynamic and depends on numerous factors including the postprandial state, TG-rich lipoprotein concentrations, HDL levels and function, energy expenditure, insulin levels and sensitivity and adipose tissue function (Boudewijn K et al. 2013).

2.9. Disorders of Lipoprotein Metabolism

Three familial forms of elevated LDL cholesterol.

a. Familial hypercholesterolemia (FH)

Heterozygous familial hypercholesterolemia. This autosomal-dominant disorder occurs in 1 of every 500 people. The defect is a mutation in the gene for the LDL receptor; a large number of mutations affecting LDL receptor function has been reported. In all of these, half the normal number of receptors are expressed. Hypercholesterolemia often is detectable at birth or shortly thereafter, and total cholesterol levels eventually rise to 350–500 mg/dL in many persons. Tendon xanthomas, especially in the Achilles tendons and the extensor tendons of the hands, are typical. FH carries increased risk of premature CHD; CHD commonly occurs in men by the fourth or fifth decade, and about 10 years later in women. Homozygous familial hypercholesterolemia occurs in only 1 in 1 million persons. LDL-receptor activity is essentially absent, and total cholesterol levels commonly run between 700 and 1,200 mg/dL. Cutaneous xanthomas form at various sites within the first few months or years of life, whereas tendon and tuberous xanthomas develop later. Atherosclerosis is severe and widespread, affecting coronary, carotid, iliac, and femoral arteries, and the aortic root.

a. Familial defective apolipoprotein B-100 (FDB)

FDB is an autosomal dominant abnormality that causes elevated LDL cholesterol. It results from a single nucleotide mutation that substitutes glutamine for arginine at amino acid position 3,500 in apolipoprotein B. This mutation reduces affinity of LDL particles for the LDL receptor; consequently, the LDL of affected individuals is cleared from plasma more slowly than normal. FDB prevalence varies among different populations. In the United States it occurs in about 1 in 700–1000 people. Serum LDL levels are often similar to those described for persons with heterozygous FH. Affected individuals can manifest premature atherosclerosis and tendon xanthomas. However, other affected individuals have a more moderate form of hypercholesterolemia, indistinguishable from polygenic hypercholesterolemia.

b. Polygenic hypercholesterolemia

LDL-cholesterol levels ≥190 mg/dL characterize polygenic hypercholesterolemia. No unique genetic defect is responsible; rather the high LDL-cholesterol level is explained by a complex interaction of environmental and genetic factors. A variety of patterns of LDL metabolism have been reported. The disorder is associated with increased risk for premature CHD. In polygenic hypercholesterolemia, the elevation in plasma cholesterol is generally milder than in heterozygous FH, and tendon xanthomas are not observed. Only about 7 percent of the first-degree relatives of persons with polygenic hypercholesterolemia have high LDL-cholesterol levels.

Three patterns for family clustering of elevated triglycerides have been identified; they are called familial combined hyperlipidemia, familial hypertriglyceridemia, and familial dysbetalipoproteinemia.

a- In familial combined hyperlipidemia, affected persons and their first-degree relatives may at various times manifest high serum cholesterol, high triglycerides, or both. Whether the underlying defect is monogenic or polygenic is not known. Metabolic studies suggest that the liver overproduces VLDL, but other metabolic defects may be present. Many persons exhibit high levels of apo B-100 (hyperapobetalipoproteinemia). There are no specific clinical features to diagnose this disorder. When total cholesterol is high, the level is typically in the range of 250–350 mg/dL. Triglyceride levels vary considerably, but about two-thirds of the persons have levels in the range of 200–500 mg/dL. Hyperlipidemia may or may not be present in childhood. Familial combined hyperlipidemia is associated with increased risk for premature CHD. In an early study, about 10 percent of persons with early onset myocardial infarction fell in the category of this disorder.

b- Family clustering of elevated triglycerides without increased serum cholesterol levels characterizes familial hypertriglyceridemia. Persons with familial hypertriglyceridemia seemingly do not carry as high a risk for premature CHD as do those with familial combined hyperlipidemia. This is not surprising because the former generally have lower levels of LDL cholesterol than the latter. Many persons with familial hypertriglyceridemia also manifest obesity, but in some, triglycerides are elevated

without obesity or any other evidence of the metabolic syndrome. These latter persons may have a defect in catabolism of TGRLP (e.g., an abnormality in lipoprotein lipase activity).

c- A third category of familial clustering of elevated triglycerides includes those with increased remnant lipoproteins (familial dysbetalipoproteinemia). This condition also has been named type 3 hyperlipoproteinemia. The defining defect in this disorder is an isoform variation in apolipoprotein E. Among the three major isoforms, E-2, E-3, and E-4, the one most often associated with dysbetalipoproteinemia is apo E-2. Affected persons usually are homozygous for apo E-2. Since apo E mediates binding of VLDL remnants and chylomicron remnants to their hepatic receptors, these remnants accumulate in plasma when the dysfunctional apo E-2 is present. The frequency of apo E-2 homozygosity in the general population is approximately 1 in 100, but the clinical syndrome of dysbetalipoproteinemia occurs much less frequently. The difference in frequency between the permissive genotype and the clinical syndrome is explained by the requirement for other factors, including age, hypothyroidism, obesity, diabetes mellitus, or the coincident presence of another genetic lipoprotein disorder, such as familial combined hyperlipidemia, to fully express the syndrome. Some persons have palmar xanthomas of the creases of the palms and fingers, but these may progress to nodules several millimeters in size. Tuberoeruptive xanthomas occur and vary from small papules to larger lesions. Premature atherosclerotic disease may present as myocardial infarction, stroke, or peripheral arterial disease. Hyperlipidemia is accentuated by concomitant glucose intolerance, diabetes mellitus, hyperuricemia, hypothyroidism, and obesity. The disorder is not commonly expressed in childhood.

2.9.1. Low HDL Cholesterol

When serum triglycerides become borderline high (150–199 mg/dL), HDL-cholesterol levels begin to fall. When triglyceride levels are greater than 150 mg/dL, HDL-cholesterol concentrations frequently are <40 mg/dL in men (or <50 mg/dL in women). Thus, the term isolated low HDL can be reserved for HDL-cholesterol levels <40 mg/dL in the presence of serum triglycerides <150 mg/dL. Causes other than elevated

triglycerides listed in Table (2.1) account for most cases of isolated low HDL. In the United States population, obesity and physical inactivity are major factors; genetic factors undoubtedly play an important role as well in many persons. In rare cases, genetic defects in metabolism of HDL alone can cause isolated low HDL. The relationship between HDL and CHD risk is complex (see Table 2.1). First, a low HDL per se may directly promote the development of coronary atherosclerosis and predispose to CHD. Several mechanisms have been implicated: impaired reverse cholesterol transport, loss of protection against atherogenicity of LDL, and reduction in HDL-carried, anti-atherogenic factors. Some persons with severe deficiency of HDL do not manifest premature CHD; this suggests that HDL is not uniquely involved in atherogenesis, as is LDL. But this finding does not rule out the possibility that HDL provides some protection against development of CHD. Second, a low HDL commonly is a marker for atherogenic dyslipidemia (lipid triad) raised triglycerides and remnant lipoproteins, small LDL particles, and low HDL. Both remnants and small LDL may have independent atherogenic properties. Finally, a low HDL cholesterol can be a marker for the metabolic syndrome; many persons with isolated low HDL have the other risk factors characteristic of this syndrome. Besides atherogenic dyslipidemia, these persons often have hypertension and insulin resistance, the latter being indicated by the presence of abdominal obesity. Prothrombotic and proinflammatory states typically are noted in persons with the metabolic syndrome. Finally, cigarette smoking reduces HDL-cholesterol concentrations and represents another factor contributing to the HDL-CHD relationship in smokers (Phillips NR et al. 1981).

Table 2.1. Low Serum HDL Cholesterol: Causes and Associations with CHD

Causes of low HDL	**Postulated Factors Associating Low HDL with CHD**
Elevated serum Triglycerides Overweight and obesity* Physical inactivity* Cigarette smoking Very high carbohydrate intake (>60% of total energy) Type 2 diabetes* Certain drugs† Genetic factors*	■ Direct atherogenic effect of low HDL Postulated mechanisms: – Decreased reverse cholesterol transport – Increased LDL oxidation – Increased LDL aggregation – Increased arterial inflammation ■ Marker for atherogenic dyslipidemia (“lipid triad”): – Higher VLDL triglycerides and remnant lipoproteins – Small, dense LDL – Low HDL cholesterol ■ Marker for metabolic syndrome – Abdominal obesity – Atherogenic dyslipidemia – Elevated blood pressure – Insulin resistance and elevated plasma glucose – Prothrombotic state – Proinflammatory state ■ Cigarette smoking – Smoking lowers HDL cholesterol

2.10. The Cholesterol Biosynthetic Pathway

Figure (2.1) takes a closer look at the cholesterol biosynthetic pathway, focusing on the enzymes that are regulated, sterol intermediates and the location of enzymes in the cell. Sterols are synthesized from the two-carbon building block, acetyl-CoA. The soluble enzyme acetoacetyl-CoA thiolase interconverts acetyl-CoA and acetoacetyl- CoA, which are then condensed by 3-hydroxy-3-methylglutaryl (HMG)-CoA synthase to form HMG-CoA. There are two forms of HMG-CoA synthase. A mitochondrial form, involved in

ketogenesis, predominates in the liver. In extrahepatic tissues, the most abundant form is a soluble enzyme of 53 kDa that is highly regulated by supply of cholesterol. Like acetoacetyl-CoA thiolase, HMG-CoA synthase has classically been described as a cytosolic enzyme because it is found in the 100,000 x g supernatant of homogenized cells and tissues. However, both enzymes contain peroxisomal targeting sequences and may reside in multiple cellular compartments. HMG-CoA reductase catalyzes the reduction of HMG-CoA to mevalonate, utilizing two molecules of NADPH. HMG-CoA reductase is a 97-kDa glycoprotein of the endoplasmic reticulum and peroxisomes. Analysis of the endoplasmic reticulum enzyme's domain structure revealed an N-terminal membrane domain with eight transmembrane spans, a short linker, and a C-terminal catalytic domain facing the cytosol. Transmembrane spans 2-5 share a high degree of sequence similarity with several other key proteins in cholesterol metabolism; this region is termed the sterol-sensing domain. Elucidation of the crystal structure of the HMG-CoA reductase catalytic domain indicated that the active protein is a tetramer, which is consistent with biochemical analysis. The monomers appear to be arranged in two dimers, with the active sites at the monomer-monomer interface. The dimer-dimer interface is predominantly hydrophobic. HMG-CoA reductase is the rate-determining enzyme of the cholesterol biosynthetic pathway and, like HMG-CoA synthase, is highly regulated by supply of cholesterol.

