EVALUATION OF THE EFFECT OF BIOTIN IN DYSLIPIDEMIA

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CERTIFICATE

This is to certify that this dissertation entitled "**Evaluation of the effect of Biotin in Dyslipidemia**" by the candidate Dr. N. Asvini for M.D. (Pharmacology) is a bonafide record of the research work done by her under the guidance of Dr. G. Hemavathy M.D. Professor, Department of Pharmacology, Stanley Medical College, during the period of study (2012 - 2015), in the Department of Pharmacology, Stanley Medical College,Chennai-01.

I also certify that this dissertation is the result of the independent work on the part of the candidate.

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CONTENTS

ABBREVIATIONS

ATP III	-	Adult treatment panel III
ACS	-	Acute Coronary Syndrome
ABCA1	-	ATP – binding cassette transporter A1
CAD	-	Coronary artery disease
CVD	-	Coronary Vascular disease
CRF	-	Coronary Risk Factors
CRP-C	-	reactive protein
CHD	-	Coronary Heart Disease
DM	-	Diabetes Mellitus
HDL-C	-	High Density Lipoprotein cholesterol
LDL-C	-	Low density lipoprotein Cholesterol
TC	-	Total cholesterol
TG	-	Triglyceride
VLDL	-	Very low density Lipoprotein Cholesterol
PEPCK	-	Phospho Enol Pyruvate Carboxy Kinase

ABSTRACT

INTRODUCTION

Dyslipidemias are an emerging global health challenge yet, they remain underdiagnosed and under treated. Many newer therapies are being discovered for treating these dyslipidemias. Studies have shown that Biotin a B complex vitamin has favourable effects on the plasma lipid profile of diabetic and non diabetic subjects.This study was conducted to find out the effect of pharmacological doses of biotin on plasma lipid profile of patients with secondary dyslipidemias.

AIM AND OBJECTIVES

The objective of this study was to determine and compare the percentage change from baseline in lipid parameters at weeks 1^{st} ,2^{nd} ,4^{th} and 6^{th} week between the two groups – control group which received Atorvastatin 20 mg per day and study group which received Atorvastatin 20 mg per day with Biotin 5 mg/day.

METHODOLOGY

60 subjects in the age group of 45-60 yrs with secondary dyslipidemia meeting the inclusion criteria were enrolled after getting a written informed consent. After performing baseline laboratory investigations and lipid profile they were randomized into two groupsThe control group. received Atorvastatin 20 mg

alone and the study group received Biotin 5mg along with Atorvastatin 20 mg for 6 weeks. Both the groups were followed up till 12 weeks.

RESULTS

As compared with monotherapy, combination therapy resulted in greater reduction in total cholesterol (36%) ($p<0.001$), greater reduction in LDL cholesterol (42%) ($p<0.001$), greater reduction in triglycerides (26.73%) ($p<0.001$) and greater reduction in VLDL (36%) ($p=0.839$) at 6 weeks. Addition of Biotin also resulted in greater rise in HDL (4.17%), but the difference was not statistically significant. The total cholesterol : HDL ratio became 3.54 in the combination group which was in the desirable level. Adverse events were also less in the combination therapy.

CONCLUSION

Combination therapy of Biotin 5mg with Atorvastatin 20 mg is more efficacious than Atorvastatin alone in terms of reduction in Total cholesterol, LDL cholesterol and triglycerides. Biotin is a safe and well tolerated adjuvant hypolipidemic agent in secondary dyslipidemias.

KEY WORDS

Biotin, hyperlipoproteimias,statins,fibrates,lipid profile,creatine kinase

INTRODUCTION

Dyslipidemias and their complications are now emerging as an important public health challenge globally.[1] It has been predicted that by 2020, cardiovascular diseases secondary to dyslipidemias will be the largest causes of disability and death in the developing countries,[2] especially in India, with 2.6 million Indians predicted to die due to cardiovascular diseases.[3]

The role of these lipoprotein abnormalities in the causation and maintanence of atherosclerosis and their primary role in cardiovascular morbidity and mortality is well known and cannot be underestimated.[4]

Lipid lowering therapies including pharmacological and non-pharmacological therapies have a significant role in the primary & secondary prevention of cardiovascular disorders.

The HMG Co-A reductase inhibitors are the first line and widely used pharmacological agents, but they still have some limitations. Patients are usually under treated due to the fear of adverse events and donot achieve their LDL goals. After prolonged use and even increased doses statins produce a ceiling dose effect due to feed back compensatory induction of HMG Co A reductase enzyme.[5]

Studies have shown that clinical use of effective pharmacological strategies for lowering LDL have reduced cardiovascular events. But still they prevent only a minority of these endpoints. Consequently other aspects of lipid profile have become tempting targets for addressing the residual burden of disease.[6]

Other newer agents in development include cholesteryl ester transfer protein inhibitors, antisense oligonucleotides and PPAR-agonists but the trial results are not satisfactory.

Recently, more vitamin mediated effects are being discovered at gene expression level.[7] Of these, Biotin has shown to regulate expression of genes which are important in intermediary metabolism.

Biotin is a B complex vitamin now being used for treating dermatitis, brittle nails, hair loss and biotin deficiency associated parentral alimentation.

Studies investigating the effect of Biotin administration in small groups of patients have concluded that pharmacological doses of biotin decrease hypertriglyceridimia.[8] It has also been shown that Biotin supplementation is effective in reducing the plasma lipoproteins of diabetic & non diabetic subjects.[9]

This study was conducted to find out the effect of pharmacological doses of Biotin on plasma lipid profile of dyslipidemic patients. If Biotin is proved to be an efficacious lipid lowering agent it would emerge as a cheaper and better tolerated adjuvant drug in the management of dyslipidemia. This will help in reducing the disease burden in the future.

REVIEW OF LITERATURE

DYSLIPIDEMIA

Synonyms:

Hyperlipidemia, Hyperlipoproteinemia.

Definition:

Dyslipidemia is a metabolic disorder of lipoproteins, which includes overproduction or deficiency of lipoproteins.[10]

It may manifest as

- elevation of total cholesterol (TC),
- elevation of low density lipoprotein (LDL) cholesterol,
- elevation of triglyceride (TG) levels,
- decrease in blood HDL levels.

Classification:

Dyslipidemias can result from genetic defects in lipoprotein metabolism **(Primary Dyslipidemias**) or from some other underlying disorder that affects the circulating levels of lipids (**Secondary Dyslipidemias).**

Epidemiology:

Dyslipidemia is a common metabolic disorder. The prevalence of dyslipidemia has increased from 1.05% in 1960 to about 8 - 9.6% in 1990 in the urban population of India.[11,12] The National Health Survey, conducted in U.S. during 1999 to 2000 showed that 25 % of adults had raised total cholesterol (> than 239.4 mg per dL) and were on lipid-lowering medication.[13]

Lipoprotein:

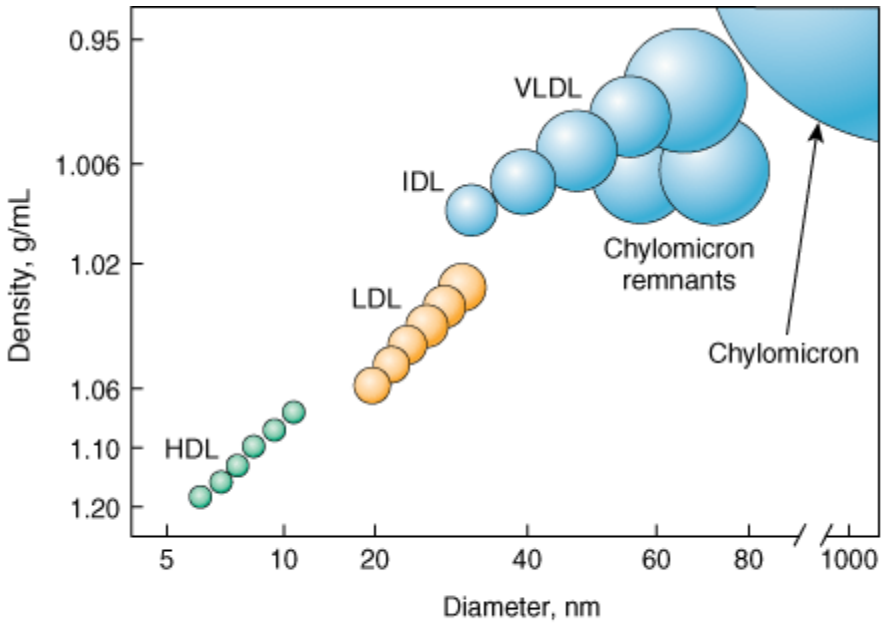
The term lipoprotein denotes a complex of lipids and protein, that are essential in the movement of cholesterol, triglycerides and fat soluble vitamins in the bloodstream. Structurally they contain a core of lipids like triglycerides and cholesterol esters which are hydrophobic, surrounded by phospholipids and unesterified cholesterol which are hydrophilic.[14]

The classification of lipoproteins is based on their relative densities. The various lipoprotein classes vary in their density, size and protein composition. The different classes of lipoproteins are

1. **The chylomicrons**
2. **The very low density lipoproteins** (VLDL)
3. **The low density lipoproteins** (LDL)

4. **The high density lipoproteins** (HDL)

The density and size distribution of various types of lipoprotein particles [15]



Source: Fauci AS, Kasper DL, Braunwald E, Hauser SL, Longo DL, Jameson JL, Loscalzo J: *Harrison's Principles of Internal Medicine*, 17th Edition: http://www.accessmedicine.com

Major lipoprotein classes[16]

LIPOPROTEIN	MAJOR LIPID COMPONENT	MAJOR APOLIPOPROTEINS	SOURCE
Chylomicrons	TG	ApoA-I, A-II, A-IV; ApoC-I, C-II, C-III; ApoB-48; ApoE	Intestine
Very low-density lipoprotein (VLDL)	TG	ApoB-100; ApoC-I, C-II, C-III; ApoE	Liver
Intermediate-density lipoprotein (IDL)	CE	ApoB-100; ApoE, ApoC	Catabolism of VLDL
Low-density lipoprotein (LDL)	CE	ApoB-100	Catabolism of IDL
High-density lipoprotein (HDL)	CE, PL	ApoA-I, A-II, A-IV; ApoC-I, C-II, C-III; ApoE	Liver, intestine, other

Of the various lipoproteins the smallest are the high density lipoproteins. They are also the most dense of all lipoproteins. The largest and least dense lipoprotein particles are the chylomicrons. The major transporters of plasma triglyceride are the chylomicrons and the very low density lipoproteins. Majority of the plasma cholesterol is transported as cholesteryl esters in the low density lipoproteins and the high density lipoproteins.

Apolipoproteins:

The protein moiety of the lipoproteins are known as apolipoproteins. The apolipoproteins act as an essential component in the structure, assembly and function of lipoproteins. They play an important role in the activation of enzymes important in lipoprotein metabolism. They also act as ligands for cell surface receptors.

Various Apo lipoproteins are

- Apo A-I, component of all HDL particles. It is synthesized in the liver and intestine.
- Apo A-II, second important component of HDL, present on approximately 2/3 of all HDL particles.

- ApoB - major structural component of chylomicrons, VLDLs, IDLs, and LDLs. Usually apoB-48 is present in chylomicrons & apoB-100 is present on VLDL, IDL or LDL. ApoB-100 is synthesized by the human liver. ApoB-48 is synthesized by the intestine.
- ApoE seen in chylomicrons, VLDL, and IDL. They play a major role in the metabolism and clearance of triglyceride-rich particles.
- C-series (apoC-I, apoC-II, and apoC-III).

Lipoprotein Metabolism:

Efficient transport of dietary lipids is carried out by the exogenous pathway of lipoprotein metabolism, through which the dietary triglycerides and cholesterol are delivered to various organs for utilization and storage.[17]

The dietary lipids are packed in the cells of the small intestine into chylomicrons - which have a low cholesterol content & high triglycerides. Then these chylomicrons are secreted into the intestinal lymph and delivered directly to the systemic circulation through the thoracic duct. Chylomicron particles in systemic circulation are then acted upon by the enzyme lipoprotein lipase which is attached to the

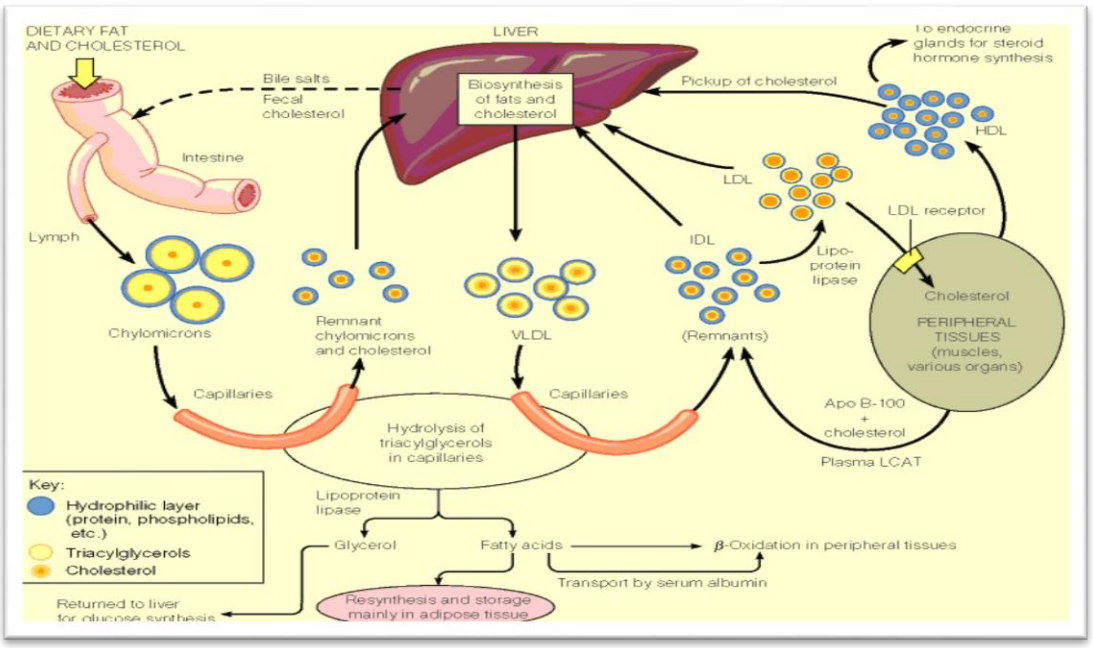
endothelial surfaces of capillaries of various tissues like adipose tissue, heart, and skeletal muscle.

The lipoprotein lipase hydrolyses the triglycerides of chylomicrons and release the free fatty acids. The released free fatty acids are taken up by adjacent myocytes or adipocytes and can be either oxidized to generate energy or can be reesterified and stored as triglyceride.

After the hydrophobic core is hydrolyzed, the triglycerides are distributed and the apolipoproteins are transferred to HDL the chylomicrons progressively become smaller and form chylomicron remnants.

These cholesterol rich chylomicron remnants then bind to apolipoprotein E receptor present on the liver and endocytosed to serve as a dietary source of cholesterol.

LIPOPROTEIN METABOLISM[18]



ENDOGENOUS PATHWAY[19]

In this pathway the apoB-containing lipoproteins are secreted from the liver and metabolised in peripheral tissues .

In the liver, the synthesized triglycerides and cholesterol esters are packed together with ApoB-100 to form VLDL. The VLDL are rich in triglycerides. During circulation in the blood the lipoprotein lipase hydrolyses the triglycerides of VLDL. This hydrolysis takes place in muscle, heart, and adipose tissue to release free fatty acids and glycerol.

After hydrolysis the VLDL remnants become intermediate density lipoproteins. The intermediate density lipoproteins contain equal amounts of cholesterol and triglyceride.

The intermediate density lipoproteins are taken up by the the liver and are acted by hepatic lipase to form low density lipoproteins. This process consists of hydrolysis of the triglycerides and transfer of all apolipoproteins except apoB-100 to other lipoproteins. The low density lipoproteins contain a relatively high cholesterol content and are atherogenic.

The cholesterol in LDL is responsible for more than one-half of the plasma cholesterol. During circulation nearly 70% of circulating LDL is cleared by liver by LDL receptor–mediated endocytosis. The absorbed LDL is hydrolysed within the lysosomes releasing cholesterol.

Another lipoprotein similar to LDL in lipid and protein composition is Lipoprotein(a). It contains apolipoprotein(a), an additional protein. The site of synthesis of apoA is the liver. ApoA is attached to apoB-100 by a disulfide linkage. The Lp(a) is cleared in the liver.