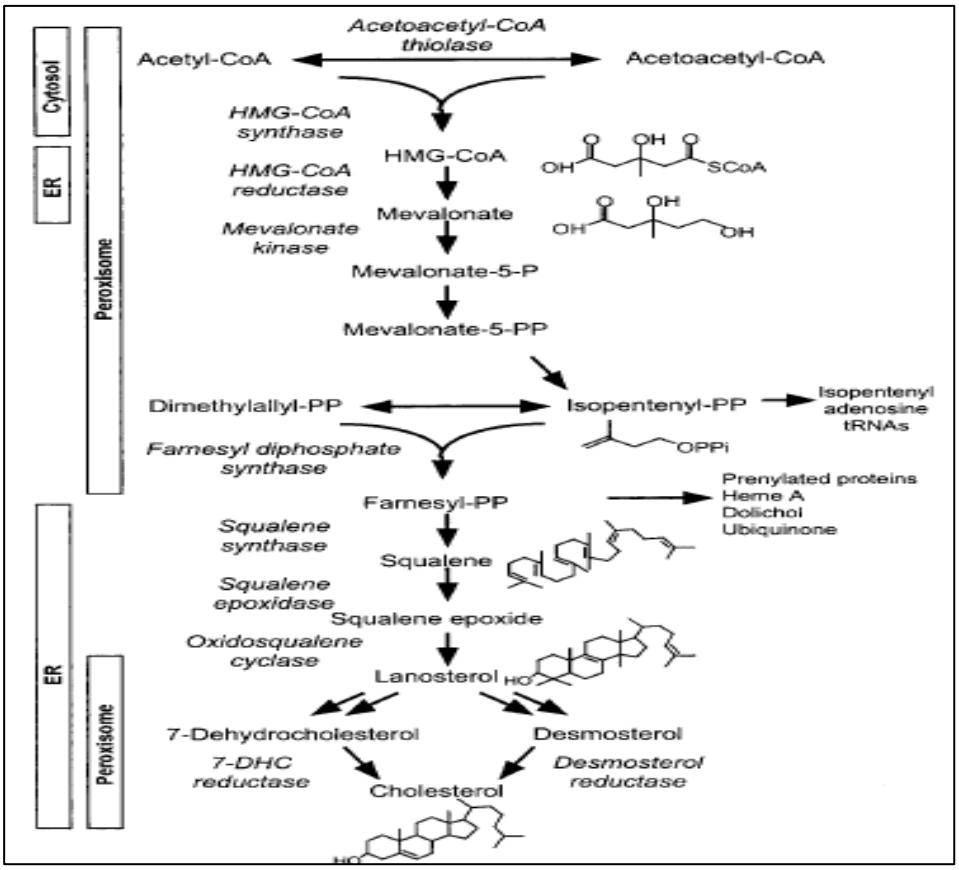

Figure 2.1. The cholesterol biosynthetic pathway. Some of the major intermediates and end-products are indicated. Enzymes in the pathway are found in cytosol, endoplasmic reticulum (ER) and peroxisomes, as noted. Figure adapted from Olivier and Krisans. HMG, 3-hydroxy-3-methylglutaryl; DHC, dehydrocholesterol

Mevalonate is metabolized to farnesyl-diphosphate (-PP) by a series of enzymes localized in peroxisomes. First, mevalonate kinase phosphorylates the 5-hydroxy group of mevalonic acid. The enzyme is a homodimer of 40 kDa that is subject to feedback inhibition by several isoprenoid intermediates. Mutations in the mevalonate kinase gene lead to the human genetic disease mevalonic aciduria. The product of mevalonate kinase, mevalonate-5-R is then phosphorylated to form mevalonic acid-5-PP, which is decarboxylated and dehydrated by mevalonate- PP decarboxylase to form isopentenyl-PE Isopentenyl-PP is in equilibrium with its isomer, dimethylallyl-PR Farnesyl-PP synthase catalyzes the head to tail condensations of two molecules of isopentenyl-PP with dimethylallyl-PP to form famesyl-PR The enzyme is part of a large family of prenyltransferases that synthesize the backbones for all isoprenoids, including

cholesterol, steroids, prenylated proteins, heine A, dolichol, ubiquinone, carotenoids, retinoids, chlorophyll and natural rubber. Squalene synthase is a 47-kDa protein of the endoplasmic reticulum and catalyzes the first committed step in cholesterol synthesis. The enzyme condenses two molecules of farnesyl-PP and then reduces the presqualene-PP intermediate to form squalene. A large N-terminal catalytic domain faces the cytosol, anchored to the membrane by a C-terminal domain. This orientation may allow the enzyme to receive the hydrophilic substrates from the cytosol and release the hydrophobic product into the endoplasmic reticulum membrane for further metabolism. Squalene synthase is highly regulated by the cholesterol content of the cell. Thus, it plays an important role in directing the flow of farnesyl-PP into the sterol or non-sterol branches of the pathway. Squalene is converted into the first sterol, lanosterol, by the action of squalene epoxidase and oxidosqualene cyclase. Lanosterol is then converted to cholesterol by a series of oxidations, reductions, and demethylations. The required enzyme reactions have been defined and metabolic intermediates identified; however, the precise sequence of reactions between lanosterol and cholesterol remains to be established. There is evidence for two alternative pathways that differ in when the A24 double bond is reduced. Both 7-dehydrocholesterol and desmosterol have been postulated to be the immediate precursor of cholesterol. One of the key enzymes in the latter part of the pathway is 7-dehydrocholesterol A7-reductase, a 55-kDa integral membrane protein. Mutations in the gene for 7-dehydrocholesterol A7-reductase cause the human genetic disease Smith-Lemli-Opitz syndrome (Vance DE. and Vance JE. 2002).

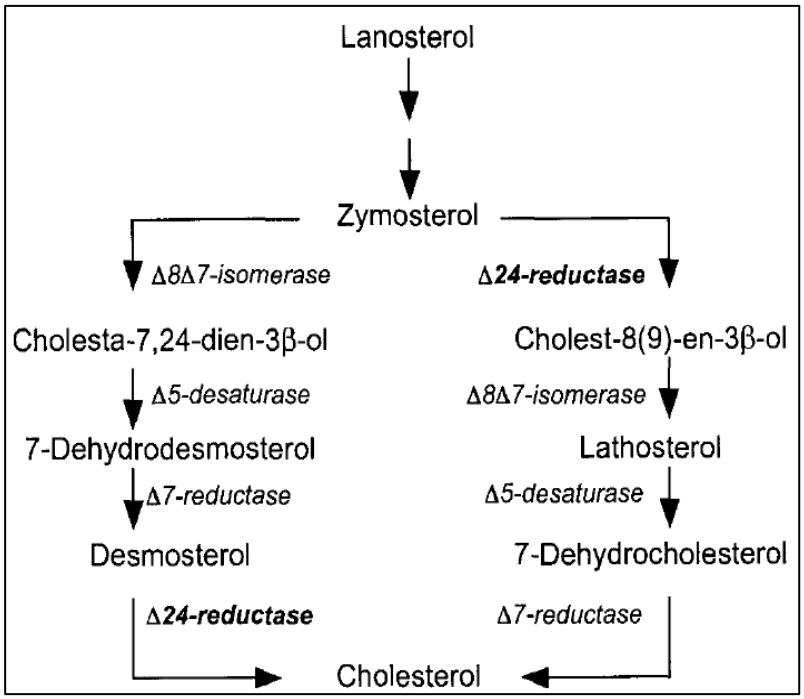

Figure 2.2. Final steps in the cholesterol biosynthetic pathway. Alternate steps have been proposed for the conversion of zymosterol to cholesterol, which differ in when the A24-reductase reaction occurs

2.11. Statins

Statins, inhibitors of 3-hydroxy-3-methylglutarylcoenzyme A (HMG-CoA) reductase, have revolutionized the treatment of hypercholesterolemia. They are the most efficient agents for reducing plasma cholesterol, being also appreciated for their good tolerance. Angiographic studies have demonstrated that these compounds reduce the progression and may induce the regression of atherosclerosis. These effects were translated in significant cardiovascular morbidity and mortality reductions in many clinical trials (WOSCOPS, AFCAPS/TexCAPS, HS, CARE, LIPID, HPS). The beneficial effects of the HMGCoA reductase inhibitors are usually attributed to their capacity to reduce the endogenous cholesterol synthesis, by competingly inhibiting the principal enzyme involved. Since mevalonate, the product of HMG CoA reductase reaction is the precursor not only for cholesterol, but also for many other nonsteroidal isoprenoidic compounds, inhibition of this key enzyme may result in pleiotropic effects. They have been divided into two categories, involving: directly lipids, or intracellular signaling pathways. The first category includes: inhibition of cholesterol biosynthesis, increased uptake and

degradation of low density lipoproteins (LDL), inhibition of the secretion of lipoproteins, inhibition of LDL oxidation, and inhibition of the scavenger receptors expression. Statins modulate a series of processes leading to reduction of the accumulation of esterified cholesterol into macrophages, increase of endothelial NO synthetase, reduction of the inflammatory process, increased stability of the atherosclerotic plaques, restoration of platelets activity and of the coagulation process. In addition, statins can inhibit tumor cells growth and enhance intracellular calcium mobilization. It was observed that inhibitors of HMG-CoA reductase induce a reduction of the formation of osteoclasts in rodents. Human subjects treated with statins have shown a reduction in the number of bone fractures. The discovery of statins has led to an important progress in the primary and secondary prevention of coronary heart disease. Although angiographic modifications following statin therapy were modest, clinical benefits that accompanied the therapy have been significant. Numerous clinical studies have correlated the reduction of blood cholesterol induced by these compounds with the reduction of the number of major coronary events, as well as general mortality in coronary patients (Camelia S. and Anca S. 2001).

2.11.1. Structure of Statins

The chemical structure of all statins, as illustrated in Figure (2.3), consists of the pharmacophore and its moiety containing a ring system with different substituents. The pharmacophore is shared among all statins, it is a dihydroxyheptanoic acid segment which is very similar to the HMGCoA substrate. The ring system consists of a complex hydrophobic structure covalently linked to the pharmacophore and it is involved in binding interactions with the HMG-CoA reductase enzyme. It has also been shown that the HMG-CoA reductase is stereoselective and as a result all statins need to have the required stereochemistry of chiral carbon atoms, C3 and C5, on their pharmacophore. The statin pharmacophore inhibits the HMG-CoA reductase enzyme and it binds to the same active site as the HMG-CoA substrate naturally would.

The statins differ from each other in their hydrophobic ring structure and its substituents, covalently linked to the HMG-like moiety. These differences in structure affect the

pharmacological properties of the statins, such as the affinity to the active site of the HMG-CoA reductase enzyme, the rates of entry into hepatic cells versus extrahepatic cells, the bioavailability of the compounds, and the biochemical metabolism and excretion mechanisms that affect the biologic half-life of the active compound. The additional groups range in character from very hydrophobic (e.g., cerivastatin) to partly hydrophobic (e.g., rosuvastatin). Rosuvastatin has a unique polar ethane sulfonomide group, which is quite hydrophilic and confers low lipophilicity. A unique polar interaction is formed between the sulfonamide group and the HMG-CoA reductase enzyme. As a result, rosuvastatin has stronger binding affinity to the HMGR enzyme compared to the other statins, which relates to its higher efficiency in lowering LDL-C. The lipophilicity of the statins is considered important since the hepatoselectivity of the statins is related to their degree of lipophilicity. The higher the lipophilicity of statins, the greater level of exposure it gets to non-hepatic tissues, while the more hydrophobic statins have a tendency to be more selective for the liver. Whereas lipophilic statins passively and nonselectively diffuse into both hepatocyte and non-heptatocyte. The more hydrophobic statins largely rely on active transport into hepatocyte to exert their effects. The bulky hydrophobic substituents of statins potentially pose a difficult binding situation, but statins exploit the conformational flexibility of HMG-CoA reductase to create a hydrophobic binding pocket near the active site. The HMG-like moiety of the statins is kinked at the O5-hydroxyl group to allow it extend into a narrow HMG-binding pocket in the enzyme where endogenous HMG-CoA is normally bound. The HMG-binding pocket is characterized by a loop, referred to as “cis loop”. Several polar interactions are formed between the HMGmoieties and residues that are located in the cis loop (Ser684, Asp690, Lys691, and Lys692). Lys691 also participates in a hydrogen-bonding network with Glu559, Asp767, and the O5-hydroxyl of the statins. Hydrophobic side chains of the enzyme involving residues Leu562, Val683, Leu853, Ala856, and Leu857 participate in van der Waals contacts with the statins (Emmanuel EAE. and Hafsa H. 2016).

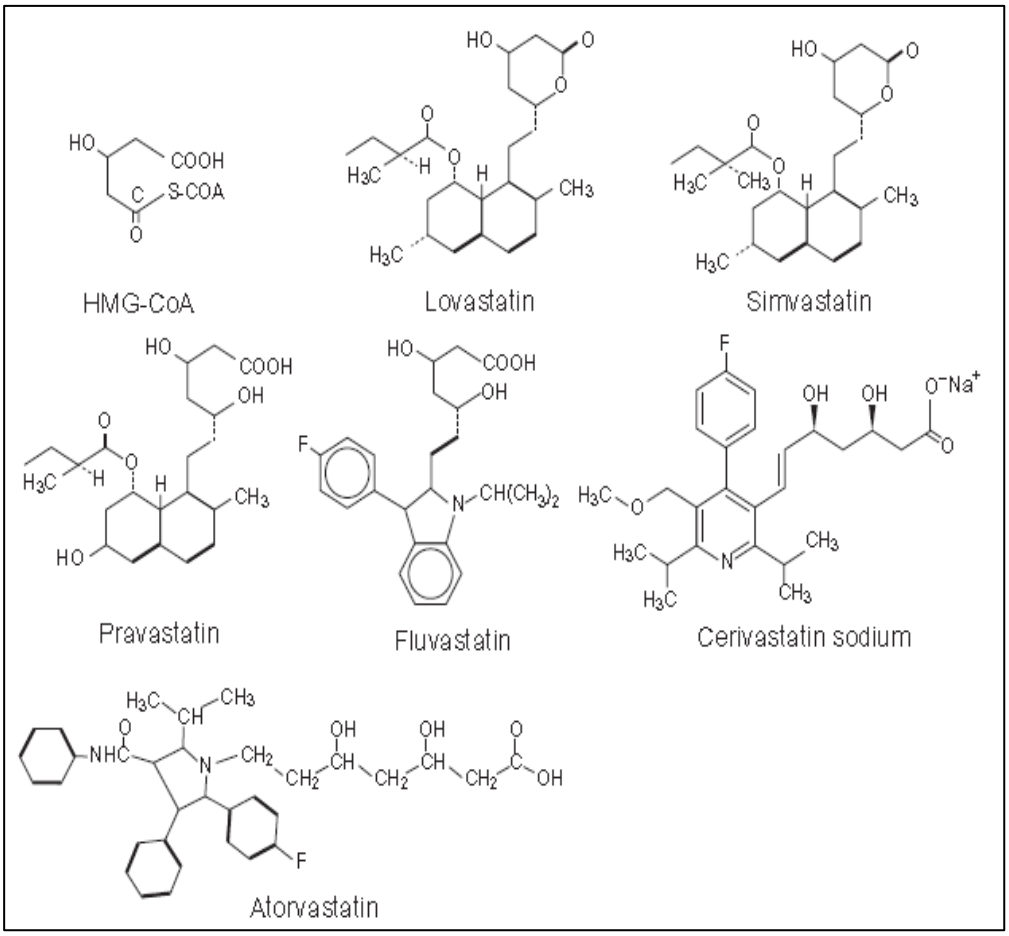

Figure 2.3. Chemical structure of the statins and HMG-CoA

2.11.2. Properties Of Statins

Commercially available statins in the U.S. can be categorized in three different ways: (1) production method, (2) chemical structure and (3) solubility. Statins can be generated by different methods. Some of them, referred as type 1 (e.g. lovastatin, simvastatin, pravastatin, mevastatin and pitavastatin), are generated by fermentation from certain fungi, while others, known as type 2 (e.g. atorvastatin, fluvastatin and rosuvastatin) are synthetically made. These two types of statins differ also in their structure. Type 1 statins have a decalin-ring structure, while type 2 statins possess a fluorophenyl group, which induces an additional interaction with HMG-CoA reductase (Istvan ES. and Deisenhofer J. 2001). Another way to categorize different statins is by their water solubility, with

pravastatin, and rosuvastatin being hydrophilic in nature, and atorvastatin, fluvastatin, lovastatin and simvastatin being lipophilic (Lutjohann D et al. 2004).

2.11.3. Specific Activity

Atorvastatin, cerivastatin, fluvastatin and pravastatin are administered as active compounds (acid form). Lovastatin and simvastatin are administered as inactive forms (lactone), which have to be enzymatically hydrolized to generate active forms (Blumenthal RS. 2000).

2.11.4. Brand Name and Derivation of Statin Drugs

Table 2.2. Brand name and Derivation of statin drugs

Statin	Brand Name	Derivation
Atorvastatin	Lipitor, Torvast	Synthetic
Fluvastatin	Lescol, Lescol XL	Synthetic
Lovastatin	Mevacor, Altocor, Altoprev	Fermentation-derived. Naturally occurring compound. Found in oyster mushrooms and red yeast rice.
Pitavastatin	Livalo, Pitava	Synthetic
Pravastatin	Pravachol, Selektine, Lipostat	Fermentation-derived.(A fermentation product of bacterium Nocardia autotrophica).
Rosuvastatin	Crestor	Synthetic
Simvastatin	Zocor, Lipex	Fermentation-derived. (Simvastatin is a synthetic derivate of a fermentation product of Aspergillus terreus.)

2.11.5. Comparative Effectiveness

An independent analysis has been done to compare atorvastatin, pravastatin and simvastatin, based on their effectiveness against placebos. It found that, at commonly prescribed doses, there are no statistically significant differences amongst statins in reducing cardiovascular morbidity and mortality. The CURVES study, which compared the efficacy of different doses of atorvastatin, simvastatin, pravastatin, lovastatin, and fluvastatin for reducing LDL and total cholesterol in patients with hypercholesterolemia, found that atorvastatin was more effective without increasing adverse events.

Statins differ in their ability to reduce cholesterol levels. Doses should be individualized according to patient characteristics such as goal of therapy and response. After initiation and/or dose changes, lipid levels should be analyzed within 1–3 months and dosage adjusted accordingly, then every 6–12 months afterwards. A link between cholesterol and cardiovascular disease, known as the lipid hypothesis, had already been suggested (Suresh P et al. 2011).

Table 2.3. Comparative effectiveness of the statin drugs

Statin Equivalent Dosages						
%LDLReduction (approx.)	Atorvastatin	Fluvastatin	Lovastatin	Pravastatin	Rosuvastatin	Simvastatin
10-20%	-	20 mg	10	10	-	5
20-30%	-	40 mg	20	20	-	10
30-40%	10 mg	80 mg	40	40	5	20
40-45%	20 mg	-	80	80	5-10	40
46-50%	40 mg	-	-	-	10-20	80
50-55%	80 mg	-	-	-	20	-
56-60%	-	-	-	-	40	-
Starting dose						
Starting dose	10–20 mg	20 mg	10-20 mg	40 mg	10 mg;5 mg if hypothyroid,>65 yo, Asian	20 mg
If higher LDL reduction goal	40 mg if >45%	40 mg if >25%	20 mg if >20%	-	20 mg if LDL >190 mg/dL (4.87 mmol/L)	40 mg if >45%
Optimal timing	Anytime	Evening	With evening meals	Anytime	Anytime	Evening

2.11.6. Mechanism Of Action Of Statins

Synthesis of cholesterol occurs in the cytoplasm and membrane of the endoplasmic reticulum of virtually all tissues in humans including the liver, intestine, adrenal cortex, and reproductive tissues. Most of the circulating cholesterol comes from internal manufacture rather than the diet. The liver produces about 70% of total cholesterol in the body and when the liver can no longer produce it, blood cholesterol levels will subsequently fall. As illustrated in Figure (2.4), the chemical pathway producing cholesterol in all cells begins with condensation of acetyl-CoA with acetoacetyl-CoA to form HMG-CoA (β-hydroxy-β-methylglutaryl coenzyme A) in a reaction catalyzed by HMG-CoA synthase. The HMG-CoA reductase enzyme then converts HMG-CoA to mevalonate, which is the first rate-limiting step in the pathway of cholesterol synthesis. After mevalonate is generated, multiple reactions follow to finally produce cholesterol Figure (2.4) (Emmanuel EAE. and Hafsa H. 2016).

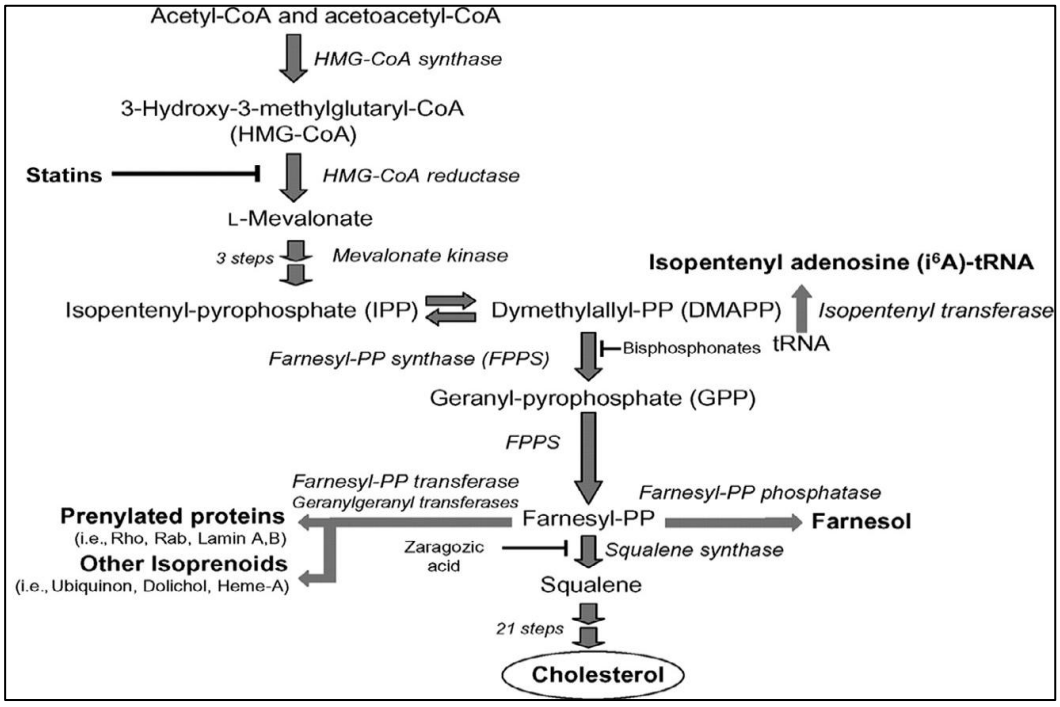

Figure 2.4. The mevalonate pathway. Statins act by inhibiting HMG-CoA reductase, the key enzyme of the mevalonate pathway. Statins could have pleiotropic effects possibly through other products of the mevalonate pathway (e.g., iA tRNA, prenylated proteins, and other isoprenoids) that play central roles in cell signaling, protein synthesis, and cytoskeletal organization

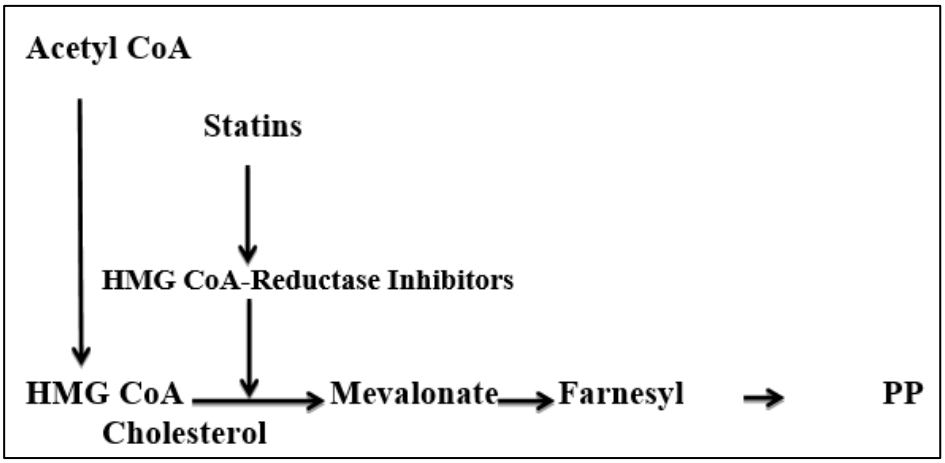

Figure 2.5. Schematic representation of Statin effect on Cholesterol synthesis (Siobhra O. 2007)

As HMG-CoA reductase inhibitors, statins are competitive, reversible inhibitors of HMG-CoA reductase, the rate-limiting enzyme of cholesterol biosynthesis. This rate-limiting step is the conversion of HMG-CoA to CoA and mevalonate by a four-electron reductive deacylation process. It is catalyzed by HMG-CoA reductase in a reaction that proceeds as follows:

(S)-HMG-CoA + 2NADPH+ $2H^+$ → (R)- mevalonate + $2NADPH^+$ +CoASH

In this step, the NADP+ is the oxidized form of nicotinamide adenine dinucleotide, NADPH is the reduced form of NADP+, and CoASH is the reduced form of CoA. Statins mimic HMG-CoA, the natural substrate molecule, and compete to bind to the HMG-CoA reductase enzyme. Production of mevalonate product slows down leads to decrease cholesterol biosynthesis downstream. Since the majority of cholesterol synthesis in the body occurs in hepatocytes, HMG-CoA reductase inhibitors mainly target the liver. Statins do more than just compete with the normal substrate in the enzyme active site; when bound, they alter the conformation of the enzyme itself, preventing HMGCoA reductase from attaining a functional structure. The high efficacy and specificity of statins are determined by their ability to make the enzyme conform their shape. Although the binding of statins to HMG-CoA reductase is reversible, their affinity for the enzyme is approximately 1000–10,000-fold stronger than for its natural substrate molecule, HMG-CoA. When the HMG-CoA binds to the enzyme, it interacts with the elongated NADP(H)

in the binding site, whereas no portion of this molecule is involved in the binding of statins. All statins are competitive inhibitors of HMG-CoA reductase with respect to binding of the substrate HMG-CoA, but not with binding of NADPH. For the statin–enzyme complexes, the Ki (inhibition constant) values range between 0.1 and 2.3 nM, whereas the Michaelis constant, Km, for HMG-CoA is 4 mM.