Role of LDL cholesterol in atherogenesis:

The term atherosclerosis is derived from Greek word for “gruel” and “hardening”. It a disease process charecterized by atheromas that protrude into the vascular lumen.[20]

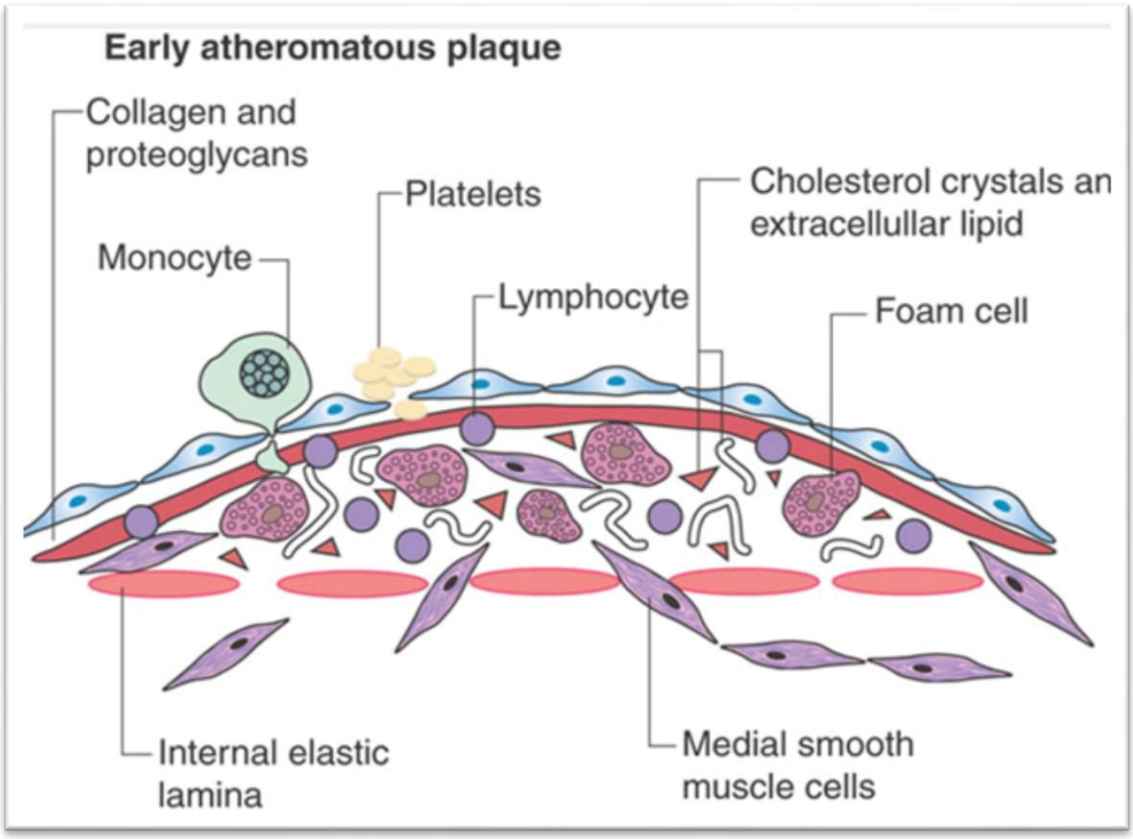
These atherosclerotic plaques obstruct the blood flow and may weaken the underlying media and rupture to cause acute catastrophic events.

Major risk factors:

- Non modifiable
    1. Higher age
    2. Male
    3. Family history of atherosclerosis
    4. Genetic abnormalities
- Modifiable
    1. Hyperlipidemia
    2. Hypertension
    3. Cigarrette smoking
    4. Diabetes mellitus
    5. C-reactive protein.

Of the major modifiable risk factors hyperlipidemia is significant in that it can initiate atherosclerosis even when present as a single risk factor.

STRUCTURE OF EARLY ATHEROMATOUS PLAQUE[21]



The plaque consists of a superficial fibrous cap which is composed of smooth muscle cells and dense collagen. There is a cellular region with more macrophages, T cells and smooth muscle cells below the fibrous cap. Below the cap is the necrotic core consisting of lipids like cholesterol and cholesterol esters, dead cell debris, organized thrombus and proteins.

Role of hyperlipidemia in atherosclerosis

The excess of LDL cholesterol in blood

1. Increases local production of reactive oxygen species which impairs endothelial cell function, accelerates decay of NO and increases local shear stress.
2. Accumulates in the intima and are oxidized to form oxidized LDL. The oxidized LDL is ingested by macrophages to form foam cells.
3. Stimulates the release of growth factors, cytokines & chemokines and increase monocyte recruitment.[22]

High Density lipoproteins:

High density lipoproteins are antiatherogenic as they help in the removal of cholesterol from the artery wall. They also inhibit the oxidation of LDL cholesterol.

HDL is synthesized and secreted from the intestine and the liver. The apolipoproteins - apoC and apo E are also synthesized from the liver. Initially the HDL particle consists of phospholipid bilayers and apoA and free cholesterol. The cholesterol which is effluxed from the cells is accepted by the HDL through the class B scavenger receptor and transported to liver for excretion. This process is called the reverse cholesterol transport.

Another important mechanism for cholesterol transport is through the ATP-binding cassette transporter A1 and G1 which help in the transport of cholesterol from the cells to the HDL.

HDL concentrations are reciprocally related to the plasma triacylglycerol concentrations and are inversely related to the incidence of atherosclerosis. This is because the HDL levels reflect the efficiency of reverse cholesterol transport.

DISORDERS OF LIPOPROTEIN METABOLISM:

Dyslipidemias that result from mutations of genes that encode defective apoproteins (defective apoB-100, apoC-II, apoA-V) or alter the lipoprotein receptors like LDL receptors on cells are called primary dyslipidemias or primary hyperlipoproteinemias.

Primary Hyperlipoproteinemias:

Fredrickson and Levy classified primary hyperlipoproteinemias into five types according to the type of lipoprotein particles that accumulate in the blood.

CLASSIFICATION OF PRIMARY HYPER LIPOPROTEINEMIAS[23]

Phenotype	Generic Designation	Elevated Lipoprotein Class	Elevated Lipid Class	Primary Genetic Disorders
I	Exogenous hyperlipemia	Chylomicrons	Triglycerides	Familial lipoprotein lipase deficiency Familial apolipoprotein C-II deficiency Unclassified
II-a	Hypercholesterolemia	LDL	Cholesterol	Familial hypercholesterolemia Familial combined hyperlipidemia Polygenic hypercholesterolemia
II-b	Combined hyperlipidemia	LDL, VLDL	Cholesterol, Triglycerides	Familial combined hyperlipidemia Unclassified
III	Remnant hyperlipidemia	β-VLDL	Triglycerides, Cholesterol	Familial dysbetalipoproteinemia Unclassified
IV	Endogenous hyperlipemia	VLDL	Triglycerides	Familial hypertriglyceridemia (mild) Familial combined hyperlipidemia Sporadic hypertriglyceridemia Tangier disease
V	Mixed hyperlipemia	VLDL, Chylomicrons	Triglycerides, Cholesterol	Familial hypertriglyceridemia (severe) Familial lipoprotein lipase deficiency Familial apolipoprotein C-II deficiency

Other primary Disorders of lipoprotein metabolism:

- Familial defective ApoB-100(FDB)
- Autosomal Dominant hypercholesterolemia due to mutations in Pcsk 9 (ADH-Pcsk9 or Adh 3)
- Autosomal recessive Hypercholesterolemia
- Sitosterolemia
- Polygenic hypercholesterolemia
- Apo –V deficiency
- Hepatic lipase deficiency.

Secondary Hyperlipoproteinemias:

Abnormal levels of lipoproteins occuring secondary to a variety of diseases are called secondary hyperlipoproteinemias.

Obesity:

Obesity is associated with more adipocyte mass and decreased insulin sensitivity. This results in

- Elevation in VLDL and Triglycerides
- Elevated free fatty acids
- Low levels of HDL

Diabetes Mellitus:

Diabetes Mellitus is a common cause of dyslipidemia. The associated abnormalities include

- Increased small dense LDL
- Increased VLDL
- Decreased HDL
- Increased triglycerides
- Enhanced oxidation of LDL
- Increased triglyceride rich lipoprotein resulting from decreased lipoprotein lipase activity.

Lipodystrophy

Lipodystrophy is associated with profound insulin resistance and elevated plasma levels of VLDL and chylomicrons. Partial lipodystropy can also present with dyslipidemia.

Thyroid Disease

In hypothyroidism there is reduction in hepatic LDL receptor function and delayed clearance of LDL. As a result there is elevation of plasma LDL, increased levels of circulating IDL and mild hypertriglyceridemia. In hyperthyroidism the plasma levels of LDL-C are reduced.

Renal Disorders

- In renal failure there is more triglyceride lipolysis and reduced clearance of remnants.
- Nephrotic syndrome is associated with pronounced mixed hyperlipoproteinemia which is due to
    - Increased hepatic production of VLDL
    - Decreased clearance of VLDLs
    - Increased LDL production

- End stage renal disease is associated with mild hypertriglyceridemia (less than 300 mg/dL).This due to the accumulation of VLDLs and remnant lipoproteins.
- In renal transplants hyperlipoproteinemia occurs due to the effect of the drugs administered for immunosuppression (cyclosporine and glucocorticoids)

Liver Diseases:

Liver is the principal site of lipoprotein metabolism. Hence liver diseases are associated with abnormalities in plasma lipid levels.

- Hepatitis is associated with mild to moderate hypertriglyceridemia due to increased VLDL synthesis
- Severe hepatitis and liver failure often leads to major reductions in cholesterol and triglycerides.
- Cholestasis is associated with severe hypercholesterolemia.

Alcohol

Alcohol consumption is associated with increase in plasma triglyceride levels. It also increases hepatic secretion of VLDL. Few studies show that regular alcohol use in smaller amounts raises the HDL.

Estrogen

Increased VLDL and HDL synthesis is seen with estrogen administration leading to rise in plasma levels of both triglycerides and HDL-C.

To minimize the effect of exogenous estrogen on lipids, low-dose preparations of estrogen or the estrogen patch can be used.

Lysosomal Storage Diseases

Cholesteryl ester storage disease (due to deficiency in lysosomal acid lipase) & glycogen storage diseases like von Gierke's disease are rare causes of secondary hyperlipidemias.

Cushing's Syndrome

Glucocorticoid excess seen in Cushing’s syndrome is associated with

- Increased VLDL synthesis,
- Hypertriglyceridemia,
- Mild rise in LDL.

Drugs associated with hyperlipidemia:

Significant alterations in lipid profile may occur secondary to many drugs. Commonly implicated drugs include

- Thiazide diuretics
- Atypical antipsychotics(Clozapine,Olanzepine)
- Cyclosporine
- Corticosteroids
- Androgens
- Betablockers
- Retinoids
- Protease inhibitors
- Estrogen
- Progesterone

DIAGNOSIS

The recommendations of NCEP ATPIII guidelines are that all adults ≥ 20 years of age should undergo lipid screening.[24] The screening includes a total lipid profile including total cholesterol, LDL cholesterol, triglycerides and HDL cholesterol which should be repeated every 5 yrs.

Patients usually have a primary or genetic cause for their lipid disorder with secondary factors contributing to the hyperlipidemia.

The first step in management is to determine the class or classes of lipoproteins that are increased or decreased in the patient. After classifying the hyperlipidemia, possible secondary causes of the hyperlipidemia should be ruled out.

The family history, drug history and diet history should be noted and physical examination should be done to rule out secondary causes like diabetes, hypothyroidism, nephrotic syndrome or liver disease.

Investigations to be done include

- urinanalysis
- serum thyroid stimulating hormone
- serum alkaline phosphatase
- glycosylated hemoglobin

The plasma lipoprotein abnormalities are firmly established risk factors of atherosclerosis and hence should be treated aggressively.

The following table lists the risk factor recognized by the **current (Adult treatment panel III) guidelines.**

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Risk factors for ASCVD (modified from ATP III)

Underlying risk factors
- Obesity
- Disinclination to exercise
- Atherogenic diet

Major (or traditional) risk factors
- High LDL cholesterol
- Low HDL cholesterol (< 40 mg/dL)
- Diabetes
- Smoking
- Hypertension (≥ 140/90 mm Hg or on BP medication)
- Family history of premature CAD in first-degree relative (male < 55 years old, female < 65 years old)
- Age ≥ 45 years old for men, ≥ 55 years old for women
- Male sex

Emerging risk factors
- Metabolic syndrome
- Triglycerides
- Lp(a)
- Lp-PLA$_2$
- Remnant lipoproteins
- Small, dense LDL
- Fibrinogen
- Homocysteine
- Urine microalbumin/creatinine ratio
- High-sensitivity CRP
- Impaired fasting glucose (110-125 mg/dL, per ATP III, or 100-125 mg/dL per American Diabetes Association)
- Measures of subclinical ASCVD
  - -Ankle brachial index
  - -Exercise testing (with or without nuclear imaging)
  - -Electron-beam tomography
  - -MRI
  - -Carotid intimal medial thickness
  - -Carotid ultrasound

According to the ATP III guidelines the patients are classified into one of the treatment strata according to the quantitative estimate of risk[24].

UPDATED ATP III GOALS AND CUT POINT FOR THERAPY[25]

Risk Category	LDL-C (mg/dL)		
	Goal	Initiation Level for TLC	Consideration Level for Drug Therapy
High risk: CHD or CHD risk equivalents (10-yr risk >20%)	<100 (optional: <70)	≥100	≥100 (<100: consider drug options)
Moderately high risk: 2+ risk factors (10-yr risk 10–20%)	<130 (optional: <100)	≥130	≥130 (100–129: consider drug options)
Moderate risk: 2+ risk factors (10-yr risk <10%)	<130	≥130	≥160
Lower risk: 0–1 risk factor	<160	≥160	≥190 (160–189: LDL-C–lowering drug optional)

The first step to achieve the LDL goal involves lifestyle changes which includes specific diet and exercise recommendations.

Diet

Dietary modification is an important aspect in managing dyslipidemia

- Restriction of saturated fat and cholesterol in patients with elevated LDL levels.

- Restriction of simple carbohydrates for individuals with hypertriglyceridemia.
- Restriction of total fat intake for severe hypertriglyceridemia.
- Plant sterol and sterol esters reduce plasma LDL-C levels by 10% when taken three times per day .
- The addition of psyllium, soy protein, or Chinese red yeast rice[26] to the diet.

Weight Loss and Exercise

Weight reduction is associated with fall in plasma triglyceride and LDL-C levels and increase in HDL levels.

Regular aerobic exercises can also have positive change on lipids in large measure due to the associated weight reduction.

Pharmacologic Treatment

In addition to therapeutic life style changes, drug therapy is usually initiated to achieve the LDL goals.

Major classes of available drugs are

Statins	Lovastatin, Pravastatin, Simvastatin, Fluvastatin, Atorvastatin, Rosuvastatin
Bile Acid Sequestrants	Cholestyramine , colestipol ,colesevelam
Fibrates	Gemfibrozil ,Fenofibrate ,Clofibrate
Nicotinic acid.	Niacin,acipimox
Cholesterol absorbtion inhibitors	Ezetimibe
Cholesterol ester transfer protein inhibitors	Torcetrapib

Phytosterols	

Statins are preferred usually for LDL reduction. Depending on the baseline LDL level, the starting dose of statin can be initiated and response checked in about 6 weeks and the dose of statin can be raised if necessary.

Other lipid risk factors are low HDL cholesterol, elevated triglycerides and small LDL particles. This lipid triad is called atherogenic dyslipidemia.

Atherogenic dyslipidemia occurs as a component of metabolic syndrome.In such conditions non – HDL cholesterol (Triglycerides, VLDL cholesterol and LDL cholesterol) represents a secondary target of therapy[27].

If after 6 weeks of therapy LDL goal is achieved and when Non HDL cholesterol goal is not achieved we can add a fibrate or nicotinic acid for triglyceride lowering[28].

Statins (HMG-CoA Reductase Inhibitors)

Statins are competitive inhibitors of the HMG-CoA reductase enzyme which is the rate limiting step in cholesterol biosynthesis.

Available statins are Mevastatin, Lovastatin, simvastatin, Pravasatatin, Fluvastatin, Atorvastatin and Rosuvastatin.

ATORVASTATIN

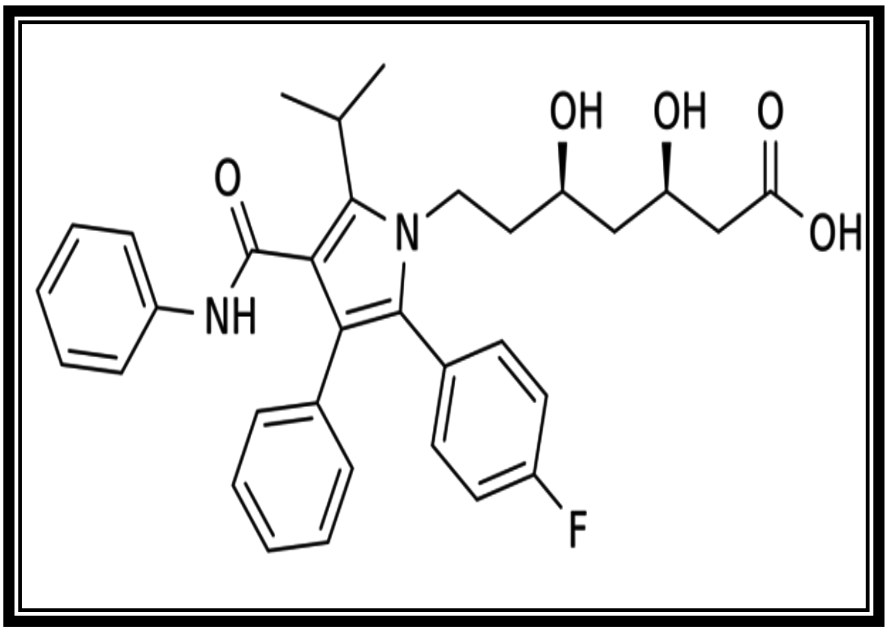
Atorvastatin was discovered by Bruce D Roth, an American chemist in 1985. Unlike other statins it is a completely synthetic compound[29].

Chemical Data:

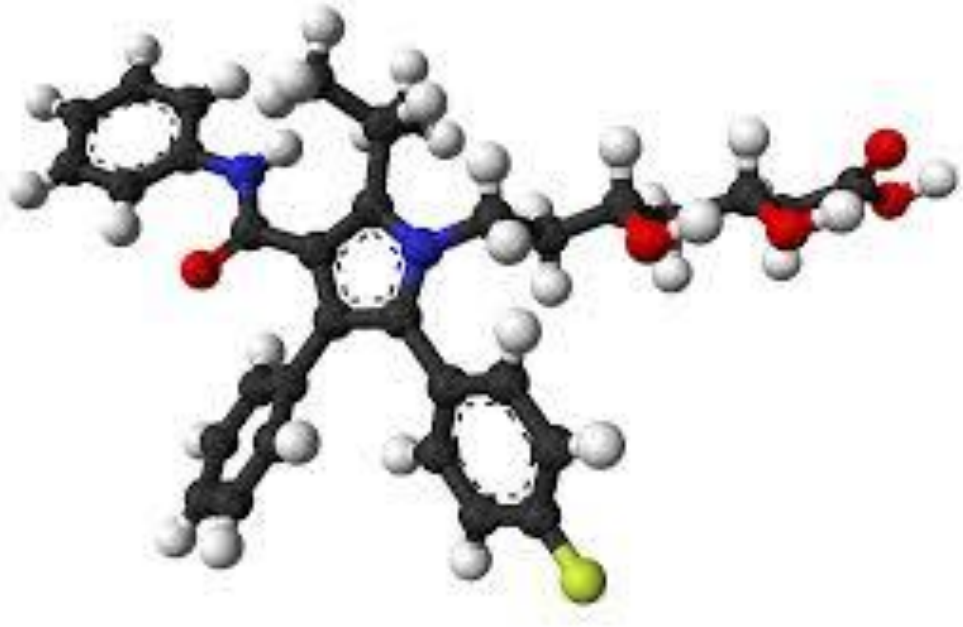
Formula	:	$C_{33}H_{35}FN_2O_5$
Molecular mass	:	558.64g/mol

Physico chemical properties:

Atorvastatin is a hydrophobic compound, administered in the open ring form**.** It contains a mevalonic acid like moiety in its structure which is responsible for inhibition of the enzyme HMG CoA reductase.Its chemical structure is as follows



3D-Ball and Stick model of the Atorvastatin molecule

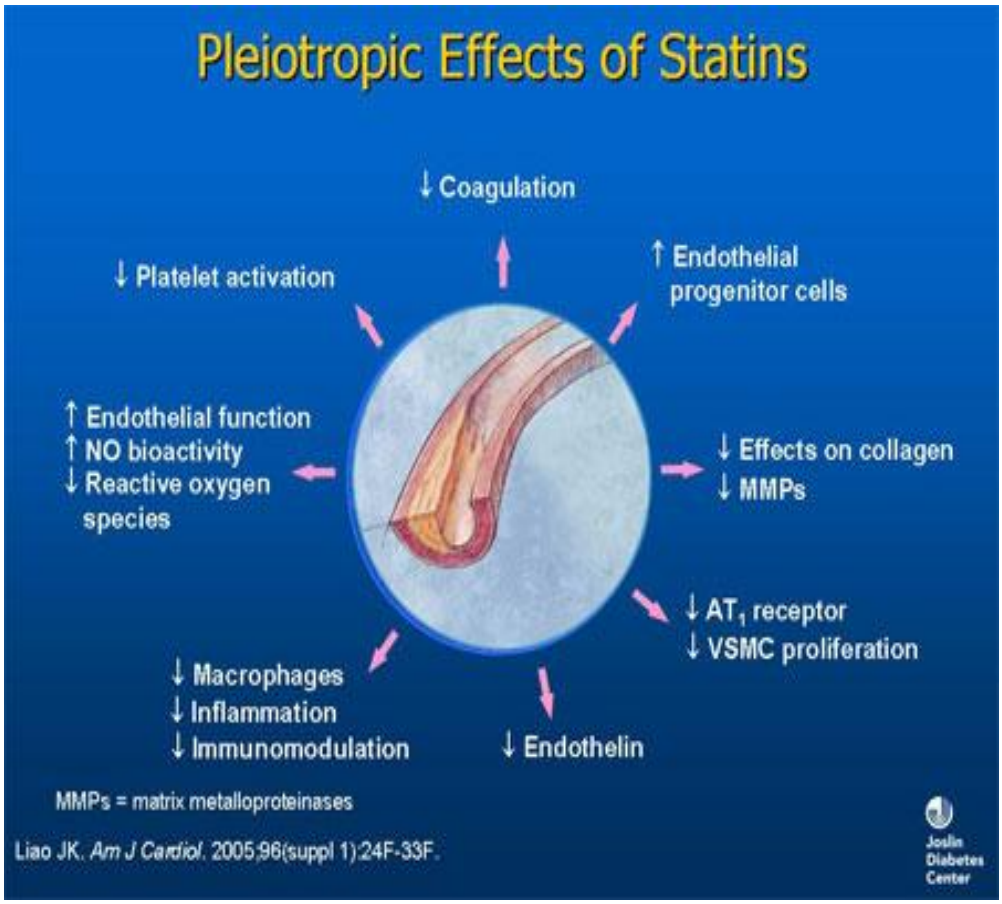


Mechanism of action[30]:

The rate limiting step in hepatic cholesterol biosynthesis is the reduction of 3-hydroxy-3-methylglutaryl-coenzyme A to mevalonate which is catalyzed by HMG-CoA reductase. Inhibition of this enzyme reduces denovo cholesterol synthesis and increases the expression of low density lipoprotein receptors on hepatocytes. This results in increased LDL uptake in the hepatocytes and reduction in LDL levels in blood. In addition atorvastatin also causes reduction in triglyceride levels and slight rise in HDL levels.

Pleiotrophic effects of Statins:

Mevalonate, the product of HMG - CoA reductase reaction, is the precursor of cholesterol and also many other nonsteroidal isoprenoidic compounds. Hence inhibition of this key enzyme may result in pleiotropic effects[30].



The term statin pleiotrophy encompasses the non LDL mediated effects of statin treatment.[31]

Currently believed nonlipid-related pharmacological properties of statins include:

- Halting of atherosclerosis by the inhibition of macrophage and smooth muscle growth and plaque stabilization (ACS implications)
- Increased post transcriptional endothelial nitric oxide (NO) synthase expression and bioavailability (cardiovascular and cerebrovascular implications), an ability to recruit endothelial progenitor cells (important in repair of ischemic injury), decreased endothelin-1 expression, and improved endothelial dysfunction secondary to injury (implications in ACS, diabetes)
- Direct and indirect antioxidant effects, decreased reactive oxygen species
- Improved thrombogenic profile, enhanced expression of tissue plasminogen activator and platelet activator inhibitor-1
- Anti-inflammatory effects; reduction of inflammatory cytokines, chemokines, adhesion molecules, and C-reactive protein (CRP) molecules , inhibition of a variety of signaling proteins (implications in the treatment of bacterial infections, sepsis)
- Inhibition of lymphocyte growth and other blood mononuclear cells (implications for leukemia)

- Immunosuppressive activity, linked to inhibition on promoter IV of major-histocompatibility class II (MHC-II) transactivating factor, leading to suppression of T-lymphocyte activation (implications of statins as immunomodulators and applicability in organ transplant)
- Inhibition of cardiac hypertrophy, using rat animal models, in vivo cardiac hypertrophy induced by angiotensin II infusion (simulating HTN) or by transaortic constriction was inhibited by simvastatin administration over four weeks

Other beneficial effects of statins:

- Mevalonate plays a key role in cell proliferation and thus selective inhibition of HMG Co A reductase can lead to a new chemotherapy for cancer disease.
- Recent experiments have shown that mevalonate pathway plays an important role in murine and osteoclast formation and bone resorption. Subjects with hyperlipidemia with increased risk of osteoporosis could benefit from statin therapy.

Pharmacokinetics

Absorbtion

Atorvastatin undergoes rapid absorbtion when taken orally and peak plasma level occurs at 2.5 hrs. Bioavailability of atorvastatin is 12% and their hepatic metabolites varies between 5% and 30% of administered doses.

Distribution

In the plasma, >95% of Atorvastatin and their metabolites are protein bound.

Metabolism

Atorvastatin is extensively metabolized by cytochrome P450, CYP3A4 hydroxylation to form active ortho and para hydroxylated metabolites which accounts for about 70% of the circulating HMG CoA reductase inhibitory activity.

Excretion

Atorvastatin and its metabolites are eliminated primarily in bile following hepatic or extra hepatic metabolism. There is no enterohepatic circulation. Less than 2% of a dose of Atorvastatin is recovered in urine following oral administration.

Uses

- Primary and secondary dyslipidemias
- Primary prevention of cardiovascular events according to ATP III guidelines
- Secondary prevention of myocardial infarction, stroke & unstable angina in patients with established coronary heart disease
- Myocardial infarction and stroke prevention in type II Diabetes Mellitus

Dosage forms

Available as 10mg, 20 mg, 40 mg tablets and in combination with other lipid lowering drugs.

Untoward effects

Major

1. Severe myopathy and rhabdomyolysis are rare (<1%) but life thereatening side effects. Severe myopathy can usually be avoided by careful patient selection, avoidance of interacting drugs and by instructing the patient to contact the physician immediately in the event of unexplained muscle pain.

In the event of muscle symptoms, the plasma creatine kinase (CK) level should be obtained to document the myopathy. Serum CK levels need not be monitored on a routine basis in patients taking statins and an elevated CK in the absence of symptoms does not predict the development of myopathy and does not necessarily suggest the need for discontinuing the drug.

The risk of statin-associated myopathy is increased in older age, frailty, renal insufficiency and by coadministration of drugs that interfere with the metabolism of statins such as erythromycin and related antibiotics, antifungal agents, immunosuppressive drugs, and fibric acid derivatives (particularly gemfibrozil).

2. Elevation in liver transaminases [alanine (ALT) and aspartate (AST)] may also occur (<1%) with therapy.

Liver enzymes should be checked before starting therapy, at 2–3 months, and then annually. Substantial (greater than three times the upper limit of normal) elevation in transaminases is relatively rare and mild-to-moderate (one to three times normal) elevation in transaminases in the absence of symptoms need not mandate discontinuing the medication.

Severe clinical hepatitis associated with statins is exceedingly rare, and the trend is toward less frequent monitoring of transaminases in patients taking statins. The statin-associated elevation in liver enzymes resolves upon discontinuation of the medication.

Other common side effects include

- Dyspepsia,
- Headaches,
- Fatigue, and
- Muscle or joint pains.

Drug interactions

Inhibition by other drugs of OATP1B1, which transports several statins into hepatocytes, and inhibition or induction of CYP3A4 by a variety of pharmacological agents provide rationales for drug-drug interactions involving statins

Most commonly statin interacts with

- fibrates, especially gemfibrozil (38%),
- cyclosporine (4%),
- digoxin (5%),
- warfarin (4%),
- macrolide antibiotics (3%),

- mibefradil (2%), and
- azole antifungals (1%) .

Other drugs that increase the risk of statin-induced myopathy include niacin, HIV protease inhibitors, amiodarone, and nefazodone .

Use in specific populations:

Geriatric Use

Atorvastatin metabolism appears to be slower in the elderly resulting in greater bioavailability and longer half life, however no dose adjustment is necessary.

Renal Dysfunction

Kidney plays no role in the disposition of atorvastatin, dosage adjustment in patients with renal impairment is not necessary.

Hepatic impairment

Patients with hepatic disease may not be able to clear the drug effectively, hence atorvastatin is contraindicated in active liver disease.

BIOTIN

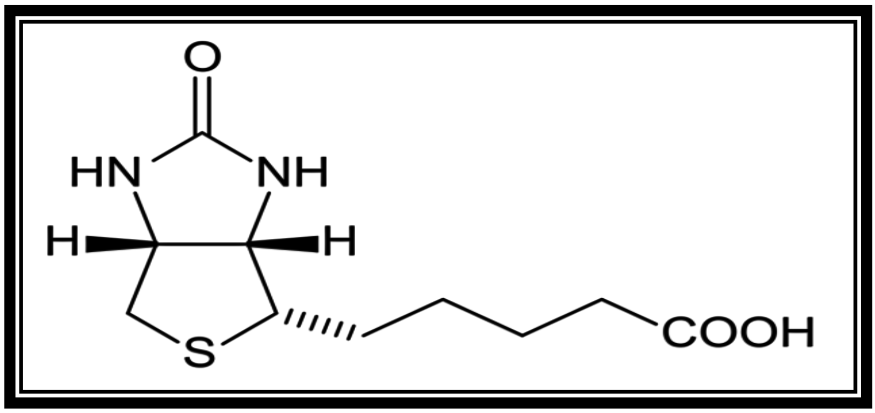
Synonyms:

Vitamin H, Coenzyme R, Vitamin B7.

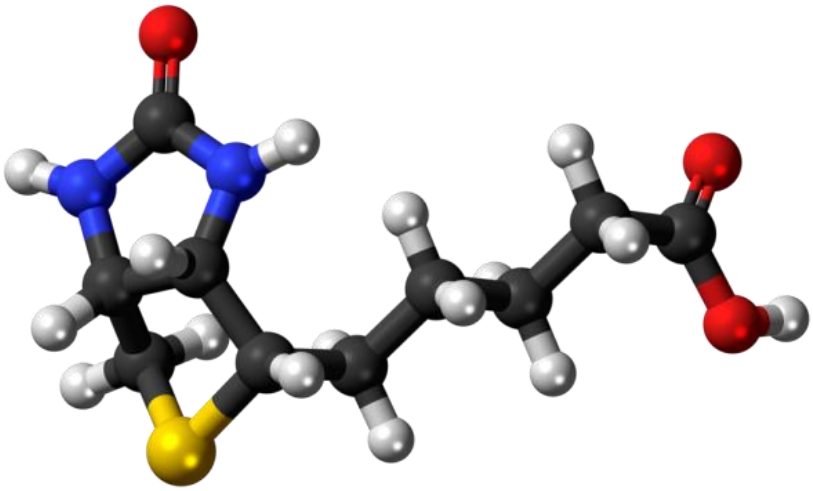
Biotin is a water soluble B - complex vitamin discovered around 70 years ago. Other name of biotin is vitamin H, the H representing “Haar and Haut”, which means “hair and skin” in German. It was originally identified as a growth factor in yeast.[32]

Chemical structure:

Chemically Biotin is *cis*-tetra hydro-2-oxothienol[3,4-d]-imidazoline-4-valeric acid. It consists of a tetrahydrothiophene ring fused with an ureido (tetra hydro imidizalone)ring. The ureido ring and the ionizable carboxyl group of biotin allow modest solubility of the white crystalline solid in aqueous solution, especially at an alkaline pH. Its chemical structure is as follows.



3D BALL AND STICK MODEL OF BIOTIN



Dietary sources:

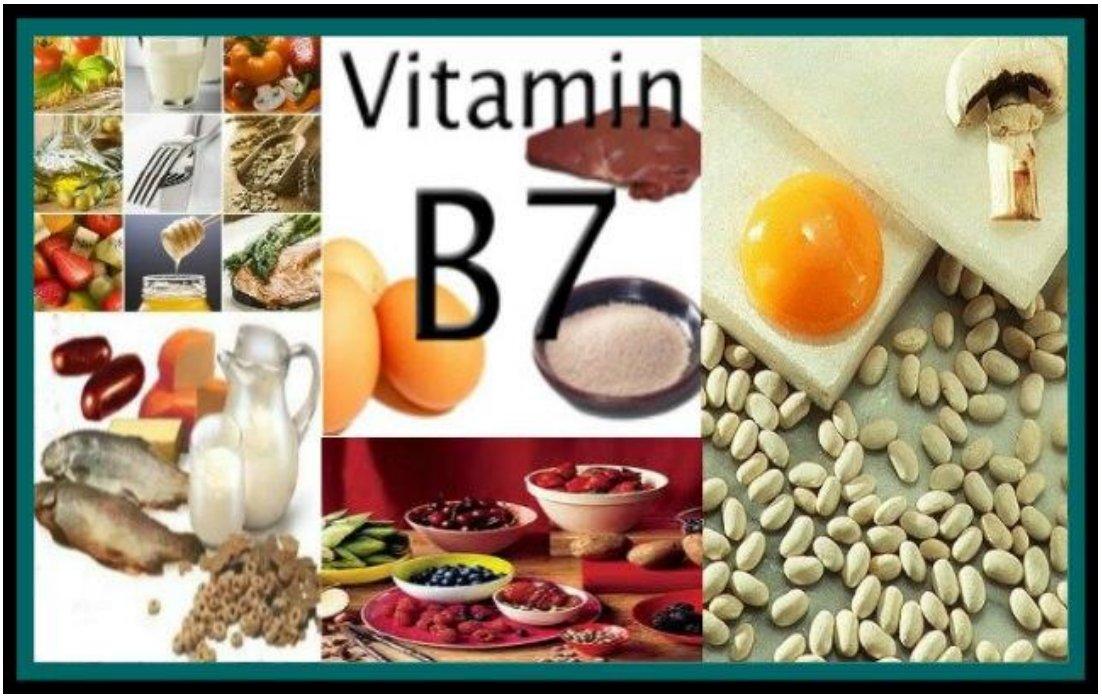
Biotin cannot be synthesized as such by humans and mammals and has be obtained from diet or synthesised by intestinal bacteria.