The fine-tuning of cholesterol synthesis is partly controlled in vitro and in vivo by the delivery of sterols to the cell via the LDL receptor pathway. Esterified cholesterol is mainly delivered into the cell by LDL; lysosomes provide the milieu for the enzymatic conversion of cholesteryl esters to free cholesterol. In addition to reducing de novo cholesterol synthesis with the use of statins, liver cells sense the reduced levels of cholesterol production, inducing the activation of protease that slices the sterol regulatory element-binding proteins (SREBPs) from the endoplasmic reticulum. In the nucleus, gene expression of LDL receptors is increased and translocation occurs into the production of LDL receptors to be displayed on the cell surface for uptake of LDL and its precursors (intermediate density—IDL and very low-density lipoproteins—VLDL). Inhibiting the cholesterol synthesis pathway created a multidimensional approach to lowering LDL, and HMG-CoA reductase had become the ideal target, as the pathway's first committed rate-limiting enzyme. Statins, therefore, result in a reduction in plasma cholesterol both by decreased cholesterol synthesis and by increased catabolism of LDL.

By inhibiting the HMG-CoA reductase, statins also inhibit production of specific prenylated protein metabolites downstream, such as geranylgeranyl pyrophosphate (GGPP) and farnesyl pyrophosphate (FPP). GGPP and FPP are lipid attachments that constitute key intermediates for posttranslational events of several cell signaling proteins, including the Ras, Rac, and Rho GTPase family members. Isoprenylation, or the attachment of these lipids, is fundamental for the activation and intracellular transport of these proteins that act as molecular switches controlling multiple pathways and cell functions such as maintenance of motility, cell shape, differentiation, factor secretion, and proliferation. Considering that the key role prenylated proteins play, it is expected that statin effects may extend beyond their LDL lowering actions (pleiotropic effects). These pleiotropic effects of statins, which are cholesterol independent, include reduction of the inflammatory process and accumulation of esterified cholesterol into macrophages,

increased stability of the atherosclerotic plaques, increase of endothelial NO synthetase, restoration of platelets activity, and of the coagulation process. The cholesterol-dependent and -independent effects of statins create significant reduction in coronary events, both as primary and secondary interventions. Statins remain the most efficient hypolipidemic compounds that have reduced the rate of mortality in coronary patients universally.

2.11.7. Timing Of Administration Of Statins

The vast majority of cholesterol synthesis appears to occur at night, so statins with short half-lives are usually taken in the evening or at bedtime to maximize their therapeutic effects. Studies have shown greater reductions in total and LDL-C in the short-acting simvastatin taken at night rather than the morning. A small study of atorvastatin, which has a long half-life, found no significant differences whether it was administered in the morning or the evening (Phillips NR et al. 1981).

2.11.8. Efficacy of Statins

2.11.8.1. Effect On LDL Cholesterol

The statins are currently the most powerful approved drugs for lowering LDL-C, with reductions in the range of 30–63%. Rosuvastatin may be somewhat more potent than atorvastatin, and both these agents may be significantly more potent than simvastatin, lovastatin, pravastatin, and fluvastatin. There may be an additive hypolipidemic effect when any of the statins is used in combination with a bile acid sequestrant, or the cholesterol-absorption inhibitor ezetimibe.

2.11.8.2. Effect On HDL

The level of HDL cholesterol is inversely related to the presence or development of ASCVD, but a causal relationship between the cholesterol content in HDL particles (HDL-C) has not been established. Furthermore, the evidence that raising HDL-C is of benefit in reducing cardiovascular events has not been established. Simvastatin (40–80

mg/day) may be more effective than atorvastatin (20–40 mg/day) for increasing serum HDL-C and apolipoprotein A–I concentrations. However, rosuvastatin may be even more effective, raising HDL-C by up to 10%. In patients with metabolic syndrome, rosuvastatin (10–20 mg/day) may be more effective than atorvastatin (10–20 mg/day) in increasing large HDL particles. Whether this is clinically important is still uncertain.

2.11.8.3. Effect On Triglycerides

The causal association between elevated triglycerides and cardiovascular risk is still uncertain due to the association between other lipoproteins and other conditions associated with increased ASCVD risk such as disorders of insulin resistance. Atorvastatin and rosuvastatin may be more effective at lowering triglycerides (14–33%) than other statins in patients with hypercholesterolemia. The effects of atorvastatin and rosuvastatin on plasma triglycerides may be dose dependent.

2.11.8.4. Genetic/Ethnic Effects

Evidence suggests that part of the variability in the response to and side effects with statins may be related to genetic differences in the rate of drug metabolism. For instance, CYP2D6 is a member of the cytochrome P450 super family of drug-oxidizing enzymes. CYP2D6 may be functionally absent in 7% of Caucasians and African-Americans, while deficiency is rare among Asians. The CYP2D6 phenotype appears to be important in patients treated with simvastatin, as it can affect both the degree of lipid lowering and tolerability. Polymorphisms in the gene coding for HMG- CoA reductase also appear to affect the LDL-C response to statins, but not the HDL-C response. Concerns have been raised that Asians may have greater responses to low doses of statins than Caucasians. Prescribing information for rosuvastatin recommends starting therapy at a lower initial dose in Asians than in other groups, given observed differences in pharmacokinetics. There is no strong evidence supporting such an approach with other statins.

2.11.8.5. Pleiotropic Effects

Several studies have shown that the benefit of statin therapy in terms of its ability to lower serum LDL-C in patients with and without clinical evidence of ASCVD. Evidence suggests that some of the benefits of statin therapy may be mediated by their pleiotropic effects, including their stabilization of atherosclerotic plaques, anti-inflammatory properties, improvement of endothelial dysfunction, and anti-thrombotic effects, which may only be partly accounted for by the cholesterol-lowering properties of these therapeutic agents. Variation in the reduction of ASCVD risk in statins trials may only partially account for the changes in LDL-C, HDL-C, and triglycerides. In fact, animal models and human studies have shown that statin therapy may reduce the rate of progression of atherosclerosis and stabilize atherosclerotic lesions. There is limited evidence on the exact timing of regression of atherosclerosis, particularly coronary atherosclerosis, after statin treatment. This issue was addressed using high-resolution MRI to assess aortic and carotid artery plaques. No changes were seen at 6 months, but progressive regression was noted at 12 and 24 months. The earliest change, seen at 12 months, was a reduction in plaque size followed by an increase in luminal area due to arterial remodeling. While lowering of LDL-C levels followed by stabilization and regression of atherosclerosis are accepted as the primary mechanism by which statins reduce ASCVD events, the observed benefit of this intervention begins within months after its initiation. This makes regression an unlikely cause for this early benefit. Other mechanisms thought to be involved include reduction of inflammation, reversal of endothelial dysfunction, reduction of oxidative modification of LDL, reduction in monocyte adhesion to the endothelium, increased mobilization and differentiation of endothelial progenitor cells, and decreased thrombogenicity. In addition to their ability to lower LDL-C and increase HDL-C, statins may cross-talk with the sphingosine-1-phosphate (S1P—a naturally occurring bioactive lysophospholipid that regulates diverse physiological functions in a variety of different organ systems) receptors, resulting in the enhancement of S1P-induced anti-inflammatory and anti-atherothrombotic effects independently of serum LDL-C, partly through the upregulation of the expression of S1P receptors and the increase of plasma levels of S1P. Statin-induced S1P signaling may, therefore, play a major role in mediating the enhanced response to HDL, and may represent a feature of the pleiotropic effects of statins through which they mediate

improvement of endothelial function, increased mobilization, and differentiation of endothelial progenitor cells, bioavailability of NO, anti-inflammatory response, inhibition of oxidation, and anti-atherogenic and anti-thrombotic actions.

2.11.9. Side Effects Of Statins

Several clinical trials have demonstrated the efficacy and safety of statin treatment. Nevertheless, a significant proportion of patients taking these drugs may experience some degree of intolerance, which in turn may result in statin discontinuation. Patient characteristics that may influence statin safety include but are not limited to: multiple or serious comorbidities, including impaired renal or hepatic function; a history of previous statin intolerance or muscle disorders; concomitant use of drugs affecting statin metabolism; a history of hemorrhagic stroke; and age >75 years. Asian ancestry may also influence the initial choice of statin intensity. Although several adverse effects are commonly reported by patients during statin therapy, only muscle toxicity and hepatic enzyme elevations are consistently statin related.

2.11.9.1. Muscle Injury

Although muscle toxicity remains a concern, severe myopathy is unusual, affecting perhaps 0.1% of patients. The clinical features of statin induced myopathy include symptoms such as muscle aches or myalgia, weakness, stiffness, and cramps. Muscle symptoms usually occur within the first 6 months of treatment, although they can begin months or even years after initiation of therapy. Statin-induced myopathy may resolve, or substantially abate, within 2 months of statin discontinuation, which may be helpful for determining the relationship of symptoms to statin use. Skeletal muscle-related adverse effects of statin therapy are most often categorized as myalgias, myopathy, or rhabdomyolysis.

2.11.9.2. Hepatic Dysfunction

Clinical studies of statins have demonstrated a 0.5–3.0% occurrence of persistent elevations in aminotransferases in patients receiving statins. This has primarily occurred during the first 3months of therapy and is dose dependent. While many drugs may cause liver disease, the evidence indicates that significant liver pathology attributable to statins is rare. These rare episodes of more severe liver injury may, predominantly, occur 3–4 months after initiation of statin therapy, with a range in one study of 1 month to 10 years. However, these are sufficiently uncommon that overall the incidence of hepatic failure in patients taking statins appears to be no different from the incidence in the general population. Thus, when the serious hepatotoxicity is encountered in a statin-treated patient, undiagnosed, and nonstatin related liver diseases should be strongly considered in the differential diagnosis. The pattern of more severe hepatotoxicity attributed to statins has included hepatocellular, cholestatic, and autoimmune injury. The most commonly reported hepatic adverse effect is the phenomenon known as “transaminitis” in which liver enzyme levels are elevated in the absence of histopathological changes. Although the underlying mechanism remains unclear, it may result from altered lipid components within the hepatocyte membrane, leading to increased permeability and subsequent “leakage” of liver enzymes. In fact, the phenomenon is observed with all classes of lipid-lowering drugs including resins, which are not absorbed. Therefore, this effect may be due to the lipid-lowering process itself and may not be specific to statins. When it occurs, it is usually hepatocellular and only very rarely cholestatic. Most cases of “transaminitis” resolve spontaneously without the need for drug discontinuation.

2.11.9.3. Renal Dysfunction

Reports of rosuvastatin-induced renal toxicity, largely proteinuria and hematuria, initially caused widespread concern. As a result, submission data for all statins were reviewed by the FDA, which eventually concluded that statins, including rosuvastatin, did not cause renal toxicity. There have been a number of reports about proteinuria with statins, particularly in patients receiving rosuvastatin or simvastatin. However, it is believed that

proteinuria with statins is a benign finding. Statins may cause proteinuria through tubular inhibition of active transport of small-molecular weight proteins.

2.11.9.4. Behavioral And Cognitive

Early research suggested that lowering cholesterol concentrations may be associated with an increase in violent or suicidal deaths. Other studies showed that both chronically low and medically lowered serum cholesterol may be associated with an increased incidence of depression. Although concerns have been raised, statins do not appear to be associated with an increased risk of suicide or depression. There have been case reports of patients developing severe irritability and aggression associated with the use of statins. It is not known whether the statin use caused these symptoms, but very rare idiosyncratic reactions of this sort may be missed in controlled trials.