Good sources of biotin are

- seafood, red meat, liver, pork and beef.
- Egg yolks,milk,curd.
- Cauliflower, lettuce, cucumber and spinach.
- Fruits like banana, avocado, cranberries, strawberries,raspberries.
- Soy products, peanut.
- Cashews, almonds, walnuts and sunflower seeds.

Cereal grains , fruits , most vegetables and meat are poor sources .

SOURCES OF BIOTIN



Absorbtion , Transport , Metabolism and Excretion[33]:

Biotin in the diet is largely protein bound and digestion of these proteins by gastrointestinal enzymes produces biotinyl peptides, which may be further hydrolysed by intestinal biotinidase to release biotin.

Avidin, a protein found in raw egg whites, binds biotin tightly and prevents its absorbtion. The peptide biocytin (€-N-bitoinyl lysine) is resistant to hydrolysis by proteolytic enzymes in the intestinal tract but together with Biotin is readily absorbed.

A Biotin carrier, the sodium dependant multivitamin transporter (SMVT) for which pantothenic acid and lipoate compete is present in the brush border membrane of intestines. This helps in the transport of biotin against a sodium ion concentration gradient. The enzyme biocytinase (biotin amidohydrolase) in plasma and erythrocyte catalyzes the hydrolysis of biocytin to yield free biotin. The clearance of Biotin is more rapid in deficient individuals. During circulation the free biotin is taken up by liver, kidney and muscle and is localized in cytosolic and mitochondrial carboxylases. About half of the absorbed biotin is excreted as the metabolite bisnorbitin, occurring from β-oxidation of the valeric side chain, and biotin sulfoxide. The urinary excretion and fecal excretion of biotin exceeds dietary intake because of microfloral biosynthesis.

Bioavailability:

The bioavailability of biotin is variable. In general it is seen in food in the proteinbound form called biocytin. This bound form should undergo proteolysis prior to absorbtion. In egg whites biotin is bound tightly to avidin which prevents its intestinal absorbtion, when consumed raw. Cooking denatures the avidin thereby assisting free biotin release and facilitating biotin absorbtion. Oxidising agents convert the thioether to sulfoxides and sulfones which donot have biotin activity.

Physiological roles:

Biotin is an essential co-factor for carboxylase enzymes in many biochemical pathways. It acquires a carboxyl substituent in its ureido group and thus functions as CO_2 carries in these reactions. Thus it is essential for cell growth and an important requisite in the synthesis of fatty acids and plays a major role in the metabolism of fats and amino acids.

Studies have proven that biotin regulates gene expression in addition to its role as carboxylase prosthetic group. Biotin has shown to stimulate genes favouring hypoglycemia like the genes coding for insulin, insulin receptor and glucokinase. It also decreases the expression of phosphoenolpyruvate carboxykinase in the liver. PEPCK is the key enzyme of gluconeogenesis. It helps in glucose production by the liver. These findings indicate that biotin is an important vitamin involved in glucose and lipid metabolism. Few studies have proved that biotin deficiency is associated with decreased utilization of glucose and.impaired glucose tolerance.[34] Few more studies have shown that pharmacological doses of biotin are effective in diabetes mellitus.

Another study conducted in a smaller number of subjects have shown that 61.4 micromol /day of biotin significantly reduced the plasma triglyceride level and VLDL levels.

Other applications:

Biotinylation is the process of attachment of the vitamin to different chemical sites. This is utilized in various laboratory techniques in biochemistry. In the study of DNA transcription, DNA replication, protein localization and protein interactions, biotin is now being used. The property of binding very tightly to avidin is also used in different biotechnological applications.

Deficiency:

Biotin deficiency in human beings is a rare occurrence as it is seen in many foods and easily synthesized by intestinal bacteria. It may be seen in severe malnutrition or inborn errors of metabolism like biotinidase deficiency , multiple carboxylase deficiency. Consumption of large quantities of raw egg whites may also cause biotin deficiency.

Low levels of biotin are also seen in those who have undergone partial gastrectomy and in patients with burns, epilepsy, atheletes and old age. Marginal biotin deficiency may occur in pregnancy and lactation.

The common deficiency symptoms are nausea, vomiting, anorexia, glossitis, depression, pallor and a dry scaly dermatitis. Symptoms of severe deficiency include erythematous skin lesions, hypotonia and

ataxia. Children with biotin deficiency present with vomiting, seizures & developmental delay.

Requirements[35]:

•For Men : 30 micrograms/day

•For Women : 30 micrograms/day

•For Pregnancy : 30 micrograms/day

•For Lactation : 35 micrograms/day

Toxicity:

In patients with biotinidase deficiency administration of doses even upto 300 times normal dietary intake was not associated with any adverse events [36].

Laboratory assessment of status:

Biotin is measured in biological samples by microbiological assay, where whole blood is first digested with papain or acid hydrolysis to release free biotin , samples of which are added to a biotin –deficient medium , inoculated with a test organism.

Other methods for measuring unbound biotin include avidin binding assays, where a competitive protein – binding radio assay is setup with H-labelled biotin, and non radioactive enzyme linked sorbent assays, using steptavidin as the binding agent.

Urinary excretion of biotin and 3-hydroxyvaleric acid appears to be a better indicator of biotin status than blood concentrations.[37]

The most sensitive indicator of biotin deficiency is measurement of Lymphocyte propionyl carboxylase measured by an optimized assay.

Reference intervals:

Reference interval values for whole blood biotin by a microbiological method are 0.5 to 2.20 nmol/l , with a mean of 1.31 nmol/l.[38]

Deficiency is considered likely below 0.5 nmol/l.

AIM

To determine the plasma lipid lowering effect of biotin, by comparing the efficacy and side effect profile of the **combination therapy of Biotin and Atorvastatin** with **monotherapy of Atorvastatin.**

PRIMARY OBJECTIVES

1. To compare the percentage change from baseline in LDL cholesterol at
   1^{st}, 2^{nd}, 4^{th} and 6^{th} week between the two groups - control and study group.
2. To compare the percentage change from base line in other lipid parameters at 1^{st}, 2^{nd}, 4^{th} and 6^{th} week between the two groups.

SECONDARY OBJECTIVE

To compare the side effect profile and laboratory parameters between the two groups during the treatment.

EVALUATION OF THE EFFECT OF BIOTIN IN DYSLIPIDEMIA

METHODOLOGY:

This study was conducted among outpatients attending the Hypertension clinic of the Department of Medicine with dyslipidemia eligible for lipid lowering therapy.

STUDY DESIGN

Single centre, prospective, open label, parallel group, comparative study conducted for a period of 12 weeks .

STUDY CENTRE

Hypertension clinic of the Department of Medicine, Government Stanley Medical College and Hospital, Chennai-1.

STUDY POPULATION

Outpatients who visited the hypertension clinic with dyslipidemia eligible for lipid lowering therapy.

SAMPLE SIZE

60 patients

STUDY PERIOD

From March 2013 to February 2014

STUDY DURATION

12 weeks for each patient

STUDY DRUGS

1) Tablet Atorvastatin 10 mg obtained from govt. supply at Stanley Hospital.

2) Tablet Biotin 5 mg (trade name ESSYVIT, Ranbaxy Pharma) purchased from retail outlets.

The batch number, date of manufacturing, bill number from retailer ,date of purchase were entered in the drug treatment form used for each patient.

SELECTION CRITERIA:

- **Inclusion criteria**
  - Age range 45-60 years
  - Both sexes

- Newly diagnosed cases of dyslipidemia with
  - ✓ Plasma total Cholesterol > 200 mg/dl
  - ✓ Plasma LDL levels = 100-189 mg/dl
  - ✓ Plasma triglyceride >150 mg/dl
  - ✓ Plasma VLDL >30 mg/dl
  - ✓ Plasma HDL <40 mg/dl
- Diabetes mellitus (Type 2) or hypertension (systolic Bp<160 mm hg and diastolic Bp<100 mm hg)
- Patients who were willing to give informed consent.

➢ **Exclusion criteria**

- Age less than 45 years or more than 60 years
- Patients with high plasma levels of LDL cholesterol alone or high plasma levels of triglyceride alone.
- Patients with very high levels of plasma total cholesterol (i.e., more than 500 mg/dl) and very high levels of plasma triglyceride (i.e., more than 500 mg/dl) .
- Patients with uncontrolled Diabetes Mellitus (i.e., Fasting Blood Sugar>140 mg/dl).

- Patients with uncontrolled hypertension (i.e., systolic BP >160 mm hg and diastolic BP >100 mm hg).
- Patients with history or clinical evidence of ischaemic heart disease or unstable angina or stable angina or cerebrovascular disease or peripheral arterial disease or neuromuscular disorders.
- Patient with history or laboratory evidence of liver dysfunction.
- Patient with history or clinical evidence of renal dysfunction.
- Patient with metabolic or hormonal disorders.
- History of smoking or alcoholism.
- History of thyroid dysfunction.
- Patients who took antiplatelet drugs.

PROCEDURE:

The study was conducted after obtaining approval from the Institutional Ethics Committee. Patients with Type 2 Diabetes mellitus or Hypertension who visited the Hypertension clinic were explained about the study purpose and procedures.

A total of 80 patients who were willing to participate in this study and willing to give written informed consent were selected for screening.

Written informed consent in their own language was obtained from the selected patients.

SCREENING:

Screening procedure consisted of a detailed medical and drug history, thorough clinical examination followed by laboratory investigations – which included fasting lipid profile, liver and renal function tests.

After screening 20 patients were excluded based on selection criteria.

A total of 60 patients of both sexes and age between 45 to 60 years who fulfilled the selection criteria were recruited for the study.

RANDOMIZATION:

The study subjects were randomly assigned using a computer generated randomization chart to either of the two groups Group A and Group B, each group consisting of 30 patients.

STUDY PROCEDURE:

Each patient was registered as a new Dyslipidemia case and a case record form was maintained for each patient.

DOSAGE REGIMEN

Group A	T.Atorvastatin 20 mg daily after dinner for 6 weeks
Group B	T.Biotin 5 mg +T.Atorvastatin 20 mg daily after dinner for 6 weeks.

At the first visit tablets were given for one week according to the above dosage regimen.

Patients were advised to continue their antihypertensive or antidiabetic medication as before. No special diet instruction was given.

Participants were instructed to come in between if any myalgia or any other adverse event occurred.

All patients were reviewed at the end of 1^{st}, 2^{nd}, 4^{th} and 6^{th} week. At each visit fasting lipid profile was done and plasma lipid levels were noted and adverse event monitoring was also done through out the study period.

After treatment period of 6 weeks the study medications were stopped and participants were referred back to their respective

departments and received Atorvastatin 20 mg daily at bedtime for 6 more weeks. The study participants were followed up till the 12th week. All investigations including plasma lipid profile were repeated at the end of 12th week.

INVESTIGATIONS

HEMATOLOGICAL

- Complete hemogram
    - Hemoglobin percentage
    - Total count
    - Differential count
    - Erythrocyte sedimentation rate
    - Platelet count

BIOCHEMICAL

- Fasting Plasma lipid profile
    - Total Cholesterol
    - LDL
    - Triglycerides
    - VLDL
    - HDL
- Liver function tests
    - SGOT,SGPT

- Renal function tests
    - Blood urea
    - Serum creatinine
- Creatinine phosphokinase

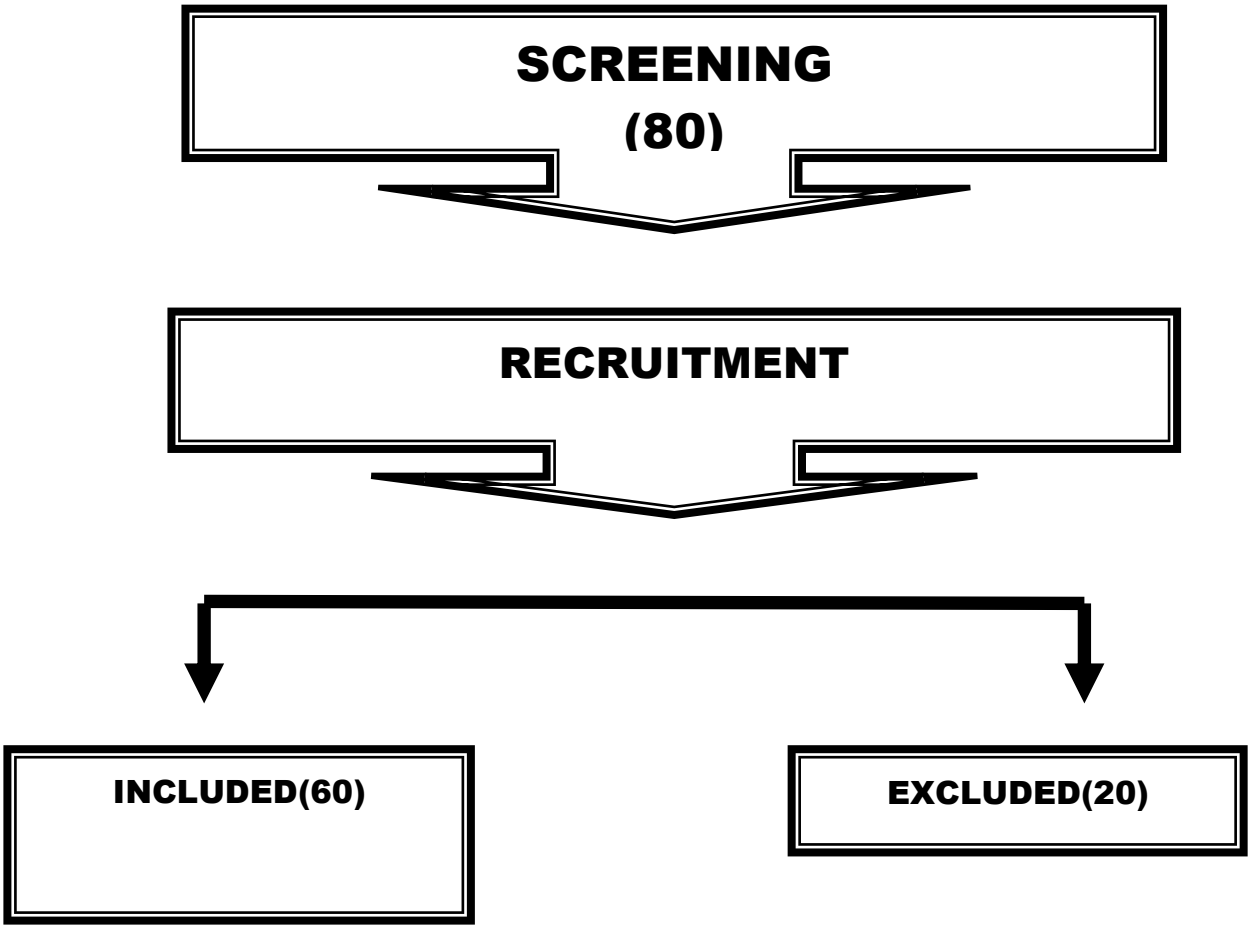
OUTCOME MEASURES

The primary efficacy measure was the percentage reduction in the plasma lipid after the 6 weeks of treatment.

Safety evaluation was based on the spontaneously reported adverse events and the changes in the liver function tests and laboratory values after the study.

STATISTICAL ANALYSIS:

Data was expressed as mean ± standard deviation. Students independant ‘ t’ test was used for comparing quantitative data between the two groups. At the end of the study the effects of Atorvastatin alone and combination of Atorvastatin with biotin on lipid profile was compared in terms of therapeutic efficacy and adverse effects.


SCREENING
(80)
RECRUITMENT
INCLUDED(60)
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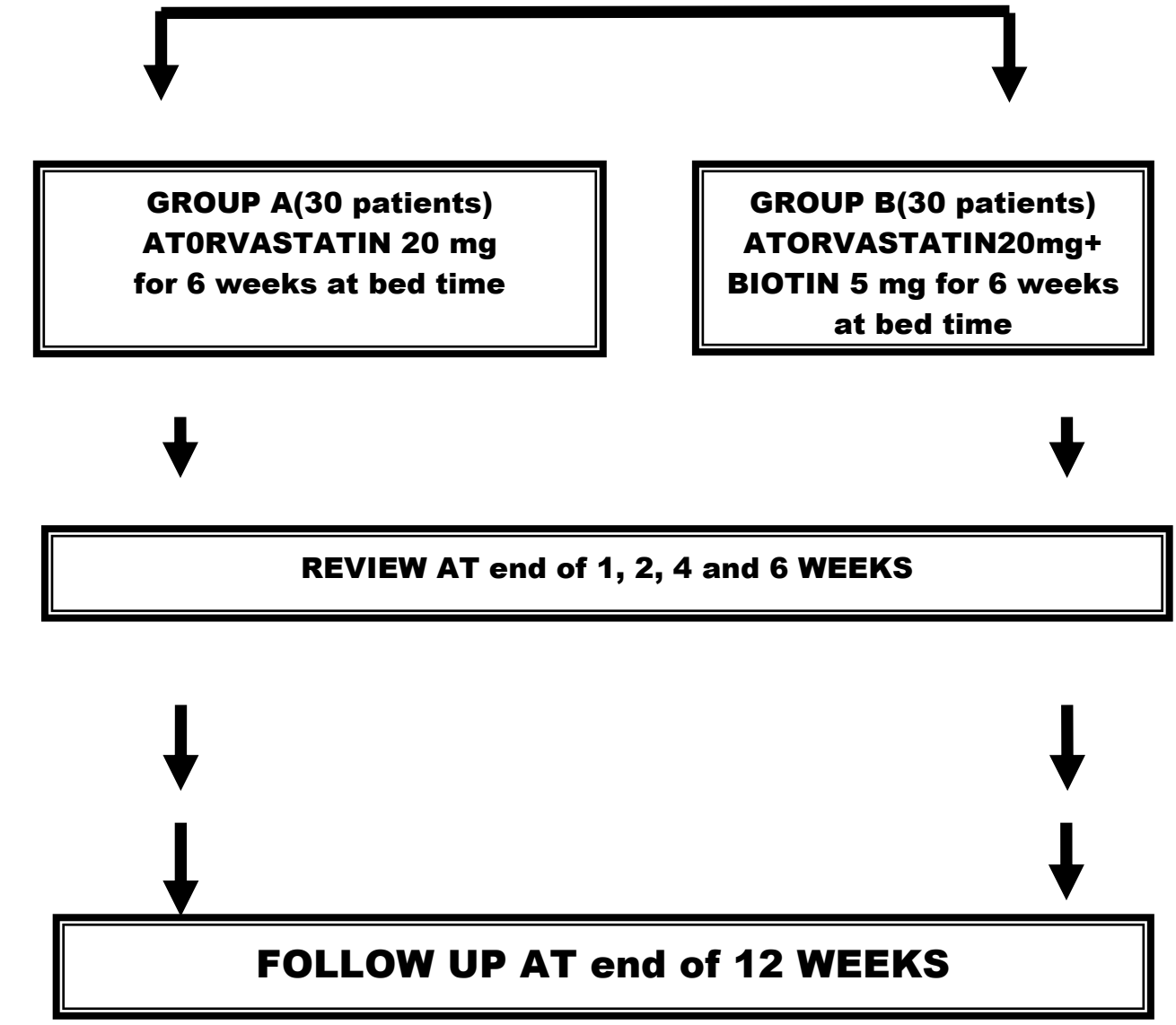


DATA COMPILATION AND ANALYSIS

RESULTS

A total of 80 dyslipidemic patients were selected and screened for the study. Based on the selection criteria, 20 patients were excluded

and the remaining 60 patients were randomly allocated into two groups of 30 patients each.There were no dropouts in each of the groups.

Baseline characteristics of both the groups including the mean age, sex distribution, associated diseases like diabetes mellitus, hypertension and baseline lipid profile were assessed and tabulated.

Plasma lipid profile was repeated at 1^{st}, 2^{nd}, 4^{th} and 6^{th} week of treatment and the percentage change in each of the plasma lipoproteins from baseline was calculated and statistically analysed by students independent 't' test.

After 6 weeks of treatment, Biotin was stopped and both the study and control groups received mono therapy with Atorvastatin in their respective departments for the next 6 weeks .

Plasma lipid profile and laboratory parameters of both the groups was repeated at the end of 12 weeks to find out any delayed effects of Biotin.

Adverse event monitoring was done for the 12 weeks of study period and the adverse events and the additional observed effects were noted and tabulated.

TABLE no. 1: BASELINE CHARACTERISTICS OF THE TWO GROUPS

BASELINE CHARACTERISTICS	CONTROL GROUP(30) MEAN	STUDY GROUP(30) MEAN
1. Mean age in yrs	50.70	50.13
2. Number of males (%) 3. Number of females (%)	13(43.3) 17(57.7)	13(43.3) 17(57.7)
4. Diabetes Mellitus (%)	7(23.3)	8(26.6)
5. Hypertension (%)	28(93.3)	27(90)
BASELINE LIPIDS		
6. Total Cholesterol (mg/dl)	263.76	286.73
7. LDL Cholesterol (mg/dl)	158.06	173.76
8. Triglyceride (mg/dl)	170.03	210.6
9. VLDL Cholesterol (mg/dl)	36.33	41
10. HDL Cholesterol (mg/dl)	46..44	47.33

Table no. 1 shows

- The baseline demographic characteristics and lipid profile of both the groups.
- The incidence of diabetes mellitus was similar in both the groups. Diabetes mellitus was present in **23.3%** of the subjects in the control group *vs.* **26.6%** of the subjects in the study group.
- The incidence of hypertension was similar in both the groups. Hypertension was present in **93.3%** of the subjects in the control group *vs*. **90%** of the subjects in the study group.
- The baseline lipid parameters were
    - The mean total cholesterol of the control group - **263.76mg/dl *vs.* 286.73 mg/dl** in study group.
    - The mean LDL of the control group - **158.06 mg/dl *vs.* 173.76 mg/dl** in study group.
    - The mean triglycerides of the control group - **170 mg/dl *vs.* 210 mg/dl** in the study group.
    - The mean VLDL of the control group - **36.33 mg/dl *vs*.41 mg/dl** in the study group.

- Mean HDL of the control group - **46.44 mg/dl *vs*.47.33 mg/dl** in the study group.

TABLE NO 2 : MEAN AGE OF BOTH GROUPS

	Group	**N**	**Mean**	**Standard. Deviation**	**Student's independent 't' –test**
Age(yrs)	Control	30	50.70	3.131	't' = 0.682 'p'= 0.498 Not significant
	Study	30	50.13	3.298	
** $p \leq 0.010$ it implies Highly Significant, * $p \leq 0.050$ it implies Significant, $p > 0.050$ it implies Not Significant					

FIGURE no: 1 MEAN AGE OF THE TWO GROUPS

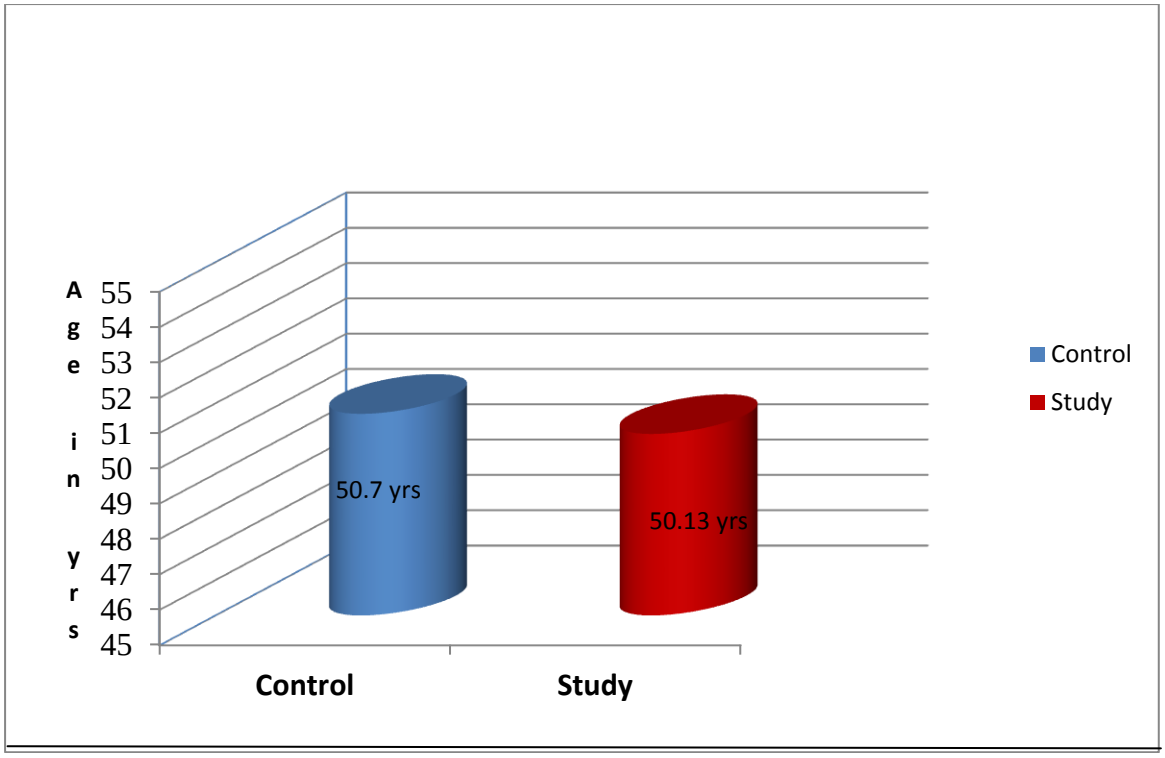


Table no 2 shows

- The comparison of the mean age of the study participants of the two groups - control and study groups.
- The mean age of patients in the control group was **50.7 yrs *vs.* 50.13 yrs** in study group.
- Statistical analysis was done by using student's independent 't' test and 'p' value was not significant p=0.498.
- There was no significant difference in mean age between two groups

Figure no 1 is a graphical representation of the data in table no 1.

- It is a bar graph showing the comparison of mean age of patients in both the groups.

TABLE NO 3 : COMPARISON OF AGE DISTRIBUTION

AGE INTERVAL	CONTROL (30)		TEST (30)	
	NO	%	NO	%
45-49yrs	11	36.66	12	40
50-54yrs	17	56.66	17	56.66
>55 yrs	2	6.66	1	3.33
TOTAL				

	30	100	30	100

FIGURE NO 2

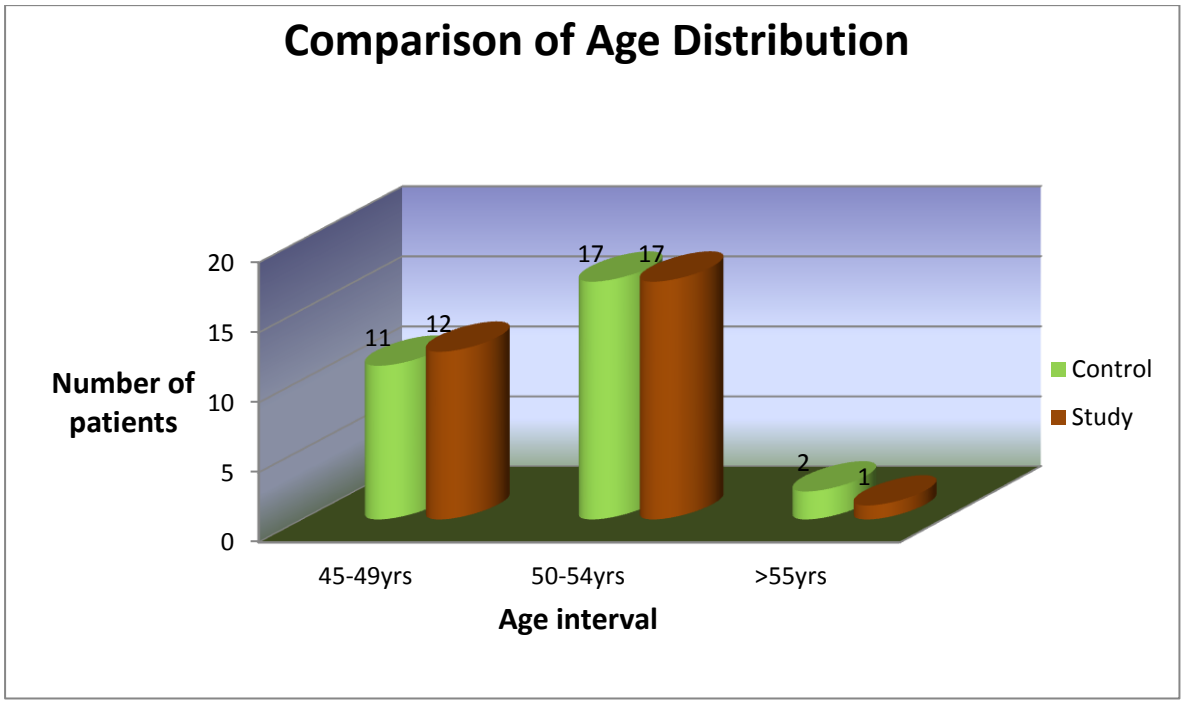


Table no. 3 shows

- The age distribution of patients of the control and study groups..
- In the control group 11(36.6%) patients were in 45-49 yrs age interval vs. 12 (40%) patients in the study group.
- In both the control and study group 17(56.6%) were in the 50-54 yrs age interval.
- In the control group 2(6.66%) patients were in the 55-60 yr age group vs. 1(3.33%) patient in study group.

- Both the study and control groups had comparable age distribution.
- There were more patients in the 50-54 yr age interval.

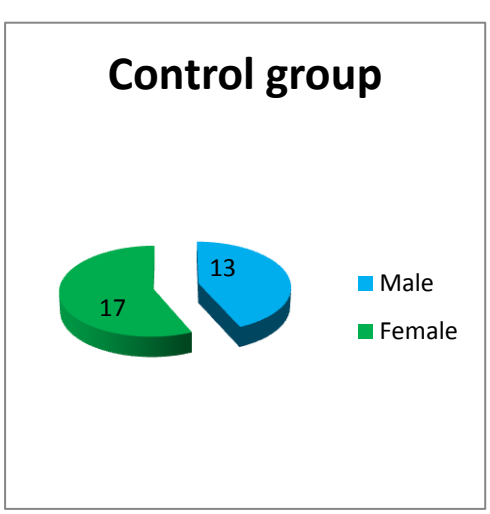
Figure no.2 is a graphical representation of the data of table no.3.It is a bar graph showing the comparison of age distribution of patients in both the groups.

TABLE no 3: GENDER DISTRIBUTION

	GROUP				**PEARSON CHI-SQUARE TEST**
	Control		**Study**		
	Number	**%**	**Number**	**%**	
Male	13	43.3%	13	43.3%	χ^2**=0.00**
Female	17	56.7%	17	56.7%	

Total	30	100	30	100	

FIGURE no 3: COMPARISON OF GENDER DISTRIBUTION



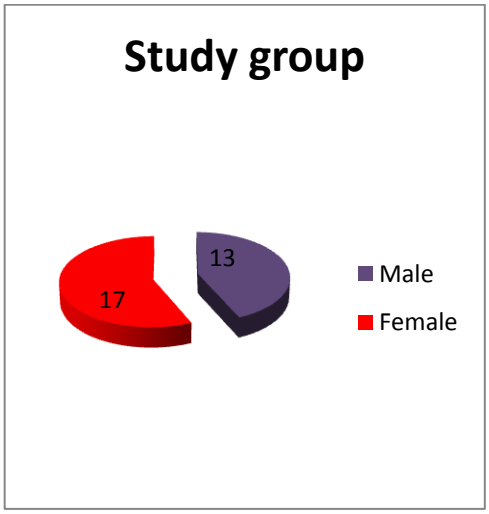


Table no 3 shows

- The gender distribution of patients in both the groups.
- The ratio of male to female in both the groups is13:17.

- Statistical analysis was done by using χ^2 test and the p value was not significant.
- There was a female preponderance in both the groups.
- There was no significant difference in the gender distribution between the two groups.

Figure no 3 is a graphical representation of the data of Table no 3.The gender distribution of the control and study groups are represented as a pie chart.

Table no 4a : Comparison of mean total cholesterol levels in both the groups

	Control	**Study**

	Mean TC level (mg/dl)	% redn	Mean TC level (mg/dl)	% redn
Baseline	263.76	-	286.73	-
1st week	221.00	16.7%	240.4	16.06%
2nd week	194.00	**26.32%**	186.2	**34.66%**
4th week	189.00	**28.33%**	167.46	**41.33%**
6th week	188.00	**28.68%**	169.8	**40.37%**
12th week	191.00	27.56%	208.3	29.96%

Table no 4b: Comparison of % reduction from Baseline in Total Cholesterol between two groups

	Control(% redn)		Study(% redn)		
	Mean	**SD**	**Mean**	**SD**	**'p' value**
Baseline	-	-	-	-	
1stweek	16.7%	5.6	16.06%	4.37	0.623
2nd week	**26.32%**	4.4	**34.66%**	8.52	**0.000****
4th week	**28.33%**	5.05	**41.33%**	7.74	**0.000****
6thweek	**28.68%**	5.56	**40.37%**	9.91	**0.000****
12thweek	27.56%	5.7	26.96%	5.02	0.671

** $p \leq 0.010$ it implies (Highly Significant), * $p \leq 0.050$ it implies (Significant) , $p > 0.050$ it implies Not Significant