Concerns have also been raised in the media and popular press about cognitive dysfunction and memory loss associated with statin use. Evans and colleagues conducted a patient survey-based analysis, to characterize the adverse cognitive effects of statins in 171 patients (age range 34–86 years) who self-reported memory or other cognitive problems associated with statin therapy while participating in a previous statin effects study. The authors found that cognitive problems associated with statin therapy have variable onset and recovery courses, a clear relation to statin potency, and significant negative impact on quality-of-life. Interestingly, a systematic review of randomized trials and observational studies found that published data do not suggest that statins harm cognition; however, the quality of the evidence was felt to be only low-to-moderate, particularly with regard to high-intensity statin therapy.

2.11.9.5. Cancer

Preclinical studies found that very high-dose statin therapy increased the risk of liver tumors in rodents. Some, but not all, observational studies have also raised the possibility that use of statins may decrease overall risk of cancer and of specific cancers. In contrast, meta-analyses of randomized trials have consistently shown no effect of statins on cancer

incidence or cancer mortality. 10-Year follow-up of the 4S trial and the West of Scotland Coronary Prevention Study (WOSCOPS) and 11-year follow-up of the Heart Protection Study (HPS) showed no increases in cancer deaths. In summary, there is no convincing evidence that statins increase or decrease the risk of cancer.

2.11.9.6. Diabetes Mellitus

Statins may have effects on glucose metabolism that might influence the development of diabetes mellitus in nondiabetics or affect glycemic control in patients with existing diabetes. Experimental evidence has been conflicting about whether statins as a group improve glucose metabolism or whether some statins show beneficial effects while others show harmful effects. It appears likely that statins may confer a small increased risk of developing diabetes, and that the risk is slightly greater with intensive statin therapy than moderate statin therapy. Recently, Mansi and colleagues conducted a retrospective cohort study of tricare beneficiaries who were evaluated between October 1, 2003 and March 1, 2012, to examine the association between statin use and new-onset diabetes, diabetic complications, and overweight/obesity. A total of 25,970 patients (3982 statin users and 21,988 nonusers) were identified as healthy adults at baseline. Of these, 3351 statins users and 3351 nonusers were propensity score matched. Statin users had higher odds of new-onset diabetes (odds ratio [OR] 1.87; 95% confidence interval [95% CI] 1.67–2.01), diabetes with complications (OR 2.50; 95% CI 1.88–3.32), and overweight/obesity (OR 1.14; 95% CI 1.04–1.25). Diabetes, diabetic complications, and overweight/ obesity were, therefore, more commonly diagnosed among statin users than similar nonusers in a healthy cohort of adults. The authors concluded that short-term clinical trials might not fully describe the risk/benefit of long-term statin use for primary prevention. Despite the above evidence, some experts still claim that the potential for an ASCVD risk reduction benefit outweighs the excess risk of diabetes in all but the lowest-risk individuals.

2.11.9.7. Others

In preclinical toxicity testing, dogs developed cataracts when given doses of statins much higher than human doses. While most large case–control and cohort studies, as well as a

small randomized trial, may not have found an increased risk of cataract, large cohort studies from England and Wales and from the United States military health system have found that statin use was associated with an increased risk of cataract. A small number of epidemiologic and case studies have suggested an association between statin and peripheral neuropathy, although this causal association has yet to be proven. Some, but not all, studies suggest that statins may lower androgen levels in men, although it appears unlikely that this effect is clinically significant. Statins may also reduce androgen levels in women, including in women with androgen excess. In the United States, statins are rated category X in pregnancy, and the recommendation is to discontinue their use prior to conception if possible. Animal studies suggest that at maternally toxic doses statins may be associated with adverse fetal outcomes, but limited human data indicate that statins may not be major human teratogens. Data on statin safety in breastfeeding are very limited. In the absence of adequate safety data, use of statins by breastfeeding mothers is discouraged (Emmanuel EAE. and Hafsa H. 2016).

2.11.10. Atorvastatin

Atorvastatin is a cholesterol-lowering medication that blocks the production of cholesterol (a type of fat) in the body. Atorvastatin reduces low-density lipoprotein (LDL) cholesterol and total cholesterol in the blood. Lowering your cholesterol can help prevent heart disease and hardening of the arteries, conditions that can lead to heart attack, stroke, and vascular disease. Atorvastatin is used to treat high cholesterol. Atorvastatin is also used to lower the risk of stroke, heart attack, or other heart complications in people with coronary heart disease or type 2 diabetes. Atorvastatin is used to treat dyslipidemias, which are disorders characterized by abnormal levels of lipids in the blood. Specifically, atorvastatin is used along with dietary therapy to decrease elevated serum total cholesterol and low-density lipoprotein cholesterol (LDL-C; so-called “bad” cholesterol), apolipoprotein B (apo B), and triglyceride concentrations. It is also used to increase concentrations of high-density lipoprotein cholesterol (HDL-C; the so-called “good” cholesterol). Familial hypercholesterolemia is an inherited condition characterized by high cholesterol levels. Atorvastatin is used to lower cholesterol in individuals as young as ten years who have familial hypercholesterolemia (LDL-C levels >than 190 mg/dl (or >than 160 mg/dl) and who have a family history of coronary heart

disease (CHD). The lipid-lowering effect of atorvastatin reduces the risk of CHD. Therefore, atorvastatin is used as primary prevention of heart attack, stroke, or angina in people who have multiple risk factors for CHD: age, smoking, high blood pressure, low HDL-C, or a family history of early CHD. Primary prevention refers to interventions that prevent the first occurrence of a disease or condition. Primary prevention of CHD is done for people who have no clinical evidence of cardiovascular disease but whom at risk. Atorvastatin is also used in primary prevention of cardiovascular events (e.g., heart attack, stroke) in people with type 2 diabetes. Atorvastatin is also used as secondary prevention. Secondary prevention refers to interventions that protect against recurrence of a disease or condition. Secondary prevention with atorvastatin is done in people who have CHD. In these people, atorvastatin is used to reduce the risk of heart attack, stroke, or hospitalization for congestive heart failure (CHF). Atorvastatin has also been shown to slow the progression of coronary atherosclerosis in patients with CHD. Atorvastatin is taken as tablets by mouth once a day, with or without food. Tablets containing 10 mg, 20 mg, 40 mg, or 80 mg are available. Low doses may be given initially, with gradual escalation depending on changes in blood lipid concentrations and the presence or absence of side effects (Suresh P et al. 2011).

3. MATERIALS AND METHODS

3.1. Subject Selection

Twenty-four subjects were participated in this study, 12 subjects (6 males, 6 females), aged between 32 and 58 years were as a case group of dyslipidemia, and 12 subjects (4 males, 8 females), aged between 30 and 49 years as a control group, who are not suffering from dyslipidemia. An overnight fasting blood samples (10-12 hrs.) were collected in the surgical specialty hospital cardiac center in Erbil city. The practical part was done from 20. 2. 2016 till 20. 4. 2016.

3.2. Determination Of Lipid Profile And Other Biochemical Parameters

For the determination of lipid profile and other biochemical parameters (Cobas c 311fully automated biochemistry analyzer, Germany, as shown in Plate (3.1) was used.

3.2.1. Determination Of Serum Lipid Profile

Lipid profile were measured with the Cobas c 311 diagnostic kits (Roche/Hitachi Cobas) by Cobas c 311 fully automated biochemistry analyzer, Germany, as shown in Plate (3.1).

3.2.1.1 Principle of Total Cholesterol (TCH) Measurement

Enzymatic, colorimetric method has been used for CH measurement. CH esters are cleaved by the action of cholesterol esterase (CE) to yield free CH and fatty acids. Cholesterol oxidase (CHOD) then catalyzes the oxidation of CH to cholest-4-en-3-one and hydrogen peroxide. In the presence of peroxidase (POD), the hydrogen peroxide

formed effects the oxidative coupling of phenol and 4-aminoantipyrine (4-AAP) to form a red quinone-imine dye.

$$\text{Cholesterol esters} + H_2O \xrightarrow{CE} \text{cholesterol} + \text{RCOOH} \quad (3.1)$$

$$\text{Cholesterol } (C_{27}H_{46}O) + O_2 \xrightarrow{CHOD} \text{cholest-4-en-3-one} + H_2O_2 \quad (3.2)$$

$$2\ H_2O_2 + \text{4-AAP} + \text{phenol} \xrightarrow{POD} \text{quinone-imine dye (red)} + 4H_2O \quad (3.3)$$

The color intensity of the dye formed is directly proportional to the CH concentration. It is determined by measuring the increase in absorbance (Cohn JS et al. 1988).

Normal ranges

Total cholesterol	mg/dL
Recommended value	< 200
Low risk	200-239
High risk	≥ 240

3.2.1.2 Principle of Triglyceride (TG) Measurement

Enzymatic colorimetric test has been used for TG measurement (Siedel J et al. 1993).

$$\text{Triglyceride } (C_{55}H_{98}O_6) + 3\ H_2O \xrightarrow{LPL} \text{glycerol } (C_3H_8O_3) + 3\ \text{RCOOH} \quad (3.4)$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{GK,\ Mg^{2+}} \text{glycerol-3-phosphate} + \text{ADP} \ldots (3.5)$$

$$\text{Glycerol-3-phosphate} + O_2 \xrightarrow{GPO} \text{dihydroxyacetone phosphate} + H_2O_2 \quad (3.6)$$

$$H_2O_2 + \text{4-aminophenazone} \xrightarrow{POD} \text{4- (p-benzoquinone-monoimino) +}$$

$$\text{4-chlorophenol-phenazone} + 2\ H_2O + HCl \quad (3.7)$$

Where,

LPL: lipoprotein lipase

GK: glycerol kinase

GPO: glycerol phosphate oxidase

POD: Peroxidase

Normal range

Triglycerides	mg/dl
Recommended value	35-160

3.2.1.3 Principle of Low Density Lipid Profile (LDL) Measurement

Homogeneous enzymatic colorimetric assay has been used for LDL measurement (Pisani T et al. 1995).

$$\text{LDL-cholesterol esters} + H_2O \xrightarrow[\text{Cholesterol esterase}]{\text{Detergent}} \text{Cholesterol} + \text{free fatty acids} \quad (3.8)$$

(selective micellary solubilization)

Cholesterol esters are broken down quantitatively into free CH and fatty acids by CE.

$$\text{LDL-Cholesterol} + O_2 \xrightarrow{\text{Cholesterol oxidase}} \Delta^4\text{-cholestenone} + H_2O_2 \quad (3.9)$$

$$2H_2O_2 + \text{4-aminoantipyrine} + \text{HSDA} + H_2O + H^+ \xrightarrow{\text{Peroxidase}} \text{Purple-blue pigment} + 5H_2O \quad (3.10)$$

(Abs. max = 585 nm)

In the presence of peroxidase, the H_2O_2 generated reacts with 4-aminoantipyrine and HSDA to form a purple-blue dye. The color intensity of this dye is directly proportional to the CH concentration and is measured photometrically.

HSDA: N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline

Normal ranges

LDL	mg/dL
Optimal	< 100
Near optimal/above optimal	100-129
Borderline high	130-159
High	160-189

Very high	≥ 190

3.2.1.4 Principle of High Density Lipid Profile (HDL) Measurement

Homogeneous enzymatic colorimetric assay has been used for HDL measurement (Matsuzaki Y et al. 1996). In the presence of magnesium ions and dextran sulfate, water-soluble complexes with LDL, VLDL, and chylomicrons are formed which are resistant to PEG-modified enzymes. The CH concentration of HDL is determined enzymatically by CE and COD coupled with PEG to the amino groups (approximately 40%). CH esters are broken down quantitatively into free CH and fatty acids by CE. In the presence of oxygen, CH is oxidized by CHOD to 4-cholestenone and hydrogen peroxide.

$$\text{HDL-cholesterol esters} + H_2O \xrightarrow{\textbf{PEG-cholesterol esterase}} \text{HDL-cholesterol} + \text{RCOOH} \quad (3.11)$$

$$\text{HDL-cholesterol} + O_2 \xrightarrow{\textbf{PEG-cholesterol esterase}} \Delta 4\text{-cholestenone} + H_2O_2 \ldots (3.12)$$

$$2H_2O_2 + \text{4-aminoantipyrine} + \text{HSDA} + H^+ + H_2O \xrightarrow{\textbf{peroxidase}} 5\,H_2O + \text{Purple blue pigment} \quad (3.13)$$

The color intensity of the blue quinoneimine dye formed is directly proportional to the HDL concentration. It is determined by measuring the increase in absorbance at 583 nm.

Normal ranges

HDL	mg/dl
Low level (Risk factor)	< 40
High level (protective factor)	≥ 60

3.2.1.5 Cholesterol / High Density Lipid Profile (CHOL/HDL) Calculation

CHOL/HDL ratio was measured with the Cobas c 311 diagnostic kits (Roche/Hitachi Cobas) by Cobas c 311 fully automated biochemistry analyzer, Germany, as shown in Plate (3.1). CHOL/HDL ratio is calculated by dividing total cholesterol to HDL number.

Normal ranges

CHOL/HDL	mg/dL
Normal or desirable	4.4
Borderline high	7.1
High	11.0

3.2.2. Measurement of Renal Function Test Parameters

Creatinine and urea were measured with the Cobas c 311 diagnostic kits (Roche/Hitachi Cobas) by Cobas c 311 fully automated biochemistry analyzer, Germany, as shown in Plate (3.1).

3.2.2.1. Principle of Creatinine Test

This kinetic colorimetric assay has been used for creatinine measurement (Jaffé M. 1986). In alkaline solution, creatinine forms a yellow-orange complex with picrate. The rate of dye formation is proportional to the creatinine concentration in the specimen. The assay uses “rate-blanking” to minimize interference by bilirubin. To correct for non-specific reaction caused by serum/plasma pseudo-creatinine chromogens, including proteins and ketones, the results for serum or plasma are corrected by -26 μmol/L (-0.3 mg/dL).

$$\text{Creatinine + picric acid} \xrightarrow{\text{Alkaline pH}} \text{yellow-orange (complex)} \qquad (3.14)$$

Normal ranges

Creatinine	mg/dl
Adults	
Female	0.50-0.90
Male	0.70-1.20

3.2.2.2. Principle of Urea Test

Kinetic test with urease and glutamate dehydrogenase has been used for urea measurement (Tiffany TO et al. 1972). Urea is hydrolyzed by urease to form ammonium and carbonate.

$$\text{Urea } (CH_4N_2O) + 2\ H_2O_2 \xrightarrow{\text{Urease}} NH_4^+ + O_2 + CO_3^{2-} \qquad (3.15)$$

In the second reaction 2-oxoglutarate reacts with ammonium in the presence of glutamate dehydrogenase (GLDH) and the coenzyme NADH to produce L-glutamate. In this reaction two moles of NADH are oxidized to NAD^+ for each mole of urea hydrolyzed.

$$NH_4^+ + \text{2-oxoglutarate} + NADH \xrightarrow{\text{GLDH}} \text{L-glutamate} + NAD^+ + H_2O \qquad (3.16)$$

The rate of decrease in the NADH concentration is directly proportional to the urea concentration in the specimen and is measured photometrically.

Normal range

Urea	(16.6-48.5)mg/dl

3.2.3. Measurement of Blood Sugar (Fasting)

Serum glucose was measured with the Cobas c 311 diagnostic kit (Roche/Hitachi Cobas) by Cobas c 311 fully automated biochemistry analyzer, Germany, as shown in Plate (3.1).

3.2.3.1. Principle of Glucose Test

For determination of fasting blood sugar (FBG) enzymatic reference method with hexokinase was used. Hexokinase (HK) catalyzes the phosphorylation of glucose to glucose-6-phosphate (G-6-P) by ATP (Tietz NW. 2006).

$$\text{Glucose + ATP} \xrightarrow{\text{HK}} \text{glucose-6-phosphate + ADP} \qquad (3.17)$$

Glucose-6-phosphate dehydrogenase (G-6-PDH) oxidizes glucose-6-phosphate in the presence of NADP to gluconate-6-phosphate. No other carbohydrate is oxidized. The rate of NADPH formation during the reaction is directly proportional to the glucose concentration and is measured photometrically.

$$\text{Glucose-6-phosphate + } NADP^{+} \xrightarrow{\text{G-6-PDH}} \text{gluconate-6-P + NADPH + } H^{+} \qquad (3.18)$$

Normal ranges

Blood sugar	mg/dl
Adult	70-105
Children	60-110
Newborn	40-60

3.3. Measurement of Erythrocyte Sedimentation Rate (E.S.R)

Erythrocyte sedimentation rate (E.S.R) was measured with the Microsed system (vital instrument) auto E.S.R analyzer, UK, as shown in Plate (3.2).

3.3.1 Principle of Erythrocyte Sedimentation Rate (E.S.R)

Ten infrared barriers vertically cover 10 test tube positions. At 2.0 mm intervals, all 10 positions on the reading plate are analyzed at the same time. As soon as the reading plate comprising ten pairs of infrared rays begins to rise, the indicating system intercepts any position occupied by samples containing the right level of blood. After approximately 3 minutes, the actual analysis begins. The computer records the zero time of each at regular intervals of 3 minutes for sample a total of 30 minutes. The instrument automatically converts the temperature to 18°C and gives the reading in 30 minutes (Gauravi D et al. 2014).

Table 3.1. Normal E.S.R (mm/hr) values according to Westergren

	Mean ESR			
Age (Yrs.)	Male	Female	Upper Limit of Normal	
			Male	Female
18-30	3.1	5.1	<7.1	<10.7
31-40	3.4	5.6	<7.8	<11.0
41-50	4.6	6.2	<10.6	<13.2
51-60	5.6	9.4	<12.2	<18.6
60 - 70	5.6	9.4	<12.7	<20.2
Over 70	5.6	10.1	<25.1	<25.1

3.4. Statistical Analysis

The data was compared by student's t-test, results were expressed as Mean ± S.E, all analyses were performed with Graph pad-prism (version 6.0). $P<0.05$ considered statistical significant.

Plate 3.1. Cobas c 311 fully automated biochemistry analyzer

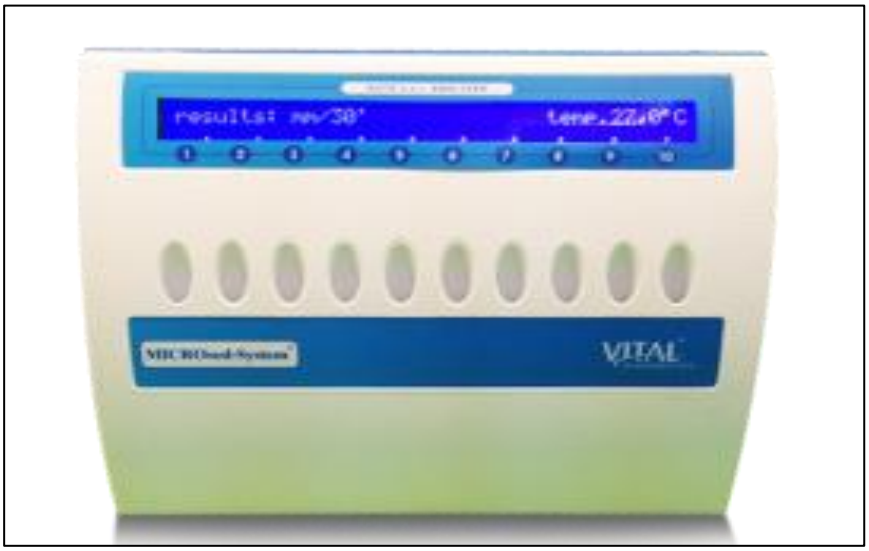

Plate 3.2. Microsed system (vital instrument) auto E.S.R analyzer

4. RESULTS

4.1. Lipid Profile

The Mean ± S.E values of TCH, TG, LDL, HDL and CHOL/HDL in control group were 162.4 ± 5.296, 101.9 ± 9.112, 89.58 ± 4.545, 45.42 ± 0.701, and 2.713 ± 0.189 mg/dl, while in dyslipidemia patient group before treatment were 270.7 ± 14.02, 172.7 ± 5.711, 127.9 ± 7.297, 34.75 ± 0.6528, and 3.050 ± 0.1283 mg/dL. The Mean ± S.E values of TCH, TG, LDL, HDL and CHOL/HDL of patients after treatment with atorvastatin were 212.9 ± 4.184, 144.1 ± 4.096, 109.0 ± 8.967, 39.58 ± 0.6450, and 2.724 ± 0.05025 mg/dL respectively (Table (4.1) and Figure (4.1), (4.2), (4.3), (4.4), (4.5). These data show significantly higher mean levels of TCH and TG in patient group before treatment with atorvastatin than that of control group, while they were decreased after treatment with atorvastatin. The mean level of LDL in patient group before treatment with atorvastatin was significantly higher than that of control group, it is decreased after treatment but not significantly. Furthermore, the HDL mean level in patient group before treatment with atorvastatin was significantly lower than that of control group, it is increased after treatment with atorvastatin. The mean level of CHOL/HDL in patient group before treatment with atorvastatin was higher as it compares with control group, but not significantly, it is decreased non-significantly after treatment with atorvastatin.

Table 4.1. Shows Mean ± S.E. of TCH, TG, LDL, HDL and CHOL/HDL in control and dyslipidemia patients before and after treatment with atorvastatin

Parameters	**Control (A) Mean ±S.E.**	**Before treatment (B) Mean ±S.E.**	**After treatment (C) Mean ±S.E.**	**Statistical evaluation P-values**	
Total cholesterol (mg/dl)	162.4 ± 5.30	270.7 ± 14.02	212.9 ± 4.18	A vs. B	<0.001
				A vs. C	<0.01
				B vs. C	<0.001
Triglyceride (mg/dl)	101.9 ± 9.11	172.7 ± 5.71	144.1 ± 4.10	A vs. B	<0.001
				A vs. C	<0.001
				B vs. C	<0.05
LDL (mg/dl)	89.58 ± 4.55	127.9 ± 7.30	109.0 ± 8.97	A vs. B	<0.01
				A vs. C	NS
				B vs. C	NS
HDL (mg/dl)	45.42 ± 0.70	34.75 ± 0.65	39.58 ± 0.65	A vs. B	<0.001
				A vs. C	<0.001
				B vs. C	<0.001
CHOL/HDL (mg/dl)	2.713 ± 0.19	3.050 ± 0.13	2.724 ± 0.05	A vs. B	NS
				A vs. C	NS
				B vs. C	NS

*p< 0.05, **p< 0.01, ***p< 0.001, NS= Non-significant

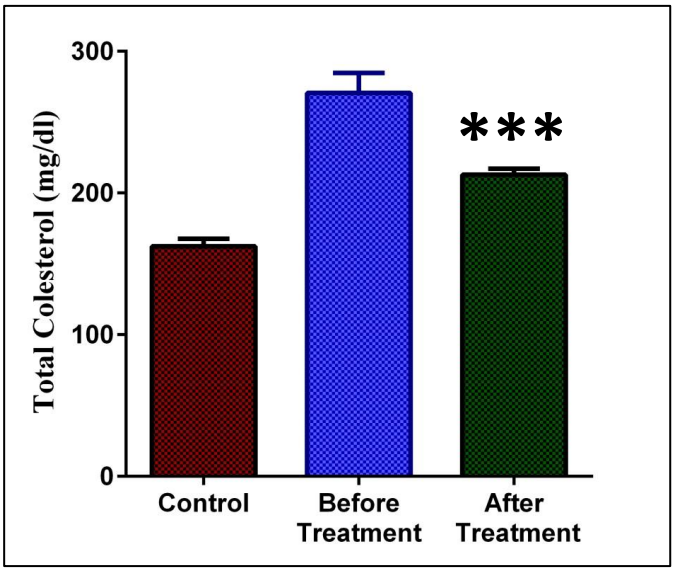

Figure 4.1. Serum TCH levels in control and patients with dyslipidemia before and after treatment with atorvastatin

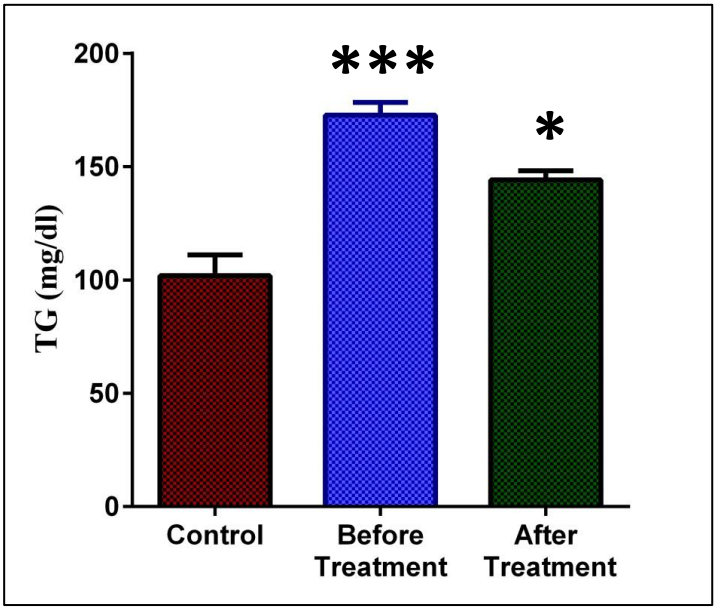

Figure 4.2. Serum TG levels in control and patients with dyslipidemia before and after treatment with atorvastatin

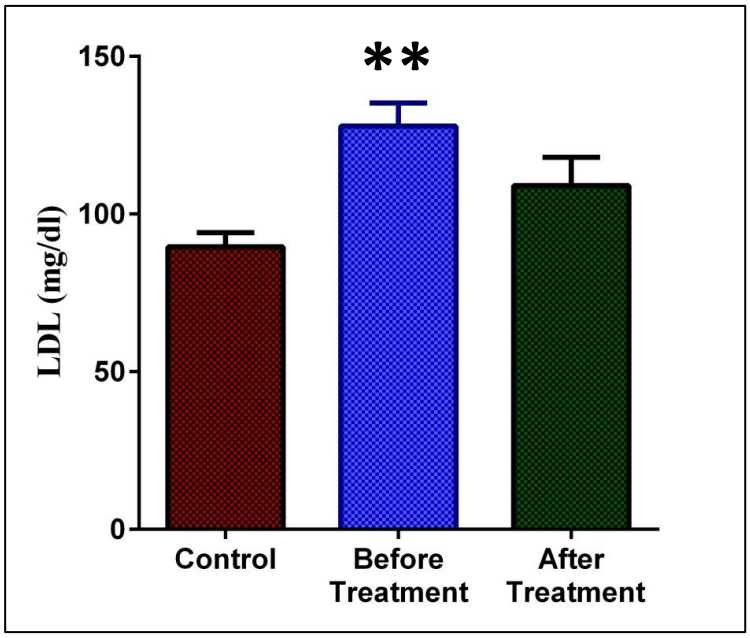

Figure 4.3. Serum LDL levels in control and patients with dyslipidemia before and after treatment with atorvastatin

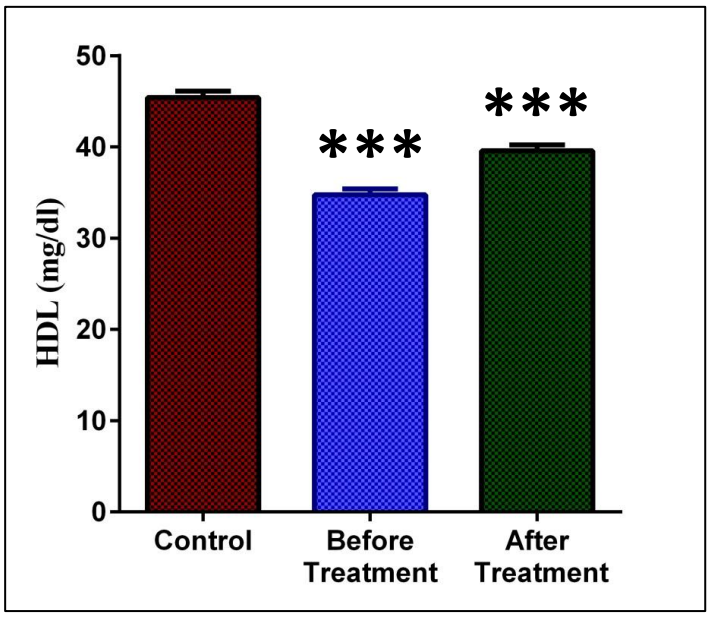

Figure 4.4. Serum HDL levels in control and patients with dyslipidemia before and after treatment with atorvastatin

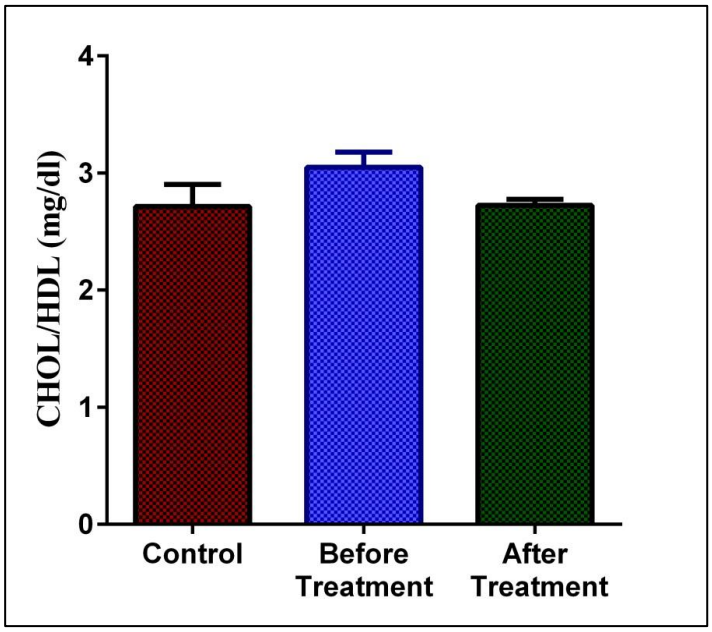

Figure 4.5. Serum CHOL/HDL levels in control and patients with dyslipidemia before and after treatment with atorvastatin

4.2. Renal Function Test Parameters

Mean levels of creatinine and urea in control group were 0.875 ± 0.032 and 19.75 ± 1.498, while in dyslipidemia patient group were 1.025 ± 0.06046 and 29.58 ± 2.726 mg/dL respectively, and after treatment with atorvastatin were 0.9917 ± 0.03362 and 28.17 ± 2.354 mg/dL, respectively Table (4.2) and Figure (4.6), (4.7). The results show that the mean level of creatinine in patient group before and after treatment with atorvastatin was higher compared to control group, but not significantly. The mean level of urea in patient group before and after treatment with atorvastatin was significantly higher compared to control group. There are no significant differences in mean levels of creatinine and urea in patients before and after treatment with atorvastatin. These data suggest that atorvastatin does not play any role in the level of serum creatinine and urea.

Table 4.2. Shows Mean ± S.E. of creatinine and urea in control and dyslipidemia patients before and after treatment with atorvastatin

Parameters	**Control (A) Mean ±S.E.**	**Before treatment (B) Mean ±S.E.**	**After treatment (C) Mean ±S.E.**	**Statistical evaluation P-values**	
Creatinine (mg/dl)	0.875 ± 0.03	1.025 ± 0.06	0.9917 ± 0.03	A vs. B	NS
				A vs. C	NS
				B vs. C	NS
Urea (mg/dl)	19.75 ± 1.50	29.58 ± 2.73	28.17 ± 2.35	A vs. B	<0.05
				A vs. C	<0.05
				B vs. C	NS

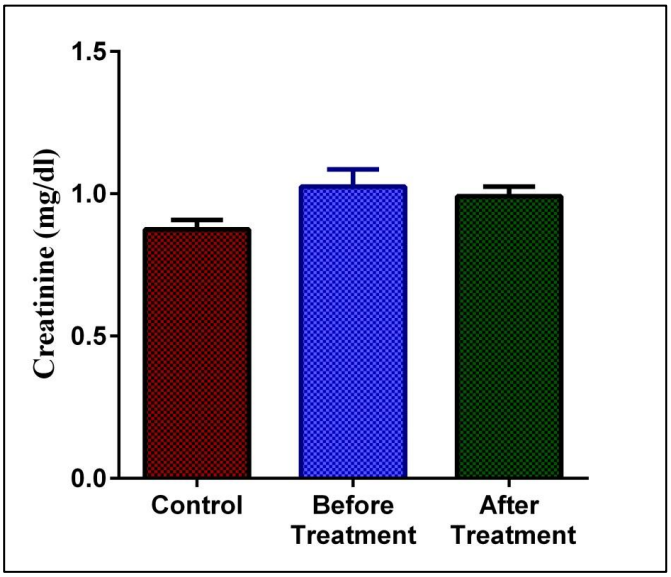

Figure 4.6. Serum Creatinine levels in control and patients with dyslipidemia before and after treatment with atorvastatin

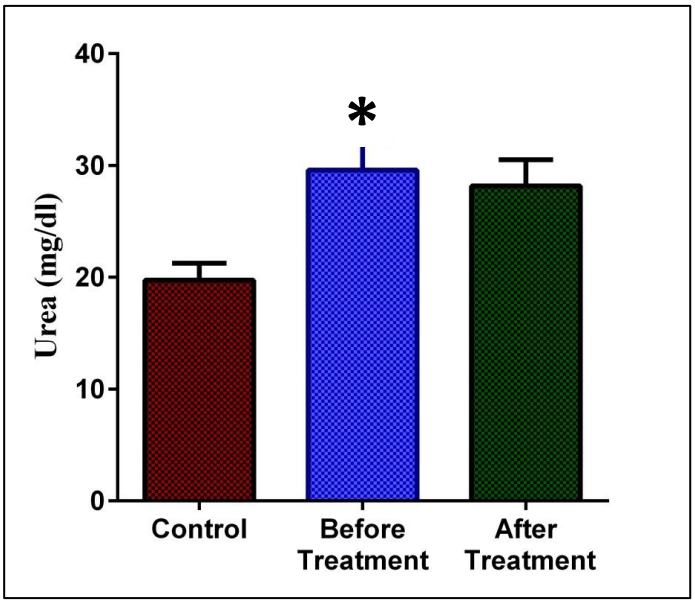

Figure 4.7. Serum Urea levels in control and patients with dyslipidemia before and after treatment with atorvastatin

4.3. Hyperglycemia

Mean serum concentration of glucose in control group and in dyslipidemia patient group before and after treatment with atorvastatin were 80.54 ± 7.743, 108.6 ± 7.689, 92.67 ± 3.076 mg/dL. This study demonstrates that serum concentration of glucose was significantly higher in patient group before and after treatment with atorvastatin in comparison with control group. It is decreased after treatment, but not significantly.

Table 4.3. Shows Mean ± S.E. of glucose in control and dyslipidemia patients before and after treatment with atorvastatin

Parameters	**Control (A) Mean ±S.E.**	**Before treatment (B) Mean ±S.E.**	**After treatment (C) Mean ±S.E.**	**Statistical evaluation P-values**	
Blood sugar (fasting) (mg/dl)	80.54 ± 7.74	108.6 ± 7.69	92.67 ± 3.08	A vs. B	<0.05
				A vs. C	<0.05
				B vs. C	NS

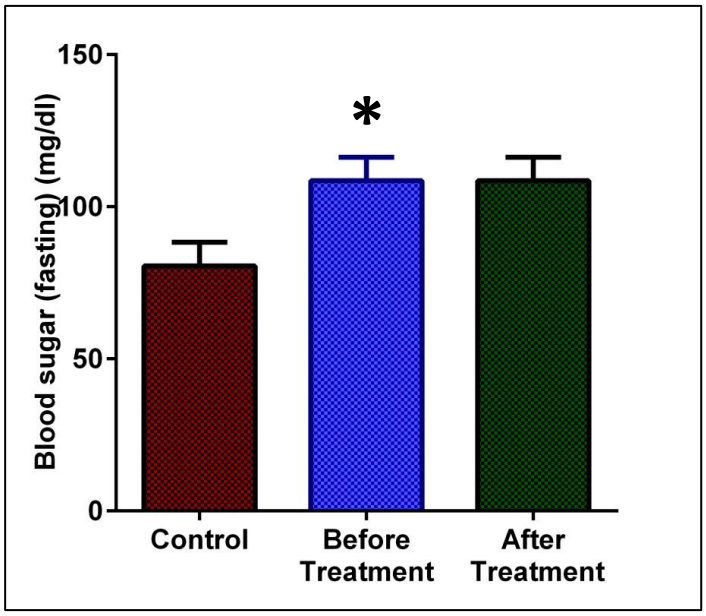

Figure 4.8. Serum Blood sugar levels in control and patients with dyslipidemia before and after treatment with atorvastatin

4.4. Erythrocyte Sedimentation Rate (E.S.R)

The Mean ± S.E value of E.S.R in control group and in dyslipidemia patient group before and after treatment with atorvastatin were 15.42 ± 0.856, 22.58 ± 1.411, 18.00 ± 0.5903 mg/dL. This study indicates that the mean value of E.S.R was significantly higher in patient group before treatment with atorvastatin as it compare with control group. It is decreased after treatment with atorvastatin, but not significantly. There is significant difference in mean levels of E.S.R in patients before and after treatment with atorvastatin.

Table 4.4. Shows Mean ± S.E. of E.S.R in control and dyslipidemia patients before and after treatment with atorvastatin

Parameters	**Control (A) Mean ±S.E.**	**Before treatment (B) Mean ±S.E.**	**After treatment (C) Mean ±S.E.**	**Statistical evaluation P-values**	
E.S.R (mm/1H)	15.42 ± 0.86	22.58 ± 1.41	18.00 ± 0.60	A vs. B	<0.001
				A vs. C	NS
				B vs. C	<0.01

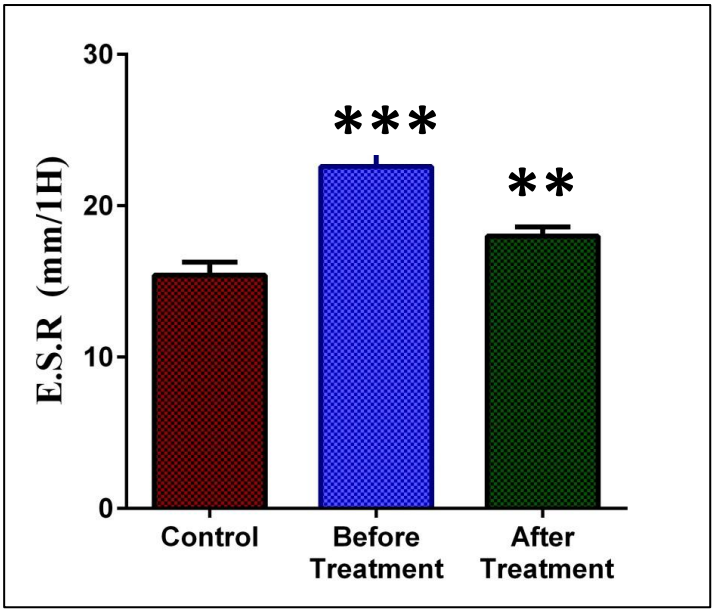

Figure 4.9. E.S.R levels in control and patients with dyslipidemia before and after treatment with atorvastatin

5. DISCUSSION

The beneficial effects of the HMGCoA reductase inhibitors are usually attributed to their capacity to reduce the endogenous cholesterol synthesis, by competingly inhibiting the principal enzyme involved (Camelia S. and Anca S. 2001).

The statins are currently the most powerful approved drugs for lowering LDL-C, with reductions in the range of 30–63%. Effective of atorvastatin (20–40 mg/day) for increasing serum HDL-C and apolipoprotein A–I concentrations. Atorvastatin effective at lowering triglycerides (14–33%) than other statins in patients with hypercholesterolemia (Emmanuel EAE. and Hafsa H. (2016).

Blood LDL-C levels were the main target of lipid lowering therapy for the prevention of CVD. To lower cholesterol, HMG-CoA reductase inhibitors or statin drugs, one of the best-studied classes of pharmaceuticals and the most prescribed of all time. The ability of 'statins' to lower LDL-C and reduce the relative risk of CHD by approximately 30 % in a variety of at-risk populations, and indeed even in those with acceptable LDL-C concentrations, and improve outcomes in both primary and secondary prevention, revolutionized the practice of cardiology. LDL-lowering with statins is not only accompanied by improved outcomes, normalization of biomarkers, reduction of plaque volume and regression of lesions, but can also reduce the incidence of unstable angina in ACS (Ferrie`res J. 2009).

HMG-CoA reductase inhibition, the rate-limiting enzyme involved in the mevalonate pathway leading to cholesterol synthesis, is believed to be the primary effect of statin drugs but, secondarily, statins lead to up regulation of LDL receptors (LDL-Rs) and enhanced LDL clearance. There are also a wide variety of beneficial 'pleiotropic', cholesterol-independent actions of statins that have received attention (Zhou Q. and Liao

JK. 2009). Some of these apply to systems other than CV, such as neuroprotective actions, and modulation of cellular senescence.

Mevalonate depletion due to HMG-CoA reductase inhibition also reduces the availability of downstream isoprenoids, notably farnesyl pyrophosphate and geranylgerany pyrophosphate, and impairs post-translational isoprenylation of proteins. These moieties function as lipid attachments or anchors for molecules, thereby enabling intracellular trafficking. Among the many affected molecules are the small GTPases. Members of this family include rho-kinases I and II ('ROCK I and II') that are crucial to organization and rearrangement of the cytoskeleton, assuming key roles in cell morphology, motility, intracellular translocation, and gene expression. ROCK functions in smooth muscle migration and plaque morphology, and regulates transcription factors involved in atherosclerosis. Rho-kinase promotes inflammation by inducing proinflammatory cytokines such as interleukin (IL)-6, monocyte chemoattractant protein-1, and macrophage migration inhibitory factor, increases endothelial expression of adhesion molecules, and promotes smooth muscle proliferation. Rho-kinase upregulates nicotinamide adenine dinucleotide phosphate-oxidase [NAD(P)H] oxidases, and joins Rac, another small GTPase, to generate reactive oxygen species (ROS). Much evidence points to statin-induced inhibition of Rho isoprenylation and ROCK activity as a principle mechanism of many pleiotropic effects, so much so that rho-kinases are considered therapeutic targets themselves (Satoh K et al. 2011).

Cholesterol has been singled out as the primary factor in development of atherosclerosis. HDL is regarded as one of the most important protective factors against arteriosclerosis. HDL's protective function has been attributed to its active participation in the reverse transport of CH (Tomás M et al. 2004). The concentration of LDL correlates positively whereas HDL correlates inversely to the development of CHD (Thakur S et al. 2011).

The strong relationship between low levels of HDL and the risk for atherosclerosis and CAD has been attributed to several distinct mechanisms that HDL transfers CH from peripheral tissues to the liver, where metabolites of the sterol are excreted into the bile; HDL accepts CH from macrophage foam cells, the cellular hallmark of the

atherosclerotic lesion. Therefore, HDL might be cardioprotective because it prevents CH accumulation in cells of the artery wall (Shao B. and Heinecke JW. 2009). An HDL-associated enzyme, paraoxonase (PON), was capable of preventing the accumulation of lipid hydroperoxides in LDL (Mackness MI et al. 1991).

Triglycerides, CH and lipoproteins are implicated in the pathogenesis of CAD, especially atherosclerosis. Reduced concentrations of HDL and increased TG have been shown to be responsible for the genesis of atherosclerotic lesions (Navab M et al. 2000). Oxidatively modified LDL contributes to the pathogenesis of atherosclerosis, increased oxidative stress and the generation of the free ORs can result in modification of LDL to oxidized LDL that could lead to atherosclerotic lesions (Mohammed O et al. 2011). LDL is a vehicle to supply CH all over the body in order to maintain cell viability and to provide CH for the synthesis of the steroid hormones. HDL plays a part in reverse CH transport and also protects LDL from oxidation (Gerald H and Daphne O. 2012).

Patients with renal failure have a high prevalence of cardiovascular disease, and it has been proposed that atherosclerosis may promote the progression of renal disease (Kasiske BL. 1987).

Several studies have shown that the benefit of statin therapy in terms of its ability to lower serum LDL-C in patients with and without clinical evidence of ASCVD. Evidence suggests that some of the benefits of statin therapy may be mediated by their pleiotropic effects, including their stabilization of atherosclerotic plaques, anti-inflammatory properties, improvement of endothelial dysfunction, and anti-thrombotic effects, which may only be partly accounted for by the cholesterol-lowering properties of these therapeutic agents. Variation in the reduction of ASCVD risk in statins trials may only partially account for the changes in LDL-C, HDL-C, and triglycerides (Egom EE et al. 2013). Diabetes is significant risk factor for atherosclerosis; the diabetic state promotes oxidative stress mediated by ROS, these consume NO and lead to endothelial dysfunction (Libby P et al. 2002).

Atherosclerosis is considered to be an inflammatory process triggered by response to injury and oxidative stress. Increasing in the plasma inflammation markers were shown to be related with the risk of vascular disease in type II diabetes patients (Emoto M et al. 2001). Atherosclerosis is an important macrovascular complication and the major cause of morbidity and mortality in patients with DM (Dogansen S et al. 2013). DM itself is a risk factor for atherosclerosis (Gleissner CA. Et al. 2007). Type II diabetes patients are increased risk for CVD that of the association with several risk factors included hypertension, hyperlipidemia, and obesity (King GL et al. 1996). Both type I and type II diabetes are powerful and independent risk factors for CAD, stroke, and peripheral arterial disease (Grundy SM et al. 1999).

The mechanism behind the relation between diabetes and atherosclerosis is not fully known. Oxidative stress, through the production of ROS has been proposed as the root cause underlying insulin resistance, type II diabetes and vascular complications, apart from inflammation and diabetic dyslipidemia. The impaired glucose metabolism and advanced glycation end products might contribute to the atherosclerotic process (Brohall G. 2007). Hyperglycemia also enhances thrombogenesis by activating platelets and reducing production of endogenous platelet inhibitors. DM promotes atherosclerotic lesion formation, increases plaque instability, and favors the formation and persistence of thrombi (González-Navarro H et al. 2007). Hyperglycemia in diabetics may induce dysfunctional endothelium which is involved in the genesis of atherosclerosis (Mcnair ED. 2006). The mean serum glucose concentration in coronary atherosclerotic patients significantly elevated level compared to the normal healthy group (Saeed RH. 2015). Hcy is an accepted risk factor for CVD. Hyperhomocysteinaemia is known to be associated with atherosclerosis, and this association is stronger in individuals with type II diabetes than in non-diabetic subjects (Hoogeveen EK et al. 2000).

Statins diminish leukocytes recruitment in postcapillary venules, stimulated by a lipid mediator (platelets activation factor-PAF or leukotriene B4) in hypercholesterolemic rats (Kimura M et al. 1997). In addition, statins are capable to inhibit transendothelial migration and chemotaxisis of neutrophiles, which can explain the antiinflammatory effect of these compounds. Another antiinflammatory effect of statins on monocytes and macrophages was the decrease of the expression of intercellular adhesion molecule -1 and

the secretion of interleukine-6 (IL-6), induced by lipopolysacharides (LPS) (Bellosta S et al. 2000).

6. CONCLUSIONS

From the present obtained results, the following conclusions could be drawn:

1. Statins have a positive effect on biochemical parameters of patients with dyslipidemia.

2. Statins have a positive effect on hematology parameter of patients with dyslipidemia.

3. Observed results present novel pleiotropic effect of statin treatment for the patients with dyslipidemia.

4. The treatments with atorvastatin induce in group of patients with dyslipidemia significant decreasing the concentrations of TCH and 21,4% or in 1,27 times in comparison with group patients before treatment.

5. The treatment with atorvastatin induces in group of patients with dyslipidemia significant decreasing the concentrations of TG about 16.6% or 1,19 times compared with group patients before treatment.

6. The concentration of HDL was significantly higher on 14,8% or in 1,17 times in group of patients treated with atorvastatin compared with group of patients before treatment.

7. The concentrations of LDL and CHOL/HDL were lower in patients after treatment with atorvastatin than that of patients before treatment but not significantly.

8. The concentrations of creatinine, urea and glucose were lower in patients after treatment with atorvastatin than that of patients before treatment but not significantly.

9. The mean value of E.S.R was statistically valid lower on 46,4% or in 1,46 times in group of patients treated with atorvastatin compared with group of patients before treatment.

RECOMMENDATIONS AND FURTHER WORKS

1. Further studies should be done to evaluate the effect of atorvastatin of patients with dyslipidemia like Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), C-reactive protein and fibrinogen.

2. Atorvastatin should be taken as a routine treatment in patients with dyslipidemia.

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