Figure no 4: Comparison of % change in Total Cholesterol between two groups

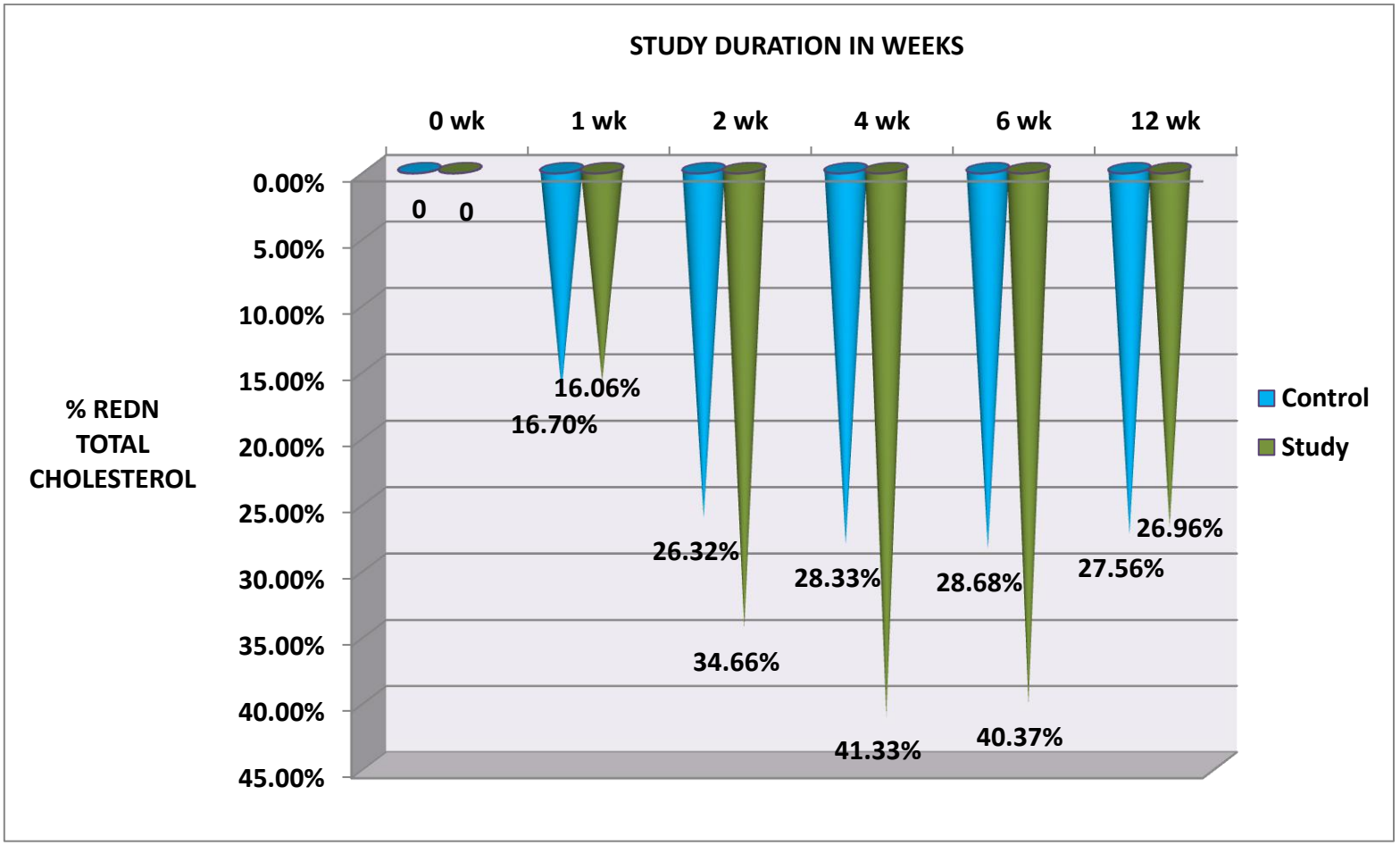


Table no 4a shows

- The mean total cholesterol levels in both the groups and the percentage reduction from the baseline at 1^{st}, 2^{nd}, 4^{th}, 6^{th} &12^{th} week.

Table no 4b shows

- The mean and standard deviation of the percentage reduction in total cholesterol levels. Statistical analysis was done by using students independent 't' test.

- By the first week of study there was16.7% reduction in total cholesterol in control group vs 16.06% in study group, but this difference was not statistically significant p=0.623.

- By second week of study there was **26.32%** reduction in total cholesterol in the control group vs **34.66%** reduction in the study group, the difference in the reduction percentages was highly significant statistically (**p=0.000****).

- At fourth week of study there was **28.33%** reduction in total cholesterol in the control group vs **41.33%** reduction in the total cholesterol in the study group. On statistical analysis the difference in percentage reduction was found to be significant (**p=0.000****).

- At six weeks, there was **28.68%** reduction in total cholesterol in the control group vs.**40.37%** reduction in the study group. On statistical analysis the difference in percentage reduction was found to be statistically highly significant **(p=0.000**).**

- At the follow up 12th week, there was **27.56%** reduction in total cholesterol in the control group vs **26.96%** reduction in total cholesterol in the study group. On analysis this difference was not statistically significant (p=0.671).

Figure no 4 is the column chart representing the data in table no 4b.

Table 5a: Comparison of the mean LDL levels in both the groups

	Control		Study	
	mean LDL level mg/dl	**% redn**	**mean LDL level mg/dl**	**% redn**
Baseline	158	-	173	-
1st week	127	**19.08%**	147	**16.90%**
2nd week	103	**34.35%**	104	**39.89%**
4th week	100	**35.89%**	89	**42.62%**
6th week	99	**36.95%**	90	**43.28%**
12th week	101	35.12%	104	36.57%

Table no 5b: Comparison of % reduction from Baseline in LDL levels between two groups

	Control(% redn)		Study(% redn)		'p' value
	Mean	**SD**	**Mean**	**SD**	
Baseline	-	-	-	-	
1stweek	**19.087%**	4.14	**16.90%**	4.04	**0.044***
2nd week	**34.35%**	1.77	**39.89%**	6.24	**0.000****
4th week	**35.89%**	6.65	**42.62%**	5.65	**0.000****
6thweek	**36.95%**	3.92	**43.28%**	5.007	**0.000****
12thweek	35.12%	4.37	36.57%	4.51	0.544**

** $p \leq 0.010$ it implies Highly Significant, * $p \leq 0.050$ it implies Significant, $p > 0.050$ it implies Not Significant

Figure no 5:Comparison of % reduction from Baseline in LDL Cholesterol between two groups

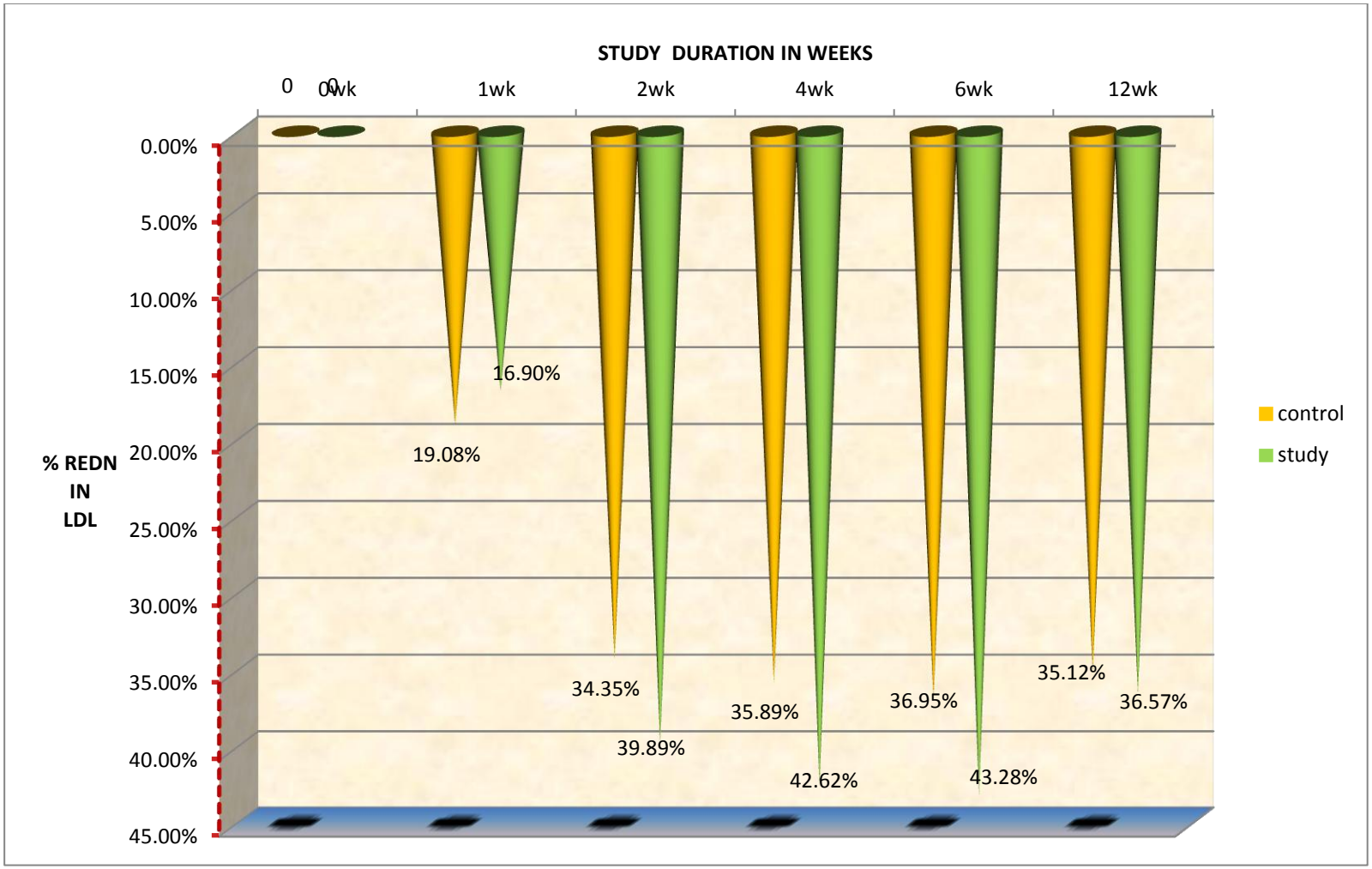


Table no 5a shows

- The mean LDL cholesterol levels of the study and control groups and the percentage reduction in LDL cholesterol from baseline at 1^{st} , 2^{nd} , 4^{th} , 6^{th} &12^{th} week.

Table no 5b shows

- The mean and standard deviation of the percentage reduction in LDL levels. Statistical analysis was done by using students independent 't' test.
- By the first week, there was a **19.08%** reduction in LDL cholesterol levels from baseline in the control group vs. **16.90 %** reduction from baseline in the study group. This difference was statistically significant **(p=0.044*).**
- At the second week, there was a **39.89%** reduction in LDL cholesterol levels from baseline in the study group vs. **34.35%** reduction in LDL cholesterol levels from baseline in the control group. On statistical analysis, this difference was statistically highly significant **(p=0.000**).**
- At the fourth week, the study group showed a **42.62%** reduction in LDL cholesterol levels from baseline vs. **35.89%** reduction from

baseline in the control group. This difference was found to be highly significant on statistical analysis **(p=0.000**).**

- At the sixth week, the study group showed a **43.28%** reduction in LDL cholesterol vs. **36.95% reduction** in control group and this difference was statistically highly significant **(p=0.000**).**
- At the twelfth week there was no statistically significant difference in the percentage reduction in LDL cholesterol levels. The % reduction in LDL cholesterol in the control group was **35.12% vs 36.57%** in the study group (p=0.544).

Figure 5 is a column chart representing the percentage reduction from baseline in LDL levels in the control and study groups at 0,1st, 2nd, 4th, 6th&12th week.

Table 4a: Mean Triglyceride levels in both the groups

	Control		Study	
	Mean TG mg/dl	**% redn**	**Mean TG mg/dl**	**%redn**
Baseline	170	-	210.6	-
1st week	160.5	**5.66**	179.6	**13.96**
2nd week	138.7	**18.34**	155.03	**25.92**
4th week	136.63	**19.88**	155.4	**25.65**
6th week	136.36	**20.66**	147.26	**28.45**
12th week	135.5	19.36	165.23	20.69

Table no 6b: Comparison of % reduction from Baseline in Triglyceride levels between two groups

	Control(% redn)		Study(% redn)		'p' value
	Mean	**SD**	**Mean**	**SD**	
Baseline	-	-	-	-	
1st week	**5.66%**	2.79	**13.96%**	5.74	**0.000****
2nd week	**18.34%**	1.26	**25.92%**	9.60	**0.000****
4th week	**19.88%**	3.61	**25.65%**	8.55	**0.002****
6th week	**20.66%**	9.26	**28.45%**	8.93	**0.002***
12th week	19.36%	9.41	20.69%	8.86	0.577

** $p \leq 0.010$ it implies (Highly Significant), * $p \leq 0.050$ it implies (Significant) , $p > 0.050$ it implies Not Significant

Figure no 6 : Comparison of % reduction from Baseline in Triglyceride levels between two groups

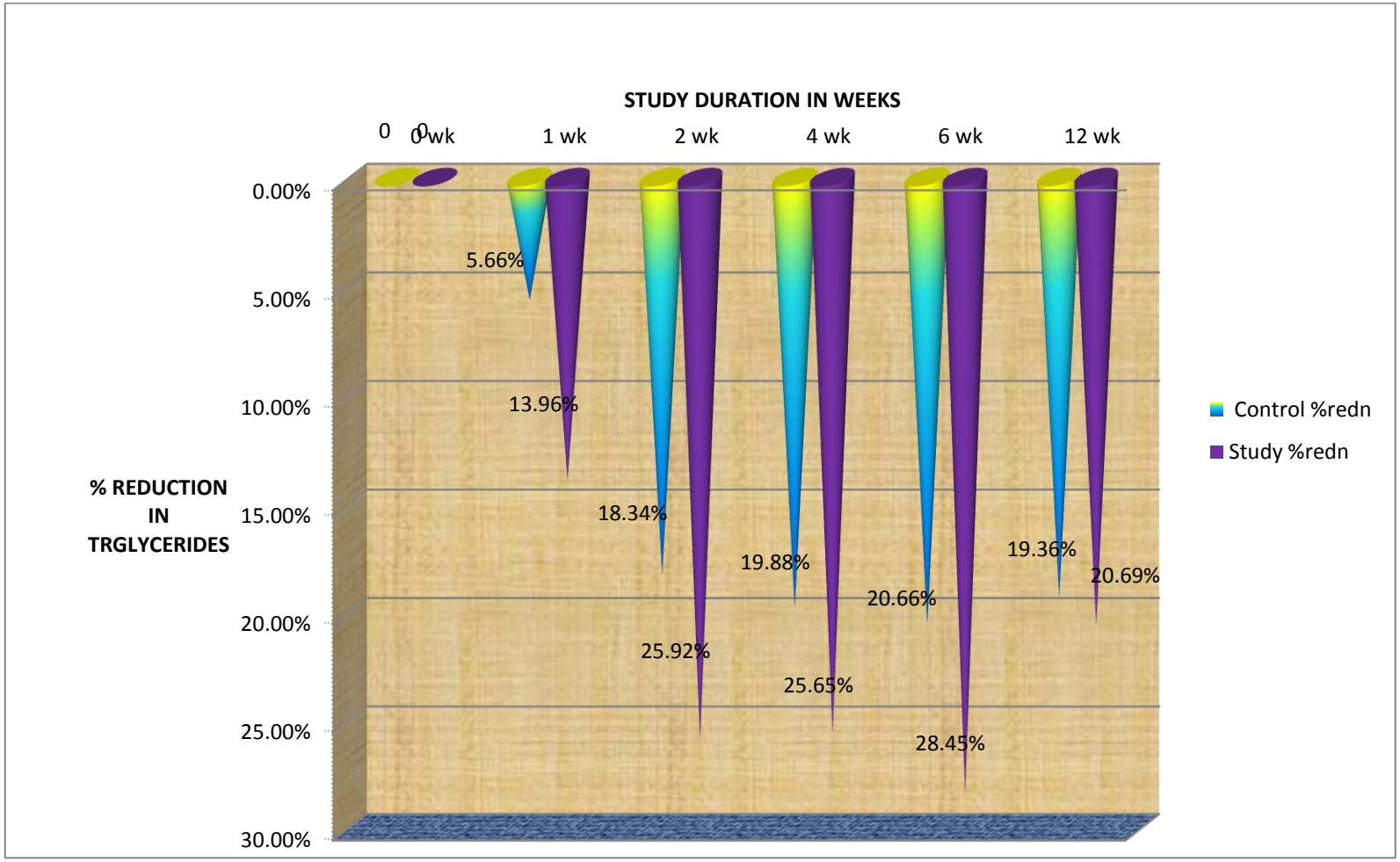


Table no 6a shows

- The mean triglyceride levels and the percentage reduction from baseline in both the groups at 1^{st} , 2^{nd} , 4^{th} ,6^{th} & 12^{th} week.

Table no 6b shows

- The mean and standard deviation of the percentage reduction in triglycerides. Statistical analysis was done by using students independent 't' test.

- By the end of the first week, the control group had **5.66%** reduction in triglycerides vs **13.96%** reduction in the study group. The difference in percentage reduction between two groups was statistically highly significant (**p=0.000****).

- By the end of the second week, there was greater percentage reduction in triglyceride levels in the study group when compared to the control group. The percentage reduction in study group was **25.92%** when compared to the control group which had **18.34%** reduction. The difference between the two groups was statistically highly significant (**p=0.000****).

- At the end of the fourth week the control group had **19.88% reduction vs 25.65%** reduction in the study group. The

difference between two groups was statistically highly significant **(p = 0.002****).

- At the sixth week the control group had **20.66% reduction vs 28.45%** reduction in the study group.On statistical analysis the difference between two groups was statistically highly significant (**p=0.002**).

- At the end of the twelfth week, there was no significant difference in percentage reduction in triglyceride levels between the two groups (p=0.422). The percentage reduction in triglyceride levels was **19.36%** in the control group **vs 20.69%** in the study group.

Figure no 6 is a column chart representing the percentage reduction in triglyceride levels from baseline at 1^{st}, 2^{nd}, 4^{th}, 6^{th} &12^{th} week of the study.

Table 7a: Comparison of the VLDL levels in both the groups

	Control		Study	
	Mean VLDL mg/dl	**% redn**	**Mean VLDL mg/dl**	**% redn**
Baseline	36.33	-	41	-
1st week	34.66	4.75%	39.23	6.2%
2nd week	27.9	23.05%	27.93	30.3%
4th week	24.56	32.03%	26.36	33%
6th week	23.36	36.16%	26	35.4%
12th week	23.63	34.56%	30.16	27.8%

Table no 7b: Comparison of % reduction from Baseline in VLDL levels between two groups

	Control(% redn)		Study(% redn)		'p' value
	Mean	**SD**	**Mean**	**SD**	
Baseline	-	-	-	-	
1st week	4.75%	3.88	6.2%	6.36	0.292
2nd week	23.05%	16.81	30.3%	15.11	0.084
4th week	32.03%	14.38	33%	15.38	0.655
6th week	36.16%	12.26	35.4%	16.12	0.839
12th week	34.56%	14.58	27.8%	19.51	0.134

** $p \leq 0.010$ it implies Highly Significant, * $p \leq 0.050$ it implies Significant , $p > 0.050$ it implies Not Significant

Figure no 7: Comparison of % reduction from Baseline in VLDL levels between two groups

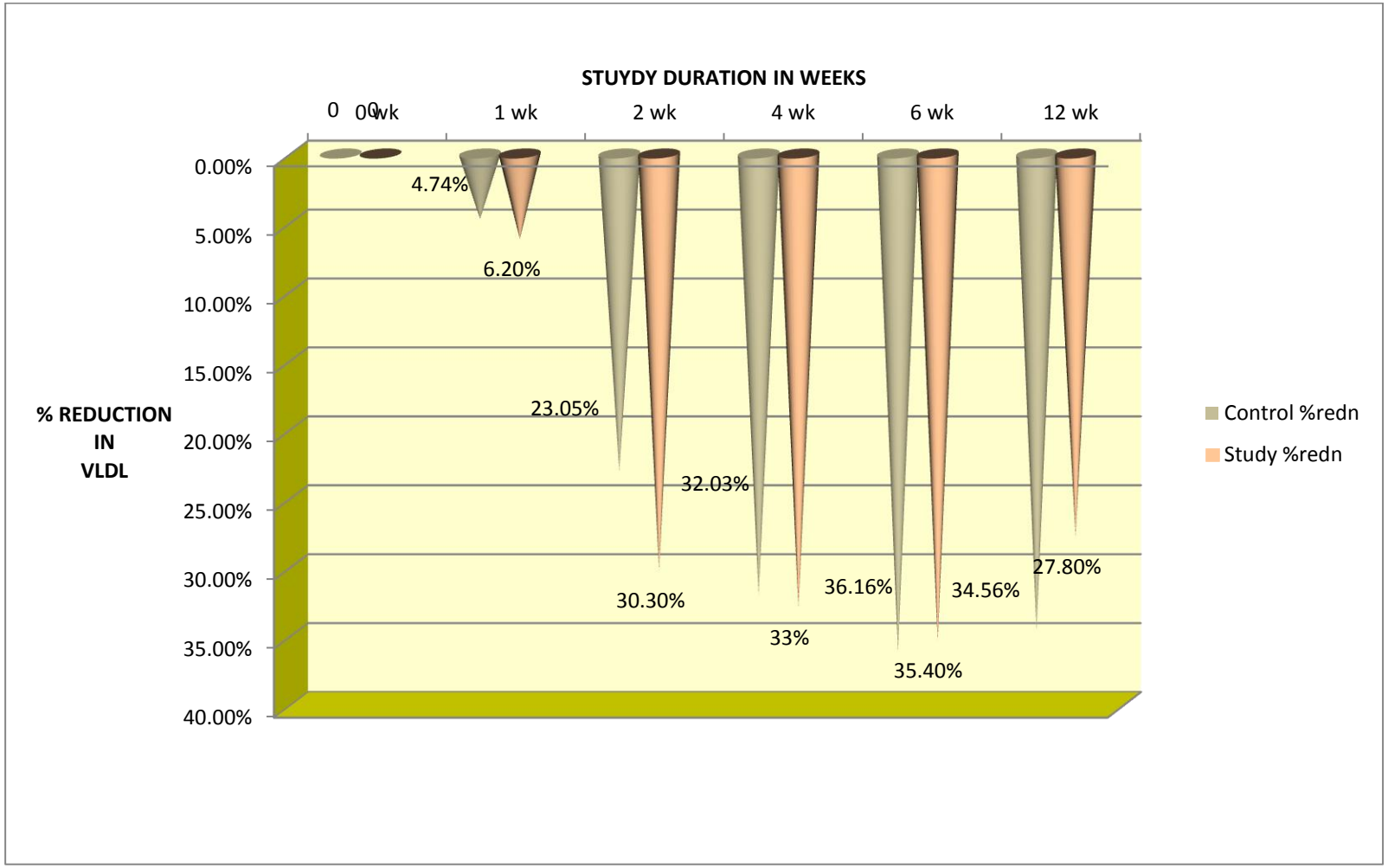


Table no 7a shows

The mean VLDL levels and percentage reduction in VLDL levels from baseline of the control and study groups at 0,1st ,2nd ,4th ,6th &12th week**.**

Table no 7b shows

- The mean and standard deviation of the percentage reduction in VLDL in both groups. Statistical analysis was done by using students independent 't' test.
- At the end of the first week, the study group had **6.2%** reduction in VLDL levels vs **4.75%** reduction in control group, this difference was not statistically significant (p=0.292).
- At the end of the second week, the study group had **30.3%** reduction in VLDL levels vs **23.05%** in control group, but this difference was not statistically significant (p=0.084).
- At the end of the fourth week, the study group had **33%** reduction in VLDL levels vs **32.03% in** the control group, but this difference was not statistically significant (p-0.655).
- At the end of the sixth week, the study group **had 35.4%** reduction percentage in VLDL level vs **36.16%** in control group.

On statistical analysis this difference was not statistically significant (p = 0.839).

- At the end of the twelfth week, there was **35.46%** reduction in VLDL levels vs **27.8%** reduction in the study group, this difference was not statistically significant (p=0.134).

Figure no 6 is a column chart showing the percentage reduction in VLDL levels of the study and control groups at 0,1st ,2nd ,4th ,6th &12th week of the study.

Table 8a: Mean HDL levels in both the groups

	Control		Study	
	Mean HDL mg/dl	**% change**	**Mean HDL mg/dl**	**% change**
Baseline	46.44	-	47.3	-
1st week	45.6	-1.26	46.22	-2.29%
2nd week	46	-0.25	47.17	-0.35%
4th week	46.26	-0.11	48.39	2.32%
6th week	47.5	2.83	47.9	4.17%
12th week	48.76	6.11	47.66	3.47%

Table no 8b:Comparison of % change from Baseline in HDL levels between two groups

	Control(% change)		Study(% change)		'p' value
	Mean	**SD**	**Mean**	**SD**	
Baseline	-	-	-	-	
1stweek	-1.26%	6.54	-2.29%	2.95	0.09
2nd week	-0.25%	8.01	-0.35%	7.36	0.620
4th week	-0.11%	5.60	2.32%	7.23	0.150
6thweek	2.83%	6.84	4.17%	9.01	0.518
12thweek	6.113%	8.80	3.47%	4.09	0.142

** $p \leq 0.010$ it implies (Highly Significant), * $p \leq 0.050$ it implies (Significant) , $p > 0.050$ it implies Not Significant

Figure no 8: Comparison of % reduction from Baseline in HDL levels between two groups

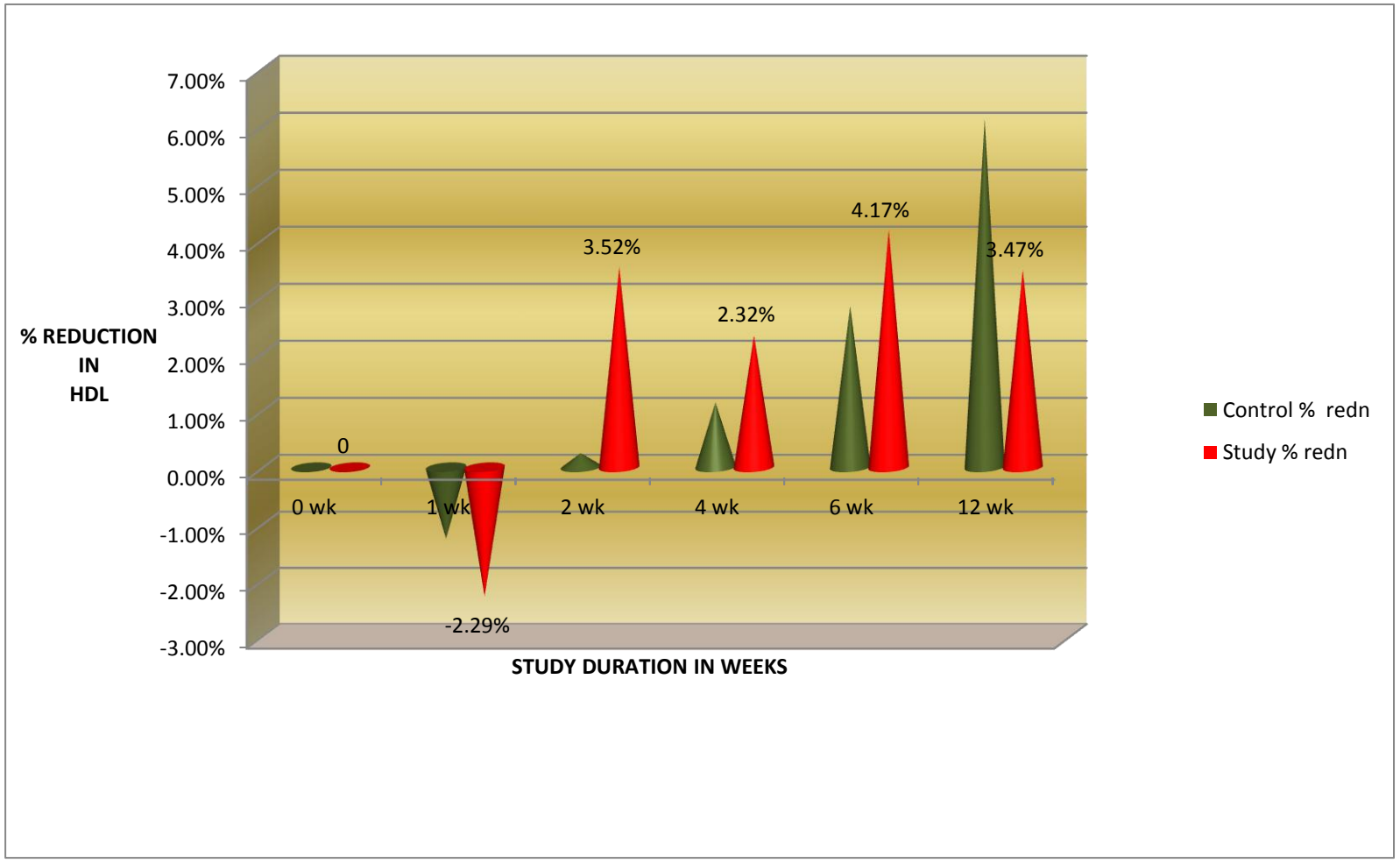


Table no 8a shows

The mean HDL levels and the percentage change from base line at 0, 1^{st} , 2^{nd} , 4^{th} , 6^{th} & 12^{th} week in both the groups.

Table no 8b shows

- The comparison of percentage change from baseline in HDL in both groups, statistical analysis was done by using students t test.
- By the end of the first week, there was a **1.26%** reduction in HDL levels in control group vs **2.2%** reduction in study group, this difference was not statistically significant (p=0.09).
- By the end of the second week. the control group had a **0.25%** fall in HDL levels vs **0.35%** fall in study group, this difference was not statistically significant (p=0.620).
- At the end of the fourth week, the control group had a **0.11%** reduction in HDL levels **vs2.32%** rise in study group. On statistical analysis this difference was not statistically significant (p=0.150).
- At the end of the sixth week, the control group had **2.83%** rise in HDL level vs **4.17%** rise in the study group but the difference was not statistically significant (p = 0.518).

- At the end of the twelfth week, the control group had **6.11%** rise in HDL levels vs **3.47%** rise in the study group, but the difference was not statistically significant (p=0.142).

Figure no 8 is a column chart showing the percentage change in HDL levels from base line at 0,1st, 2nd, 4th, 6th &12th week of both the groups.

Table no 9: Comparison of Total cholesterol: HDL-cholesterol ratio between study and control groups

	Mean total CH:HDL CH ratio	
	Control group	**Study group**
Baseline	5.68	6.06
1st week	4.84	5.09
2nd week	4.21	3.84
4th week	4.08	**3.46**
6th week	3.95	**3.54**
12th week	3.91	4.36

Figure no 9 :Comparison of the total: HDL ratio of both the groups

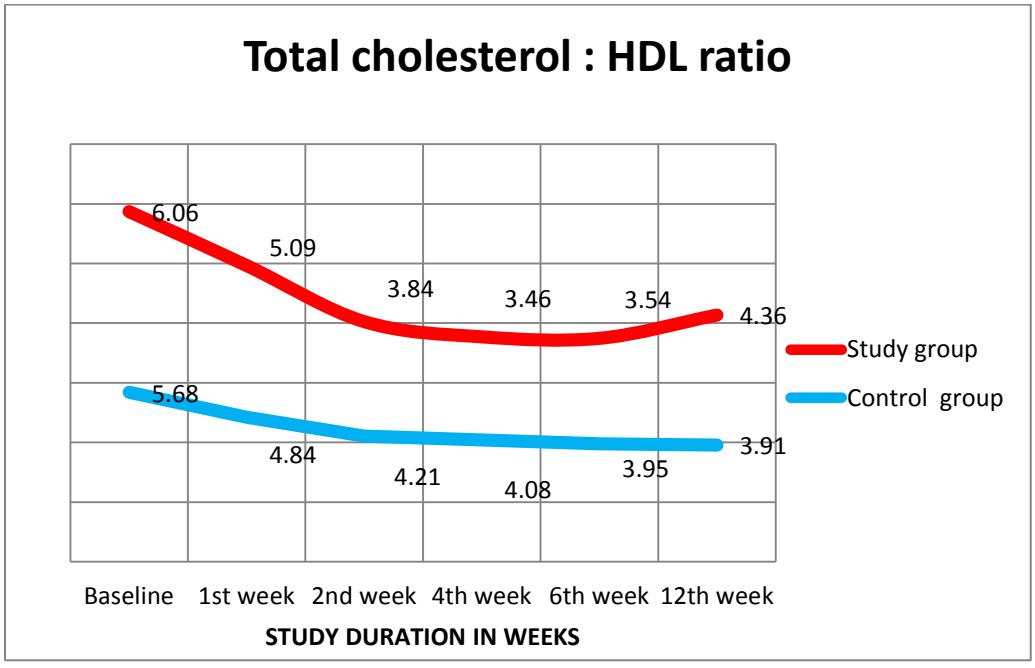


Table no 9 shows

- The ratio of total cholesterol and HDL cholesterol in both the groups during the study.
- In the control group the total cholesterol : HDL cholesterol ratio was 5.68 at baseline, thereafter it reduced to 4.84 at 1^{st} week, 4.21 at 2^{nd} week, 4.08 at 4^{th} week, 3.95 at 6^{th} week and 3.91 at 12^{th} week.
- In the study group the total cholesterol: HDL cholesterol ratio was 6.06 at baseline, later it reduced to 5.09 at 1^{st} week and 3.84 at 2^{nd} week. From the 4^{nd} week onwards the ratio was in the desirable level of ≤ 3.5.
- After the discontinuation of study drug, the ratio became 4.36 in the study group.

Figure no 9 is a line diagram representing the data of the table no 9.

TABLE no 10: BASIC HEMATOLOGICAL INVESTIGATIONS BEFORE AND AFTER THE STUDY

Parameter	**Group**	**Baseline value**		**End of study**		**Student paired t-test**
		Mean	SD	Mean	SD	
Hb (g/dl)	Control	10.95	.96	11.13	1.05	P=0.45
	Study	10.93	.95	11.28	1.10	P=0.15
Total WBC count(/mcl)	Control	9769.29	1628.98	9592.86	1514.60	P=0.67
	Study	9724.14	1615.47	9534.48	1583.23	P=0.56
ESR	Control	11.68	1.39	11.54	1.60	P=0.69
	Study	11.38	1.21	11.66	1.47	P=0.34
Urea (mg/dl	Control	20.79	2.692	21.29	.79	P=0.35
	Study	20.69	2.41	20.83	2.54	P=0.82
Serum Creatinine(mg/dl)	Control	.78	.14	.77	.12	P=0.84
	Study	.79	.14	.81	.13	P=0.69
AST (U/L)	Control	17.64	4.52	17.50	4.04	P=0.90
	Study	17.86	4.34	17.41	4.36	P=0.71
AST (U/L	Control	16.62	4.14	17.79	5.19	P=0.29
	Study	17.00	4.02	18.00	4.59	P=0.36
Ck (IU/L)	Control	82.4	2.34	83	2.7	P=0.45
	Study	83	2.4	82.6	2.6	P=0.56

*P≤0.05 significant, ** P≤0.01 highly significant, *** P≤0.001 very high significant

Table no 10 shows

- The hematological and laboratory parameters of both the groups before and after the study. There was no significant difference in the laboratory parameters before and after the study in both the groups.
- There was no significant difference in the mean SGOT and SGPT levels and CPK levels before and after the study in both the groups.

Table no 11 : INCIDENCE OF ADVERSE EFFECTS BEFORE AND AFTER THE STUDY

S.No	Adverse effect no(%)	Study Group (30)	Control group (30)
1	Nausea	2(6.66%)	2(6.66%)
2	Dyspepsia	1(3.33%)	1(3.33%)
3	Myalgia	2(6.66%)	3(10%)
4	Joint pain	3(12%)	2(10%)
	Total	8	10

Table no 12: ADDITIONAL OBSERVED EFFECTS OBSERVED DURING THE STUDY

S.No	Beneficial effect	Study Group (30)	Control Group(30)

1	Reduced hair fall	**1**	**0**

Figure no 10: Adverse effects observed during the study.

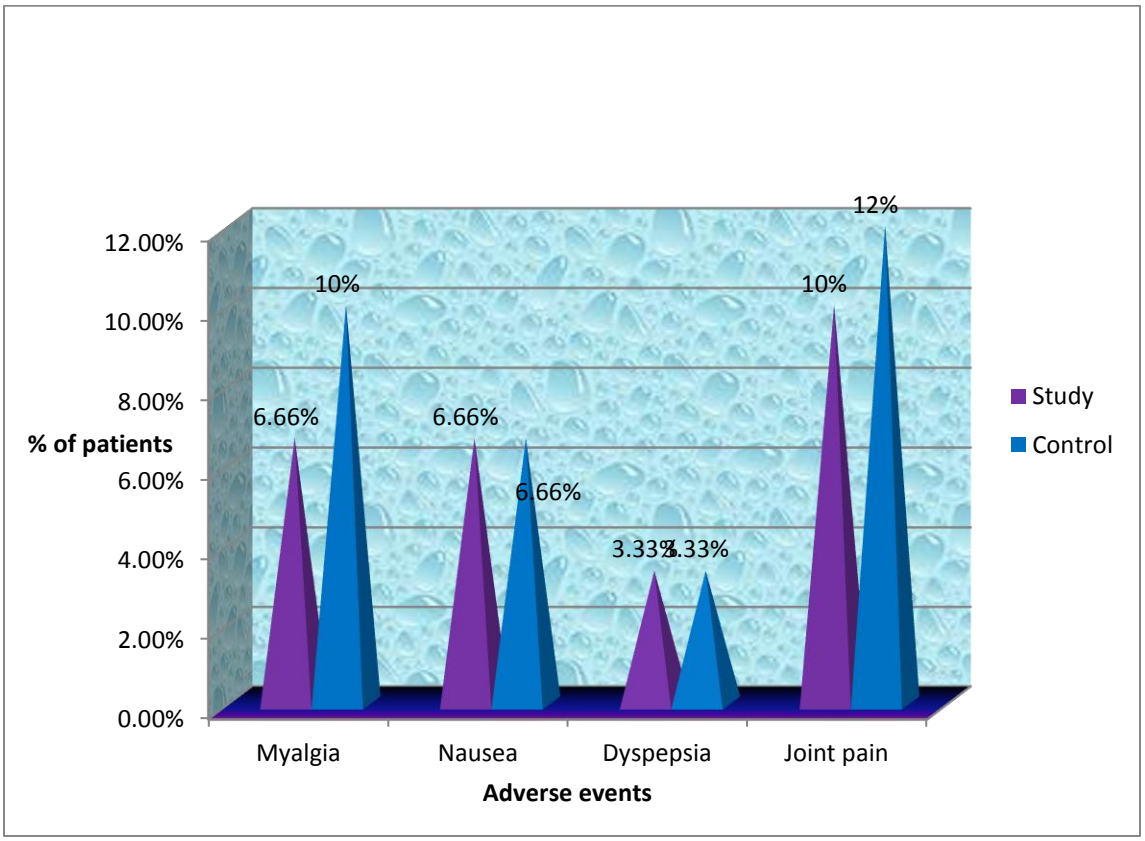


Table no 11 shows

- The incidence of adverse events observed in both the groups during the study.
- Out of 30 patients 10 patients in the control group vs 8 patients in the study group experienced minor self limiting side effects.

Figure no 10 shows a column chart representing the data of the Table no 11.

Table no 12 shows

The additional observed effect of biotin in the study and control group during the study.

In the study group which received biotin one male patient reported reduced hair fall by the end of the fourth week of the study.

DISCUSSION

Dyslipidemias are one of the major causes of coronary heart disease, ischemic cerebrovascular disease and peripheral vascular disease which are induced by atherosclerosis.

Ever since the identification of hypercholesterolemia as a major risk factor for cardiovascular morbidity and mortality, drugs that modify cholesterol levels have been under constant research. Statins were introduced in the late 1980s

and now are one of the widely used drugs. In clinical practice adherence to statins is mostly sub optimal due to myalgia and other side effects like weakness, fatiguability and cognitive impairment.[38] There are not much therapeutic options for these statin intolerant patients.

Other newer non statin lipid lowering drugs are under clinical trials for these statin intolerant patients. They include newer drugs like lomitapide (a microsomal transfer protein inhibitor), mipomersen (an antisense inhibitor of apolipoprotein B), and proprotein convertase subtilisin/kexin(PCSK) 9 inhibitors like evolocumab.

Biotin is a water soluble vitamin that acts as a prosthetic group of carboxylases.[39] Biotin also regulates intermediary metabolism at the genetic level. Studies in mice have proved that of biotin given in pharmacological concentrations reduces serum triglycerides and the expression of lipogenic genes[40]. These evidences and the lack of toxic effects of the vitamin at higher doses suggest that biotin could be used in the management of hyperlipidemias.

In this clinical study the therapeutic effect and side effect profile of combination of Biotin 5mg with Atorvastatin 20 mg was compared with the monotherapy of Atorvastatin 20 mg for 6 weeks.

Patients with primary dyslipidemias will be symptomatic at an early stage and have associated cardiovascular complication. By excluding patients less than 45 yrs of age at the screening, the possibility of inducting primary dyslipidemias is low. Moreover age more than 45 in males and 55 in females is considered as a serious risk factor for atherosclerosis. Thus, we have included only newly diagnosed cases with secondary dyslipidemias at high risk of atherosclerosis in the study.

In our study the mean age of the patients in the **study group and control group were 50.13 yrs and 50.7 yrs** respectively (vide table no 1 & figure no 1).

Though we have included patients of both sexes in the study, there was a **female preponderance (57%)** in both the groups. The sex distribution of the patients of both the groups were found to be same (vide table no 3 & figure no 2).

In our study all the patients had associated hypertension but it was of moderate severity and was well treated. We have included patients with type 2 diabetes mellitus in the study and the incidence of diabetes mellitus was **26.6% in control group and 23.3% in study group** (vide table no 1).

Statistical analysis has showed both the groups to be comparable in terms of mean age, age distribution, sex distribution, associated co-morbid diseases.

Patients compliance was good in both the groups with all patients coming for regular visits. In the study group patient follow up was good and there were no dropouts and all the patients completed the study.

Effect on total cholesterol :

Monotherapy with atorvastatin 20 mg resulted in greater 16.7% reduction in total cholesterol at the end of 1^{st} week when compared to the 16.06% reduction in the combination therapy group, but this was not statistically significant, which implies that there are no early effects of biotin in the study group.

Therafter monotherapy resulted **in 26.32% reduction in total cholesterol at the end of 2nd week, 28.33% reduction at the end of 4th week and 28.68% reduction at the end of 6th week**. These reduction rates are similar to those observed in other studies by Wilinski et al.[41]

Combination therapy group showed a **34.66% reduction in total cholesterol at the end of the 2nd week, 41.33% reduction in total cholesterol at the end of the 4th week and 40.37% reduction in total cholesterol at the end of the 6th week respectively** (vide table 4a&4b,figure 4).

This shows that the combination of biotin 5 mg with atorvastatin 20 mg resulted in a greater reduction in total cholesterol from the 2nd week onwards which was well maintained till the 6th week, this difference was also **statistically significant.**

At the follow up visit the percentage reduction came down to 29.96% in the study group which was similar to the 27.56% reduction in control group implying that Biotin has no delayed effects on plasma lipoproteins.

Effects on LDL cholesterol:

In the control group there was a 19.08% reduction in LDL levels at the first week, later the percentage reduction plateaued at around **34.35%, 35.89% and 36.95% at 2nd, 4th and 6th week** respectively. These reduction rates are similar to previous studies showing a 25-40% reduction after treatment with Atorvastatin 20 mg for 12 weeks.[42]

The study group had a 16.90% reduction at 1st week followed by a **39.89% reduction in LDL levels at the end of 2nd week, 42.62% reduction at the end of the 4th week and 43.28% reduction at the end of the 6th week.** On statistical analysis the difference in percentage reduction in LDL levels between the study group and the control group was statistically significant at 2nd, 4th, and 6th week (vide table 5a&5b,figure 5).

At the follow up visit both the groups had similar reduction percentages 35.12% in control vs. 36.57% in study group implying the absence of delayed effects of biotin on LDL levels.

Effect on triglycerides:

The combination therapy resulted in a significantly greater reduction in triglyceride level from the first week onwards. The mean triglycerides were reduced by **13.96% at the end of 1st week, 25.92% at the end of 2nd week, 25.65 at the end of 4th week and 28.45% at the end of 6th week in the study group** when compared to the control group which resulted in **a 5.66% reduction at the end of 1st week, 18.34% reduction at the end of 2nd week, 19.88% at the end of 4th week and 20.66% at the end of 6th week**.

On statistical analysis the difference in reduction perecntages were statistically significant from the first week till the sixth week.

At 12 weeks after Biotin was discontinued the percentage reduction of the study group was similar to the control group, it was 20.69% in the study group as against 19.36% in the control group.

Effect on VLDL:

The combination therapy of biotin and atorvastatin resulted in a greater reduction in VLDL when compared to monotherapy with atorvastatin but the difference was less and not statistically significant.

Effect on HDL:

After the 2nd week of study both the groups had a rise in HDL levels . Though there was a greater rise in HDL percentage in the combination group it was not statistically significant.

Effect on Total Cholesterol : HDL ratio:

The total cholesterol :HDL ratio is an important predictor of coronary artery risk and levels ≤ 3.5 are considered desirable and levels > 4.5 are associated with higher risk. In our study the combination therapy group was able to achieve desirable levels of **total cholesterol : HDL ratio of ≤ 3.5 at 4th and 6th weeks**. In the monotherapy group the total cholesterol : HDL ratio was > 3.5 through out the study.

Both the groups tolerated the medications and had few minor side effects. The incidence of adverse effects in the control group was similar to those seen in previous studies with atorvastatin 20 mg.[43] In the control group 3 out of 30 patients (10%) complained of myalgia and 4 out of 30 patients (12%) complained of general body weakness. The study group had less myalgia (6.66%) and body weakness(10%).The occurrence of gastrointestinal distress was similar in both the groups. In addition one male patient reported reduced hair fall during the study. This observation correlated with studies showing the effects of Biotin in androgenic alopecia by Fameneni et al.[44,45]

Biotin is a very safe vitamin and levels upto 300 times the normal have shown to be non toxic. Thus the addition of Biotin had resulted in fewer side effects and some additional observed effects also. None of the patients had any significant abnormality in the laboratory investigations performed.

From this study we can infer that combination therapy of biotin and atorvastatin produced significantly more reduction of total cholesterol, LDL and triglyceride level than the monotherapy with atorvastatin. This lipid lowering effect of biotin is attributed to the regulation of genes associated with intermediary metabolism and maintanence of glucose and lipid hemostasis.[46]

Biotin can thus be an effective and safe add on drug with Atorvastatin. It alone can be a valuable therapy in patients with dyslipidemia to prevent coronary artery disease and cerebrovascular disease and administration of biotin alone can be considered for further trials in dyslipidemias.

CONCLUSION

It can be concluded that combination therapy of Biotin 5 mg with Atorvastatin 20 mg is more efficacious than Atorvastatin 20 mg alone in terms of reduction in Total cholesterol, LDL cholesterol and triglycerides with less side effects. Thus, Biotin is a safe and well tolerated adjuvant hypolipidemic agent in secondary dyslipidemias.

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Appendix 1

LIPID PROFILE ESTIMATION:

Lipid profile testing consists of estimation of Total Cholesterol, Triglycerides, High Density Lipoproteins, Low Density Lipoproteins and Very Low Density Lipoproteins. Lipid profile estimation was done in the Department of Biochemistry. Blood samples for lipid profile estimation was taken after 12 hrs of fasting at the beginning of the study, end of 1, 2, 4, 6 and 12 weeks.

A. ESTIMATION BY CHOD-PAP METHOD:

Cholesterol is measured enzymatically in two reactions that hydrolyze cholesteryl esters and oxidize the 3-OH group of cholesterol. One of the reaction byproducts H2O2 is measured quantitatively in a peroxidase catalyzed reaction that produces color. Absorbance is measured at 500 nm. The colour intensity is proportional to cholesterol concentration.

Step 1: In the prescence of Cholesterol oxidase (CHOD)

Cholesteryl ester+H_2O $\Longrightarrow$ Cholestenone+Hydrogen peroxide

Step 2: In the prescence of Phenol enzyme peroxidase(PAP)

Hydrogen peroxide+4-amino phenazine $\Longrightarrow$ Coloured complex+water

B. ESTIMATION BY GPO-PAP METHOD

Triglycerides are measured enzymatically in serum or plasma using a series of coupled reactions in which triglycerides are hydrolysed to produce glycerol. Glycerol is then oxidized using glycerol oxidase and absorbance of H_2O_2 one of the reaction products, is measured at l500 nm.

Step 1: In the prescence of enzyme Esterase

Triglycerides +Water ⟹ Glycerol + Carboxylic Acid

Step 2: In the prescence of enzyme Glycerol Kinase

Glycerol + ATP ⟹ Glycerol -3-Phosphate +ADP

Step 3: In prescence of enzyme Glycerol-3-Phosphate Oxidase (GPO)

Glycerol-3-phosphate + Oxygen ⟹ Dihydroxy Acetone Phosphate + Hydrogen Peroxide.

Step 4 :In the prescence of enzyme Peroxidase

Hydrogen peroxide + Chlorophenol + 4-AminoPhenazone (PAP)
⟹ Coloured Complex.

C. HDL CHOLESTEROL:

HDL was measured by Direct method .

Principle: The apoB containing lipoproteins in the specimen are reacted with a blocking reagent that renders them non reactive with the enzymatic cholesterol reagent under conditions of the assay. The apoB containing lipoproteins were effectively excluded from the assay and only HDL cholesterol was detected.

The method uses sulfated alpha-cyclodextrin in the presence of Mg^{+2}, which forms complexes with apoB containing lipoproteins and polyethylene glycol – coupled cholesteryl esterase and cholesterol oxidase for the HDL-cholesterol measurement.

(1) ApoB containing Lipoproteins + a-Cyclodextrin + Mg^{+2} + Dextran SO_4 forms soluble non-reactive complexes with apoB-containing lipoproteins.

(2) HDL – cholesteryl esters in prescence of PEG – cholesteryl esterase forms HDL – unesterified cholesterol + fatty acid .

(3) Unesterified cholesterol + O_2 PEG in prescence of Cholesterol Oxidase forms cholestenone + H_2O_2

(4) H_2O_2 + 5-aminophenazone + N-ethyl-N-(3-methylphenyl) – N '- succinyl ethylene diamine + H2O + H^+ peroxidase forms qunoneimine dye + H_2O .

Absorbance was measured at 600 nm.

D.**LDL CHOLESTEROL:**

LDL cholesterol was calculated from measured values of total cholesterol,triglycerides and HHDL cholesterol according to the relation ship

(LDL – cholesterol) = (Total cholesterol) - (HDL – cholesterol) - TG/5

where (TG)/5 is an estimate of VLDL cholesterol and all values are expressed in mg/dl.

CASE RECORD FORM

Name Age and Sex Hospital number

Complaints

Present History

Past History

Personal History

Treatment History

General examination

*Pulse

*BP

*Anaemia

*Lymphadenopathy

*Pedal edema

*Jaundice

Systemic examination

*CVS, RS

*Abdomen

*CNS

INVESTIGATIONS : Complete blood count

Blood urea, Serum creatinine

Plasma lipid profile(LDL,CHOLESTEROL,VLDL,TRIGLYCERIDE)

Liver Function tests(SGOT,SGPT)

CPK

BASELINE CHARECTERISTICS AND BIOCHEMICAL PARAMETERS :

S NO						
1		Name of the patient				
2		Age				
3		Sex				
4		IP/OP No				
5		Address for communication				
6		Drug given				
		Batch no				
		Strip no				
		Date of purchase				
7		Drug given				
		Batch no				
		Strip no				
		Date of purchase				
S no		INITIAL 1 DAY	1^{ST} WEEK	2 WEEKS	4WEEK	6WEEK(% Redn
1	LDL					
2	TRIGLYCERIDE					
3	VLDL					
4	HDL					
5	CHOLESTEROL					
6	TOTAL PLASMA LIPID					
7	SGOT					
8	SGPT					
9	CPK					

Any adverse event or additional observed effect:

CASE FOLLOW UP FORM

Name Age and Sex Hospital number

Complaints

General examination

*Pulse

*BP

*Anaemia

*Lymphadenopathy

*Pedal edema

*Jaundice

Systemic examination

*CVS ,RS

*Abdomen

*CNS

INVESTIGATIONS :

- Complete Blood count.
- Blood urea, Serum creatinine.
- Liver Function tests(SGOT,SGPT).
- CPK.
- Fasting plasma lipid profile
    - Total cholesterol
    - LDL cholesterol
    - Triglycerides
    - VLDL
    - HDL

Any Adverse event or additional observed effect: