A STUDY ON THE EFFICACY OF HOMOEOPATHIC TREATMENT IN HYPERLIPOPROTEINEMIA

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DOCTOR OF PHILOSOPHY IN LIFE SCIENCE

By

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DECLARATION

I, ABDURAHIMAN.T, do hereby declare that this thesis entitled **"A STUDY ON THE EFFICACY OF HOMOEOPATHIC TREATMENT IN HYPERLIPOPROTEINEMIA"**, is a bonafide record of research work done by me and that no part of this thesis has been presented for the award of any degree, diploma, or other similar title.

Place : University of Calicut
Date : 1.7.05

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CERTIFICATE

Certified that this thesis entitled **"A STUDY ON THE EFFICACY OF HOMOEOPATHIC TREATMENT IN HYPERLIPOPROTEINEMIA"** is a record of bonafide research work done independently by Sri. ABDURAHIMAN.T, under my guidance and supervision for the award of PhD Degree of the University of Calicut and that no part of this has been previously formed the basis for the award of any degree, diploma other similar title in any University.

He is permitted to submit the thesis to the University.

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Chapter - 1

INTRODUCTION

Lipids are heterogenous group of compounds having common property of being relatively insoluble in water and soluble in non- polar solvents such as ether, chloroform and benzene. Because of their insolubility, they are transported in the plasma in macromolecular complexes called lipoproteins. They are spherical articles with non – polar lipids (triglycerides and cholesterol) in their core and more polar lipids (phospholipids and free cholesterol) oriented near the surface

According to the increasing density, lipoproteins are classified as chylomicrons (CM), chylomicron remnants (CMR), very low-density lipoproteins (VLDL), intermediate density lipoproteins (IDL), low-density lipoproteins (LDL) and high-density lipoproteins (HDL).

The low-density lipoprotein contains 60-70 % of total serum cholesterol and both are directly related with risk of coronary heart disease. High-density lipoproteins normally contain 20-30% of total cholesterol and their levels are inversely correlated with coronary heart disease risk. Very low-density lipoprotein contains 10-15% of the total serum cholesterol along with most of the triglycerides in fasting serum. VLDL are precursors of LDL, and some forms of VLDL particularly VLDL remnants, appears to be atherogenic.

The most common lipoprotein abnormalities lead to the accumulation of excessive lipids in plasma. These diseases are collectively known as hyperlipidemias or more properly hyperlipoproteinemias.

Primary hyperlipidemias characterized by severe hypertriglyceridemia predisposes to acute pancreatitis, whereas those disorders characterised by hypercholesterolemia, apart from hyperalphalipoproteinemia, are associated with an increased risk of premature vascular disease.

Secondary hyperlipidemia is common and occur frequently in disorders such as obesity, alcoholism, diabetes mellitus, hypothyroidism, liver and renal diseases, and as a side effect of drug therapy, particularly of hypertension.

A large body of the epidemiologic evidence suggests a direct relationship between the level of serum LDL cholesterol (or total serum cholesterol) and the rate of coronary artery disease [CAD].

Various epidemiologic, genetic and animal investigations strongly support a causal link between elevated LDL cholesterol and risk of coronary heart disease, in primary as well as secondary prevention. Several recent trials employing angiographic assessment have revealed that cholesterol-lowering therapy slows progression in a substantial portion of both men and women and produces regression of coronary atherosclerosis in some individuals.

Coronary heart disease is the leading cause of death for both men and women in the United States and accounts for about 500,000 deaths a year. [1] In India Cardio –vascular diseases accounted for 30.2 % of mortality in 1998,

compared to 25.5% in 1990. Cardiovascular mortality in India accounted for 16.9% of cardio vascular disease death worldwide. [2]

It was reported that 1.5 million deaths occurred in India in 1998 due to ischaemic heart disease. [3] Cross sectional surveys of prevalence of coronary heart disease suggest higher burden, especially in urban areas (urban 80-120 and rural 30-60 / 1000 adults). [4]

Indians around the globe have highest rates of morbidity and mortality from coronary artery disease despite the fact that nearly half of them are life long vegetarians. When compared to white and other Asians, coronary artery disease rate among Indians are 2 to 4 fold higher over all and 5-10 fold higher in those younger than 40 years of age, irrespective of gender, region or social class. During the past 30 years, coronary artery disease rates in most developed countries have declined by 30-60% while the incidence doubled in India.

During the last 25 years, cholesterol levels decreased by about 25 mg / 100 ml in United States (from 230 to 205 mg/100ml) while, it increased by 25 mg/100ml in Indians (from 165 to 190mg/100ml). Kerala has almost doubled the rate of adults with high cholesterol (> 240 mg/100ml) as in United States (Kerala 32 % vs US 18%). [5]

In a study conducted at a medical college hospital in Kerala, Mammi et al., reported that incidence of acute myocardial infarction increased from 200 cases in 1969 to 5284 in 1987. [2]

From the study conducted among the residents of an urban housing settlement in Thiruvananthapuram, Aleyamma et al., reported mean serum total cholesterol of 223 ± 45.3 mg/100ml; 223.7 ± 44.9 mg/100ml among males and 223.7 ± 45.8 mg/100ml among females. [6]

Clinical trials of relatively short term duration indicates that a 2% reduction in coronary heart disease rate results from each 1% reduction in serum cholesterol and epidemiological studies suggests that the reduction achieved with long term cholesterol lowering may even be greater, perhaps as well as 3% for each 1% reduction in serum cholesterol. Several primary prevention trials have demonstrated that the blood cholesterol lowering leads to reduction in rates of myocardial infarction and death from coronary heart disease. [1]

Although the incidence of coronary artery disease in India is increasing at an alarming rate, no effective measures have yet been taken to address this health hazard. Hyperlipoproteinemia, except hyperalphalipoproteinemia, is one of the modifiable risk factor of coronary artery disease.

Homoeopathic system of medicine is based on the doctrines, laws and rules of practice that are formulated, named and systematically set forth by Dr. Samuel Hahnemann, in his book "Organon of rational art of healing". This system of medicine is based on the doctrine of "similia similibus curentur" – meaning "let likes be treated by likes". The drugs are proved on healthy individuals and the effects produced in the psychic and physical plane are noted. This picture is compared to the psycho-physical effects produced in the disease. If

the changes produced in the disease corresponds to any drugs having similar changes – similarity -, then that drug is selected for the treatment of patient.

It is to be noted that biochemical changes produced by drugs during its action on healthy human beings are not studied.

Yet it has been clinically verified that many Homoeopathic drugs are effective in various clinical conditions including metabolic disorders. Biochemical values are found to be modified under Homoeopathic method of treatment. Various metabolic disorders including Diabetes, Gout, psychosomatic disorders including Hypertension, endocrine disorders including thyroid disorders, angina pectoris, and other systemic disorders are found to be responding well to the Homoeopathic treatment. The names of the drugs and their employment in above clinical conditions are already available in Homoeopathic literature. But no reference was yet known to be given to the biochemical changes effected by the action of drugs in relation to cholesterol metabolism disorders.

Homoeopathic method of treatment is emerging as an alternative system of medicine, having wide acceptance and Government patronage in various state in India, especially in Kerala.

More over, the cost under Homoeopathic method of treatment is less when compared to other methods of treatment.

In the light of above factors and the effectiveness of Homoeopathic medicines on clinical conditions closely related to the cholesterol metabolism, it was decided to conduct a study on the effectiveness of Homoeopathic treatment on hyperlipoproteinemia.

Chapter - 2

REVIEW OF LITERATURE

2.1 Lipids

Lipids are ubiquitous in the body tissues and have an important role in virtually all aspects of life - serving as hormones or hormone precursors, aiding in digestion, providing energy storage and metabolic fuels, acting as functional and structural components in cell membranes, and forming insulation to allow nerve conduction or to prevent heat loss

The term *lipid* applies to a class of compounds that are soluble in organic solvents and nearly insoluble in water. Chemically, lipids are either compound that yields fatty acids on hydrolysis or complex alcohols that can combine with fatty acids to form esters. Some lipids are more complex, containing non lipid groups such as sialic, phosphoryl, amino or sulphate groups. The presence of these groups gives them the property (amphipathic) of having an affinity for both water and organic solvents, which is important in the formation of membranes.

2.2 Fatty acids

Fatty acids are one of the simpler forms of lipids. They are generally indicated by the chemical formula R- COOH, where "R" stands for an alkyl chain. Fatty acid chain length varies and is commonly classified according to the number of carbon atom present. They may be short chain, medium chain or long chain fatty acids. Those of importance in human nutrition and metabolism are of long chain class containing an even number of carbon atoms.

Fatty acids are further classified according to their degree of saturation. Saturated fatty acids have no double bonds between carbon atoms, monounsaturated fatty acids contain one double bond, and polyunsaturated fatty acids contain more than one double bond. The double bonds in polyunsaturated fatty acid of both animal and plant origin are usually three carbons apart. The fatty acids commonly found in human tissue are Lauric, Myristic, Palmitic, Palmitoleic, Stearic, Oleic, Linleic, Linoleinic, Arachidic and Arachidonic acids.

Most fats in the human body are derived from the diet, which on average contains up to 40% fat, 90% of which are triglyceride. In addition, human can synthesise most fatty acids, including saturated, monounsaturated, and some polyunsaturated fats. However, some fatty acids cannot be synthesised. As these are important for growth and development, they are called essential fatty acids. They are Linoleic acid and linoleinic acids.

Much of the fatty acids in plasma exists as either esters with cholesterol or glycerol or is transported as fatty acid-albumin complex or fatty acid – prealbumin complex.[7]

Fatty acids have four major physiologic roles:

1. They are the building blocks of phospholipids and Glycolipids.
2. Many proteins are modified by the covalent attachment of fatty acid, which targets them to membrane locations.
3. Fatty acids are fuel molecules. They are stored as triacylglycerols, which are charged esters of glycerol.
4. Fatty acid derivatives serve as hormones and intracellular messengers.

2.3 Triacylglycerols

Triacylglycerols are high concentrated stores of metabolic energy because they are reduced and anhydrous. They are very non polar, and so they are stored nearly anhydrous form, where as proteins and carbohydrates are much more polar and hence more highly hydrated. In mammals the major sites of accumulation of triglycerides is the cytoplasm of adipose cells. Droplets of triacylglycerol coalesce to form a large globule, which may occupy most of the cell volume. Adipose cells are specialized for the synthesis and storage of the triacylglycerols and for their mobilization into fuel molecules that are transported to other tissues by the blood.

In human nutrition, triglycerides are the most prevalent glycerol esters encountered. They constitute 95% of tissue storage fat and are the predominant form of glycerol ester found in plasma. The fatty acids residues found in mono, di, or triglycerides vary considerably and usually include combinations of the long chain fatty acids.

Triglyceride from plants (e.g. Corn, sunflower seed, and safflower oils) tends to have large amounts of C18: 2 or Linoleic residues and are liquid at room temperature. Triglycerides from animals, especially ruminants, tend to have C_{12}:0 fatty acid residues (saturated fats) and are solid at room temperature. Some plant triglycerides, such as, coconut oil are highly saturated.

2.4 Cholesterol

The word cholesterol is derived from Greek: *chole = bile; ster = solid* and *ol = alcohol.*

Cholesterol is an alicyclic compound whose structure includes:

a). the perhydrocyclopentanophenanthrene ring,

b). a single hydroxyl group at C- 3,

c). an unsaturated center between C5 and C6

d). an eight - membered branched hydrocarbon chain attached to the D ring at position 17, and

e). a methyl group (designated C-19) attached at position 10 and another methyl group (designated C-18) attached at position 13.

In terms of physical properties, cholesterol is a lipid with very low solubility in water; at 25^0 C, the limit of solubility is approximately 0.2 mg/100ml, or 4.7μM. The very high solubility of cholesterol in blood is due to the presence of proteins called plasma lipoproteins that have ability to bind and thereby solubilise large amount of cholesterol.

Actually, only about 30% of the total circulating cholesterol occurs in free form as such; approximately 70% of the cholesterol in plasma lipoproteins exists in the form of the cholesterol esters where some long chain fatty acid, usually Linoleic acid, is attached by an ester bond to the OH group on C-3 of the A ring The presence of the long - chain fatty acid residue enhances the hydrophobicity of cholesterol

Cholesterol can be obtained from the diet or it can be synthesized *de novo.* More than 80% of the cholesterol in the body is synthesised by liver and less than 20% comes from food. [8] *An* adult on low cholesterol diet typically synthesise about 800 mg of cholesterol per day. Foods that are derived from the animal products contain cholesterol. The foods, which are particularly rich in cholesterol, include eggs, diary products such as butter, cheese, and cream, and most meat. Some of the cholesterol that is contained in these animal products is in the form of cholesteryl esters. Therefore, the ordinary diet contains a mixture of cholesterol and cholesteryl esters. Humans can readily absorb the cholesterol contained in the diet. Most people in western societies eat between 400 and 800 mg /day of cholesterol and absorb from 300 - 400 mg / day. When the dietary intake is relatively small, absorption is efficient. However, when the dietary intake exceeds approximately 500 mg /day, cholesterol absorption become somewhat less efficient.

Although *de novo* synthesis of cholesterol occurs virtually in all cells, this capacity is greatest in liver, intestine, adrenal cortex, and reproductive tissues, including ovaries, testes, and placenta

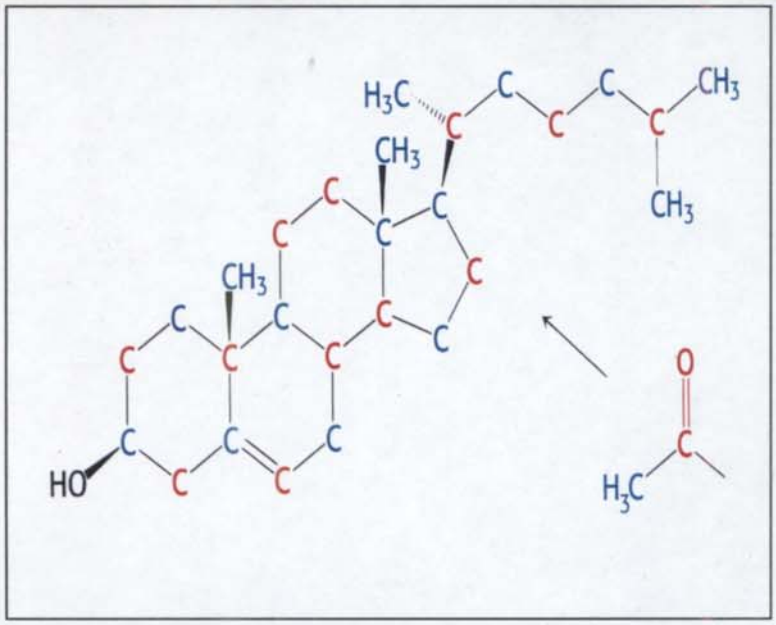


Figure 1: Labeling of Cholesterol. The result of isotope labeling experiments reveals the source of carbon atoms in cholesterol synthesised from acetate labeled in its methyl group (blue) or carboxylate atom (red). [9]

Cholesterol is synthesised from Acetyl Co A, which can be derived from carbohydrates, amino acids or fatty acids. The liver is the main site of cholesterol synthesis, but the intestine also is an important site of synthesis in humans. In addition, cholesterol is synthesised in glands that produce steroid hormones, for example, the adrenal cortex, testes, and ovaries. All the synthetic reactions occur in the cytoplasmic compartment of the cell, but some of the required enzymes are bound to the membranes of the endoplasmic reticulum.

All 27-carbon atoms of cholesterol are derived from *acetyl CoA* in a three stage synthetic process:

1. Stage one is the synthesis of the isopentenyl *pyrophosphate*, an activated isoprene unit that is the key building block of cholesterol.

2. Stage two is the condensation of six molecules of isopentenyl pyrophosphate to form *Squalene.*

3. In stage three, Squalene cyclises in an astounding reaction and tetracyclic product is subsequently converted to cholesterol.

The rate of cholesterol formation is highly responsive to the cellular level of cholesterol. This feed back regulation is mediated primarily by changes in the amount and activity of HMG CoA reductase.

1) The rate of synthesis of Reductase *m*RNA is controlled by the sterol *regulatory element binding protein (SREBP).*

2) The rate of *translation of Reductase* mRNA is inhibited by non-sterol metabolites from mevalonate as well as by dietary cholesterol.

3) The degradation *of the Reductase* is stringently controlled. The enzyme is bipartite-its cytosolic domain carries out catalysis and its membrane domain sense signals that lead to degradation.

4) *Phosphorylation* decreases the activity of reductase. This enzyme is switched off by an AMP –activated protein kinase.

All four regulatory mechanisms are modulated by receptors that sense the presence of cholesterol in the blood.

5). *Circadian rhythm*: Cholesterol synthesis also varies at different times during the day. This effect occurs predominantly in liver.. Synthesis reaches a peak about 6 hour after dark and passes through a minimum about 6 hours after re-exposure to light. The activity of hepatic HMG CoA reductase exhibits an identical diurnal variation – highest at midnight and lowest at noon. Therefore the circadian variation in cholesterol synthesis is secondary to changes in the activity of HMG CoA reductase.

Cholesterol homoeostasis is crucial for the optimal performance of multiple biochemical pathways and it is maintained by a delicate equilibrium between the cholesterol derived from dietary intake and *de novo* synthesis on one side and its utilization on other side. The free cholesterol pool in liver is maintained by the input of cholesterol from:

a). Peripheral tissues to liver via high density lipoprotein

b). Low density lipoprotein pathway

c). Non receptor mediated pathway

d). *De novo* synthesis of cholesterol

e). Absorption from gut, and

f). Re-absorption from entero-hepatic circulation and utilization through:

a). Synthesis of bile salt and bile

b). Conversion to cholesterol ester.

c). Synthesis of various lipoproteins

d). Synthesis of various steroid hormones, and

e). Synthesis of vitamin D.

The energy available in fatty acids needs to be distributed through out the body from the site of fatty acid absorption, biosynthesis or storage to the functioning tissues that consume them. This transport is closely integrated with transport of other lipids, especially cholesterol.

Human body uses three types of substances as vehicles to transport lipid based energy:

1. Chylomicrons and other plasma lipoproteins in which triglycerides are carried in protein-coated droplets, the latter also containing other lipids.

2. Fatty acids bound to serum

3. The so-called "ketone bodies"

These three vehicles are used in varying proportions to carry the energy in the blood stream via three routes:

1. Transport of dietary fatty acids as chylomicrons through out the body from the intestine after absorption.

2. Transport of lipid based energy processed by or synthesised in the liver and distributed either to adipose tissue for storage or other tissues for utilisation. In this case they used "ketone bodies" and plasma lipoproteins other than chylomicrons.

3. The transport of energy released from storage in adipose tissue to the rest of the body in the form of fatty acids that are bound to serum albumin.

2.5 Lipoproteins

Lipids synthesised in the liver and the intestines have to be transported to the various tissues to accomplish their metabolic functions. Because of their

insolubility, they are transported in the plasma in macromolecular complexes called ***Lipoproteins.***

Lipoproteins are spherical particles with non-polar lipids (Triglycerides and cholesterol esters) in their core and more polar lipids (phospholipids and free cholesterol) oriented near surface. They also contain one or more specific proteins, called ***apolipoproteins*** that are located on their surfaces. The protein components of these macromolecular aggregates have two roles- they solubilise hydrophobic lipids and contain cell targeting signals. The association of the core lipids with phospholipids and protein coat in non-covalent, occurring primarily through hydrogen bonding and vander Waals forces. The binding of lipid to protein is loose enough to allow the ready exchange of lipids among the plasma lipoproteins and between cell membrane and lipoprotein, yet strong enough to allow the various classes and sub classes of lipoprotein to be isolated by a variety of analytical techniques.

There are 5 major classes of lipoproteins – chylomicrons; very low density lipoprotein, intermediate density lipoprotein, low density lipoprotein and high density lipoproteins.

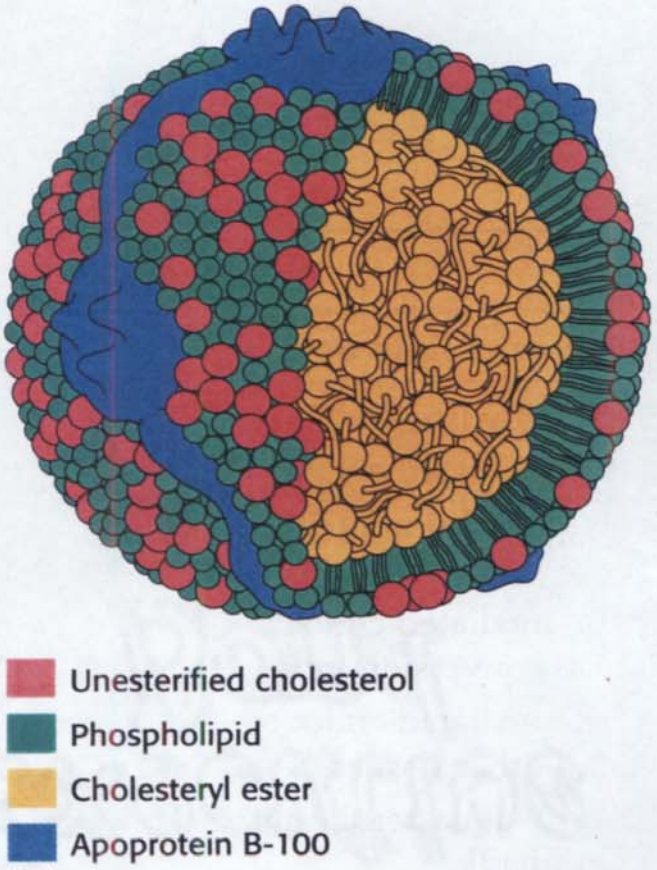


Figure 2: Schematic Model of Low-density Lipoprotein – [9]

Lipoproteins carry out three main functions:

1. Transport of the dietary fat from intestinal mucosa, to other tissues. Chylomicrons and chylomicron remnants perform this function,

2. Transfer of triglycerides from liver to other tissues. VLDL carries out this function. After the VLDL transfer their triglyceride to the tissues, their remaining constituents are returned to liver in the form of LDL and IDL, and

3. Reverse cholesterol transport - This involves HDL and LDL, returns excess cholesterol from extra-hepatic tissues to the liver.

Pure fat is less dense than water, it follows that as the proportion of lipid to protein in a lipoprotein increases, the density decreases. This property is utilized for separation of plasma lipoprotein by ultracentrifugation. Four major bands of lipoproteins can be visualized by plasma electrophoresis:

1. One band remains at the origin and contain chylomicrons.
2. Second band migrates with beta globulin and contains Low density lipoprotein.
3. Third band migrates in front of the beta region and is called prebeta lipoprotein.
4. Fourth band, called alpha lipoprotein, migrates with the alpha globulins and contain High-density lipoprotein.

Any Intermediate density lipoprotein that is present migrates with beta lipoproteins. Chylomicron remnants spread over the beta and prebeta region.

Nuclear magnetic resonance spectroscopy subdivides the lipoproteins in plasma into the following subclasses:

1. VLDL - V6 – V_1 with V_6 the largest and V_1 the smallest
2. IDL -
3. LDL - L3-L_1 with L_1 being the smallest and densest, and
4. HDL - H5 – H_1 with H_5;H_4;and H_3 being the largest particles [10]

2.6 Apolipoprotein

The protein moiety of the lipoprotein is known as apolipoprotein, constituting nearly 70% of some HDL and as little as 1% of chylomicrons. Some apolipoproteins are integral and cannot be removed, whereas others are free to transfer to other lipoproteins. The apolipoproteins have 3 functions:

1. Provide the structural element to lipoprotein particles and these are important in maintaining stability,

2. Act as ligands for specific receptors, and

3. Act as activators or inhibitors of specific enzymes involved in lipoprotein metabolism.

Individual apolipoproteins may display one, two or all of these functions. Nomenclature of apolipoproteins has been made on the basis of electrophoretic mobility of lipoproteins. – Apo - A being the apolipoprotein derived from HDL (alpha lipoprotein) and Apo- B being derived from LDL (beta lipoprotein). With the discovery of other apo- lipoproteins, succeeding letters of the alphabet have been allocated in turn. [11]

Each class of lipoprotein has a variety of apolipoproteins in differing proportions, with the exception of LDL, which contain only Apo-B100. Apolipoprotein AI and AII constitute about 90% of total HDL protein. The ratio of Apo lipoprotein AI to AII in HDL is about 3:1. In addition to being an important structural component of HDL, Apolipoprotein AI is a co factor for Lecithin Cholesterol Acyl Transferase (LCAT), the enzyme responsible for

forming cholesterol esters in plasma. Some evidence suggests that Apolipoprotein AI may inhibit LCAT and activate hepatic triglyceride lipase.

Apolipoprotein A-I is one of the first apolipoprotein to be identified and characterised. The sequence of Apolipoprotein A-I was determined by Brewer *et al.,* and followed by cloning and characterisation of its c-DNA and genomic DNA. The gene encoding apolipoprotein A-I is a member of the apolipoprotein multigene super family, which includes genes encoding exchangeable apolipoproteins (apolipoprotein A-I; A-II; Cs and E).

Apo A-I can form discoidal as well as spherical complexes, depending on the conditions of association. In vitro constituted discoidal Lipoprotein- A-I complexes usually have a pre β – HDL migration on agarose gel electrophoresis and change to an α- mobility following incubation with plasma factors (d>1.21 g/mL) or LCAT alone. Mutations in N-terminal domain have been identified and these mutations are often associated with amyloidosis. Though several mutations in the central domain of apolipoprotein A-I were described, only few of them have been associated with clear defects in lipid binding properties. Only very few mutations has been described in the C-terminal domain of apolipoprotein A-I. Funke *et al.,* reported corneal opacity and reduced plasma LCAT activity in one case. Han *et al.,* identified a new mutant associated with low HDL cholesterol level, possibly because of an increased turnover of the mutant protein, which may be due to a reduced lipid binding affinity. [12]

Apo A-II is also synthesised in liver and is structural component of HDL. Its role *in vivo* is unclear; although *in vitro* it has been demonstrated to activate hepatic triglyceride lipase.

Apolipoprotein A-IV is a component of newly secreted Chylomicrons; VLDL; LDL and HDL. Majority of this protein in plasma is not associated with lipoproteins, but exists in the free form. It has suggested that Apolipoprotein A-IV is necessary for maximal activation of lipoprotein lipase by Apolipoprotein CII, shown to activate LCAT in vitro and plays a role in the transport of cholesterol from peripheral tissues to liver.

Apolipoprotein -B exists in two forms: Apo B-100 and Apo B-48. The two proteins are known to be translocation product of single structural gene. Apolipoprotein B-100, a single polypeptide of over 4500 amino acids, is the full length translation product of ApoB gene. One gene located on chromosome 2 codes for both apo B-48 and ApoB-100. In the small intestine the *m*RNA derived from this gene is specifically modified at codon 2153, the replacement of uracil residue by cytosine changing into a stop codon. Apo B48 does not contain the LDL receptor binding domain present in Apo B100 and as a result, it is not recognised by the LDL receptor [11]

In human, **ApoB-100** is made in the liver and secreted to the plasma as a part of VLDL. Apo B-100 is the major Apolipoprotein of the LDL, the end product of the VLDL catabolism. Each VLDL particle contains one molecule of Apo B-100. In the fasting state, most of the ApoB in plasma is ApoB-100. Unlike

other apolipoptrotein, ApoB-100 can not move from one lipoprotein to another and VLDL ApoB-100 remains with Lipoprotein as it is catabolised to LDL.

Apo B - 48 contains 2152 amino acids and is identical to the amino acid terminal portion of Apo B-100, i.e.: amino terminal 48% of apo B-100 forms apoB-48.[11] Apo B48 results from the post transcriptional modification of internal Apo B-100 *m*-RNA, in which a single base substitution produces a stop codon corresponding to the residue 2153 of apo B100. Apo B-48 is made in the intestine and is the major Apo - B component of chylomicrons. Both Apo B-100 and Apo B-48 play important roles in the secretion of the VLDL and chylomicrons respectively. [7] Apo B-100 contains several hydrophobic areas that probably serve as strong lipid binding domains. It also has several domains that could serve as binding sites for heparin like molecules and form the basis for some of the cell surface interactions of the Apo-B containing lipoproteins. In addition, it contains a LDL receptor-binding domain (amino acid 3400), which allows the specific uptake of LDL by the cellular receptor. [12]

There are three different **Apolipoprotein C**, which are synthesised in liver. In plasma they transfer between the Triglyceride rich lipoproteins (Chylomicrons, VLDL, and their remnants) and HDL.

Apo C-I is the smallest of C apolipoproteins forms a minor component of VLDL, IDL and HDL and has been reported to activate LCAT in vitro.

Apo C-II occurs in Chylomicrons and VLDL, in which it functions as an activator of lipoprotein lipase and hence plays an important role in the metabolism

of triglyceride rich lipoprotein. (Chylomicrons and VLDL). It is also found in IDL and HDL.

Apo C-III forms major structural component of VLDL, but is also present in Chylomicrons, IDL and HDL [11] Because of the difference in sialic acid content, apo C-III exists in at least three polymorphic forms. It appears to have two functions. It inhibits Lipoprotein lipase and it also inhibits hepatic uptake of chylomicrons and VLDL receptor particles possibly by preventing interaction of ApoE on these remnant particles with hepatic receptors.

Apo D is a minor component of HDL, VLDL, and IDL. Its function is unknown. [11].

Apo E is the plasma lipoprotein that is formed primarily in Chylomicrons, VLDL, HDL and, chylomicron and VLDL remnants. **Apo E** is synthesised and secreted by a variety of tissues, but primarily by hepatocytes. Normal concentration of apoE is in the range of 30-70 μg/mL. The major physiological role of apoE is to mediate the interaction of lipoproteins with lipoprotein receptors, including LDL receptor and chylomicron receptors. Because it serves as a ligand for these receptors, apo-E plays a central role in determining the metabolic fate of plasma lipoproteins and therefore of cholesterol.

Apo E gene locus has multiple alleles that give rise to protein polymorphism that can be detected by isoelctric focusing. Uterman demonstrated this for the first time and Breslow further demonstrated two types of polymorphism:

1. A genetically determined polymorphism, and

2. Polymorphism caused by post translational glycosylation.

Three major isoforms of apoE termed as apoE2, apoE3 and apo E4, that results from three alleles ε2,ε3 and ε4 respectively. These alleles occur with a frequency of 8%, 77% and 15% respectively. Six common phenotypes, three homozygous (E 2/2; E 3/3; E4/4) and three heterozygous (E 4/3; E3/2; and E4/2) result from the expression of any two major three alleles.

The molecular basis for the genetically determined polymorphism is single amino acid differences at two sites in apo-E protein. Because apo-E 3 is the most frequently occurring of these three, it is considered as wild type, and apo-E 2 and apo-E 4 are considered as variants. Both apo E2 and apoE4 differ from E3 by one amino acid substitution (residue 158 in apo E2 and residue 112 in apoE4) that accounts for the charge differences among three isoforms.

The three major apoE alleles and their resultant protein product have a significant impact on normal variation of plasma lipid and lipoprotein parameters. Compared with ε3 allele, the ε2 allele is associated with lower levels of plasma cholesterol, LDL- cholesterol and LDL apoB and with slightly higher levels of plasma triglycerides and significantly higher levels of Apo- E. In contrast, ε4 allele has just opposite effect on all these parameters.

ApoE molecule is organised as two distinct structural domains, that also have different functions. – an amino terminal domain and a caboxy terminal domain. Apo-E3 and apo E4 binds normally to LDL receptor, but apo E2 has less

than 2% of normal binding ability. Substitution of Cystine for Arginine at residue 158 may be responsible for the binding defect of apo-E2 and hence important in the development type III hyperliporoteinemia. Carboxy terminal domain of apoE represents major lipid binding region. Apo–E4 has a greater preference for association with triglyceride rich lipoproteins, compared with apo-E2 and apo-E4, both of which prefer HDL. [13]

Apolipoprotein (a) is a high molecular weight glycoprotein which exhibits remarkable size heterogeneity with phenotypes ranging in size from 280000 – 700000 Daltons. It reveals striking similarities with plasminogen. It is postulated that Apo(a) gene originated via gene duplication of the plasminogen gene The apo(a) gene is located in close proximity on chromosome 6.[14].

Apolipoprotein J is a glycoprotein present in HDL_2. It is synthesised by foam cells in atheroma plaques, but not by intestinal cells. Human apo-J gene has been mapped to chromosome number 8. Apo-J inhibits macrophage mediated cell damage. [15]

2.7 Transport of Lipoprotein

Most of the cholesterol in plasma is transported as three major lipoprotein classes – VLDL cholesterol, LDL cholesterol and HDL cholesterol. The total cholesterol is the sum of all the cholesterol carried by these three lipoproteins. [10].

The pathway of lipoprotein metabolism can be divided into exogenous and endogenous pathways depending on whether they carry lipids from intestinal or hepatic origin.

Exogenous pathway: Chylomicrons are synthesised in the intestinal mucosa. Since the triacylglycerol are water insoluble, whereas the digestive enzymes are water soluble, triacylglycerol digestion takes place at lipid – water interfaces. The rate of triacylglycerol digestion therefore depends on the surface area of the interface, a quantity that is greatly increased by the churning of peristaltic movements of the intestine combined with the emulsifying action of the bile acids. Pancreatic lipase catalyses the hydrolysis of the triacylglycerol at their 1 and 3 positions to form 1, 2-diacylglycerol and 2-acylglycerols. The enzymatic activity of pancreatic lipase greatly increases when it contacts the lipid – water interface, a phenomena known as interfacial activation.

The mucosal cells in the small intestine absorb the products of digestion and the lipid contained in the bile. A mixture of fatty acids and 2-monoglycerides enters through the villi and triglycerides are synthesised from the fatty acids and monoglycerides in smooth endoplasmic reticulum . Peter O Kwiterovich reports that enterocytes also absorb free cholesterol from the gut. [10]. Meera Penumetcha, Nadya Khan Merchant, and Sampath Parthasarathy suggest that oxidized fatty acids, by enhancing the solubilisation of luminal cholesterol increases the uptake of cholesterol .[16]

After re-esterification, cholesteryl ester and triglycerides are incorporated into the core of the chylomicron particles. Enterocytes synthesise apo B48, apo A-I; Apo A-II, and Apo A-IV, which together with phospholipids, forms surface layer of chylomicron particles. Apo B-48 is essential for the chylomicron secretion. There is only one apo B-48 molecule per chylomicron particle and it

remains with the particle through out the life, until it is taken up by the liver as chylomicron remnant. Chylomicron is released from the intestinal cell by fusion of secretary vacuole with the cell membrane. Chylomicrons passes into the spaces between the intestinal cells, eventually making the way into the lymphatic system (lacteals) draining the intestine. The newly secreted chylomicrons pass intestinal lymph and gain access to the vascular system via thoracic duct. From the time of secretion they undergo constant modifications, gaining apo-CII, apo-CIII apo-E, phospholipids, and cholesterol from HDL. Acquisition of apoCII allows chylomicrons to interact with Lipoprotein lipase, which is sited in the endothelial surface of the blood vessels of the peripheral tissues, especially adipose tissues and muscles. [11]

Human LPL is a glycoprotein, mainly synthesised in adipocytes. LPL is anchored on the cell surface with a prteoglycan chain.. LPL plays an important role in regulation of plasma triglyceride concentration by hydrolysing triglycerides in chylomicron and VLDL as the first step in their metabolism. In this reaction, LPL require apoCII as an essential factor and produces chylomicron remnants and VLDL remnants, thereby releasing free fatty acids, which are either used for energy or re-esterified for endogenous triglyceride storage in adipocytes. . Apo C-III acts as an inhibitor of LPL. The resulting lipoprotein remnants appears to be additionally processed by the hepatic triglyceride lipase. Small amount of lipoprotein lipase and hepatic triglyceride lipase [HTGL] are detected in the plasma as inactive forms, whereas intravenous injection of certain amount of heparin (10-100 iu/kg of body weight) release significant amount of both lipases into circulation, as active form. Plasma obtained after injection of the

heparin (post heparin plasma) is used as a clinical sample for measuring LPL or hepatic triglyceride lipase. Analysis of LPL in post heparin plasma is usually performed as an important diagnostic measure in order to elucidate the underlying aetiology of the impaired clearance of chylomicrons and VLDL in patients with hypertriglyceridemia, which is thought to elevate the risk of Coronary artery disease. [17]

LPL acts extracellularly to hydrolyse triglycerides within chylomicron core, the fatty acid thus released can be either utilized as an energy source or re-esterified and stored in adipose tissue as triglycerides. As hydrolysis proceeds, the chylomicron core reduces in size and excess surface components, free cholesterol, phospholipids, apo C-II and apo C-III are transferred back to HDL. Apo C-III may exert a modulating effect as LPL catalyses chylomicron hydrolysis. Both apo C-II and LPL are necessary for normal chylomicron catabolism. Continuing loss of apoC-II result in either a change in its orientation or its mass is reduced to a critical level such that interaction with LPL occurs no more. Thus chylomicron remnant particles are generated which have a relatively high content of Cholesteryl ester and apo-E. These remnant particles are taken up by liver by one or more, yet unidentified receptor dependant pathways. A specific receptor has been postulated, Apo-E appears to be the ligand for this receptor mediated uptake mechanism. Remnant particles accumulate in the plasma of the subjects who are homozygous for the apo E2 isoform or who lack apo E. The cholesteryl ester delivered to the liver by the remnant particle may be utilized for the synthesis of bile acids or membrane or may be secreted in VLDL, while apo B-48 undergo degradation.[11]

Endogenous pathway: The hepatocytes are the originator and often also the acceptor of particles involved in the endogenous pathway. Hepatocyte has the ability to synthesise triglycerides from carbohydrates and fatty acids. In addition, when dietary cholesterol acquired from the receptor mediated uptake of chylomicron remnants is insufficient, hepatocytes also synthesise their own cholesterol by increasing the activity of HMG CoA reductase. ApoB-100 is synthesised in the ribosomes of the rough endoplasmic reticulum, which is the main site of triglyceride synthesis. Lipoprotein passes through Golgi apparatus, where carbohydrate residues are added to lipoprotein. The endogenously made triglycerides and cholesterol are packaged in secretory vesicles in Golgi apparatus, transported by exocytosis into the extra cellular space and introduced into the circulation through he fenestrae of the hepatic sinusoidal epithelium in the form of nascent VLDL. This triglyceride rich particle contains apo B100, apoE, and small amount of apoC at its surface. Additional cholesterol and apolipoproteins are transferred after secretion from HDL .The size of the VLDL particles secreted by the liver varies according to the hepatic availability of triglycerides. In situations where there is an excess (obesity, Diabetes, alcohol excess) large VLDL particles are secreted. Large VLDL particles are also secreted in familial hypertriglyceridemia, where as in familial combined hyperlipidemias and hypobetalipoproteinemia the rate of VLDL secretion is increased but a relative scarcity of triglycerides ensures that the individual VLDL particles are smaller.[11] Apo C-II present in the surface of the VLDL activates LPL on endothelial cells, which leads to hydrolysis of VLDL triglycerides and results in the release of free fatty acids. Rate of hydrolysis of VLDL triglyceride is

significantly lower than that of chylomicron triglycerides. The average of residence time of VLDL triglyceride is 15-16 minutes, compared with the 5-10 minute of chylomicron triglyceride. This difference may be attributed to the fact that VLDL are smaller particles and bind to fewer LDL molecules than chylomicrons.

During the hydrolysis of VLDL triglycerides, the apo-C are transferred back to HDL, VLDL particles are thus converted to VLDL remnants, some of which are taken up by liver and the rest, converted to smaller, denser particles called IDL (Intermediate density lipoprotein)

Binding of the triglyceride rich lipoprotein (produced from chylomicrons and VLDL) to liver is probably mediated by heparin sulphate proteoglycans and / or LPL as well as lipoprotein receptors (LDL receptor; LDL receptor related protein {LRP}, or VLDL receptor). VLDL receptor has been described as a new member of the LDL receptor super gene family that specifically binds VLDL in *vitro* via apo-E and LPL.

LRP is also called as alpha 2-macroglobulin receptor. LRP is a multi-ligand receptor whose ligands include ApoE containing lipoproteins, chylomicrons, LPL, protease / inhibitor complexes and toxins. An additional ligand, called receptor associated protein, is an intracellular protein that appears to function as a chaperone for LRP.

Junghan Song *et al.,* reported significant inter racial distribution of remnant receptor polymorphism and a significant association with Lipoprotein-(a), suggesting that Lp(a) metabolism in part mediated by the uptake through

remnant receptor.[18]. Large IDL particles, which also have several molecules of apo-E, bind to hepatic remnant receptor and are removed by hepatocytes. Surface materials from IDL, including some phospholipids, free cholesterol and apolipoproteins are transferred to HDL or form HDL *de novo* in circulation. Lipoprotein remnant take up appears to be mediated by receptors specific for apo-E. Both LDL receptor and a remnant receptor specific for apo-E receptor take part in remnant uptake. One candidate for remnant receptor is LRP. The net result of coupled lipolysis and cholesteryl ester exchange reaction is replacement of much of the triglyceride core of the original VLDL with cholesteryl esters. IDL undergoes a further hydrolysis in which most of the remaining triglycerides are removed and all apolipoproteins, except apoB-100, are transferred to other lipoproteins. Further hydrolysis of the IDL by hepatic triglyceride lipase (HTGL) results in the formation of LDL. HTGL has a dual role of acting as a ligand for lipoprotein and hydrolysis of triglycerides and phospholipids. In subjects who lack apo-E or are homozygous for 2/2 isoform, IDL accumulate in addition to chylomicrons. IDL also accumulates in subjects deficient in HTGL.

LDL is the major cholesterol carrying lipoprotein in the plasma and usually accounts for 70% or more of the total plasma cholesterol. Virtually, the only protein contained in the LDL particle is a simple molecule of apoB100 and this act as a ligand for the LDL receptor. LDL receptors are present on hepatocytes as well as peripheral tissues. Approximately 50% of plasma LDL uptake by the LDL receptor mediated mechanism is hepatic. The major determinant of plasma LDL concentration is the number of functional LDL receptor. In subjects, who lack functional receptors (homozygous familial

hypercholesterolemia) the LDL level is markedly elevated and LDL removal from blood is entirely dependant on non – receptor mediated mechanism.[11]

If LDL is oxidised, it can enter the macrophage through the scavenger receptors, on the surface of macrophage.[10]

2.8 LDL Receptor

LDL receptor is a glycoprotein present on the surface of most cells that mediates the uptake and degradation of LDL. It is responsible for the 80% of the clearance of LDL from circulation, the bulk of which occurs in liver, and its activity has a major influence upon the plasma cholesterol concentration. LDL receptor is discovered by Golstein and Brown in 1974.

LDL receptor has to carry out several quite distinct functions and it shall have different structural features on protein that are required for each of these functions. Thus the protein must contain:

1. Sufficient information to ensure that it is correctly glycosylated and then transported to and inserted in the cell membrane after its synthesis in endoplasmic reticulum.

2. Specific extra-cellular high affinity binding sites for its ligands apo E and ApoB and there may be additional structural information to facilitate interaction between receptors.

3. As endocytosis of ligand and receptors occurs by movement of receptors into clathrin-coated pits, the receptor must contain some recognition signal for a protein in the coated pits.

4. Structural feature required for recycling while ensuring that the ligand is delivered to appropriate site for degradation

5. Regulatory sites, as the activity of receptor are regulated by the flux of free cholesterol.

The LDL receptor protein consists of 5 structural region or domain: First domain of the LDL receptor encompasses the N-terminal 292 amino acid residues of the protein and comprises seven copies of a Cystine rich, negatively charged 40 amino acids in length that shares strong homology, particularly in the spacing of the Cystine residues, with a 40 amino acid sequence found in the terminal complement component C9. This domain contain ligand binding site.

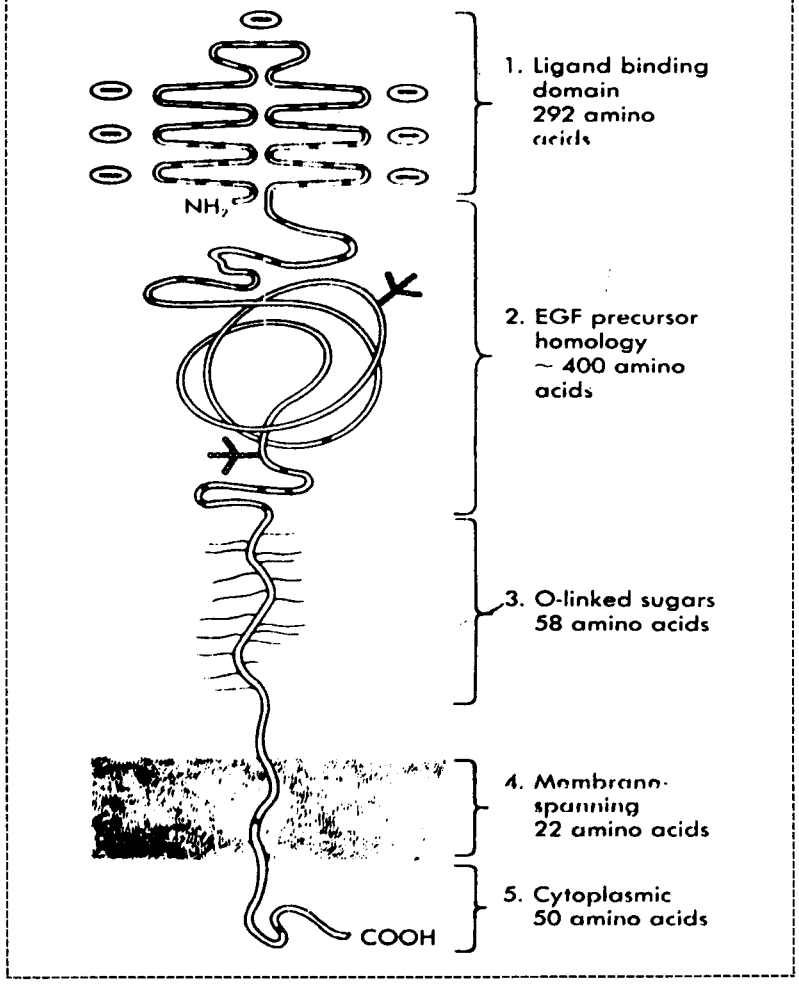


Figure 3: Structure of LDL Receptor

Second domain comprises approximately 400 amino acids with remarkable homology to the precursor of epidermal growth factor (EGF), including another three Cystine rich repeats known as growth factor like receptor. Even the introns – exon arrangement is identical in these two genes.

The third domain consists of 88 amino acid enriched in serine and threonine residues. The majority of the 18 hydroxylated amino acids are glycosylated, confirming that this part of the receptor is extra-cellular. These sugars are added co-translationally and are modified as the precursor protein undergoes maturation by passage of Golgi apparatus to the cell surface. This

change in carbohydrate residue is responsible for the apparently anomalous increment in apparent molecular weight that occurs as protein matures.

Fourth domain of the protein is a 22 amino acid stretch of hydrophobic residues bordered by charged residues that is believed to anchor the receptor in the cell surface by spanning the membrane.

Fifth domain is a COOH terminal cytoplasmic tail of 50 amino acid residues.

Once the LDL receptor protein has been transported to the cell surface, its insertion into the membrane appears to depend solely on the presence of an intact membrane-spanning domain.

Depending on the ability of the receptors to bind LDL and facilitate internalization and degeneration, and on the characteristic behavior of newly synthesised LDL receptor protein, four classes of mutation was described.

Class I mutations are defined as those in which no immuno-precepitable LDL receptor protein can be detected. This group is more correctly subdivided into:

a. Mutations, which result in no detectable *m*RNA for the receptor.

b. Those mutations in which *m*RNA is produced, but no protein.

These mutations invariably produce a receptor negative phenotype.

Class II mutations are those in which the newly synthesised precursor form of the receptor does not undergo the normal process of maturation and translocation into the cell surface. If the defect in processing is absolute, class II mutants also produce a receptor negative phenotype, but if at least some of the mutant protein reaches the cell surface and functions to some extent, then the phenotype will be receptor deficient.

Class III mutation are those in which an apparently normal protein is synthesised, but transported to the cell surface but fails to bind the ligand normally.

Class IV mutations are a group in which the receptor protein is synthesised and appears normally on the cell surface where it is fully capable of binding ligand. However the mutant receptors fail to cluster in the coated pits and are not internalised.

Changes in the amount of the LDL receptors in the cell are associated with corresponding changes in the rate receptor synthesis. The *m*RNA for the receptor is most abundant in tissues known to express high LDL receptor activity and its concentration has been shown to be increased by cholesterol depletion, in wide varieties of cultured cells. LDL receptor synthesis and *m*RNA content in cultured cells are decreased by free cholesterol or cholesterol delivered in LDL or other lipoproteins. The most potent inhibitor are oxysterols, either present as impurity or formed from cholesterol in the cell that is the metabolically active agent.

Liver expresses significant numbers of receptor and is responsible for the majority of the receptor-mediated clearance of the LDL from circulation. In cells

such as fibroblasts, regulation of the expression of LDL receptor is part of mechanism for maintaining the cholesterol homoeostasis and providing cholesterol for cell growth division. In hepatic G2 cells inhibition of cholesterol esterification greatly enhances the suppression of LDL receptor and HMG CoA reductase activity by LDL.

Synthesis of receptor and *m*RNA content has been shown to be increased by the stimulators of steroid hormone production such as ACTH and chorionic gonadotrophin in their target cells. Oestrogen treatment can produce ten fold increases in LDL receptor protein in rat liver. Hypothyroidism leads to reduction in hepatic receptor content of the rat liver, thyroxin can stimulate LDL uptake in isolated rat hepatocytes. LDL uptake by fibroblasts is decreased by epinephrine and increased by insulin. A similar effect is seen by insulin in hepatic G2 cells [19].

As LDL receptor is able to bind apolipoprotein ligands, apoB100 and apoE, it is sometimes referred as B-100 or E receptor.

Uptake of LDL, via LDL receptor is mediated through apo B100. IDL binds to LDL receptor via apoE and not via apoB100. The subclass of HDL containing apoE can also bind to LDL receptor. Lipoproteins that contain multiple copies of apoE bind to LDL receptor with much greater affinity than does LDL. [11].

Once the LDL receptor reaches the surface of the cell, they cluster in specialized thickened and indented regions of the plasma membrane called *coated pits*. Coated pits are distinguished by the presence of a polygonal matrix of proteins that coats their endoplasmic surfaces. These proteins include the major

structural protein, called clathrin, and a complex of clathrin associated protein. The presence of a defined short sequence of amino acids in the cytoplasmic domain of LDL receptor serves as a signal to direct its clustering into coated pits. The LDL receptors cluster even in the absence of their ligands, LDL. The LDL / LDL receptor complex initially enter the cell because of the invagination and pinching off of the cell surface of the coated pits. The vesicle formed by this process are called coated *endocytic vesicle.* The coat of proteins dissociates from the coated vesicles and the vesicles form the structure called *endosomes.* Proton pumps acidify the endosomal lumen. Under the mildly acidic conditions in the endosome, LDL dissociates from its receptor. This key step permits the spatial separation of the LDL and its receptor. The receptor can then be transported back to the cell surface, where it again clusters in the coated pits and can bind and internalize more LDL. This recycling of the LDL receptor permits efficient internalization of large amounts of LDL by use of relatively few LDL receptors, LDL itself is transferred into another intracellular organelle, the lysosome. Within the low pH milieu of the lysosome, an array of acid hydrolases degrades the LDL particles into its component parts – proteins are digested to amino acids, and cholesteryl esters in the core of LDL particles are converted into free cholesterol and fatty acids. Finally the cholesterol is transported out of the lysosome and enters the pool of metabolically active cholesterol in the cell, where it can be used for many purpose.[19] The released free cholesterol is available for further metabolic transformation as well as to regulate, probably via the formation of the oxysterols, the transcription and / or translation of HMG Co A reductase and LDL receptor genes. The cholesterol may be re-esterified by Acyl Coenzyme A:

Cholesteryl Acyl Transferase (ACAT) and stored, or may be utilized for bile acid, steroid or membrane synthesis depending upon tissue and cellular requirement.[11].

Hepatic catabolism of the LDL via non- LDL receptor pathway are also suggested. LDL receptor independent pathway was estimated to account for 42% of LDL cholesterol clearance in human under normal physiological conditions. Such activity could be mediated by the lipolysis stimulated receptor, a receptor that binds and degrades LDL; however the receptor is detectable only in the presence of free fatty acids. Freshly arrived free cholesterol or derivative thereof modulates the activity of enzyme that ensures cell cholesterol homoeostasis- HMG CoA reductase, acyl CoA: cholesterol acyl transferase and cholesterol 7 alpha hydrolase (CYPY). When cellular free cholesterol reaches a threshold, ACAT is activated and conjugate excess free cholesterol with long chain fatty acid to cholesterol ester. The synthesis of bile acids is catalysed by the rate limiting enzyme CYPY, that is expressed only in the liver and which expression is under control of diurnal cycle, hormone regulation and entero-hepatic circulation. High levels of bile acids returning to liver via the entero-hepatic circulation were shown to suppress CYPY activity. Many studies have shown that cholesterol induces the activity of CYPY, whereas some provided the data that dietary cholesterol does not have a stimulatory effect on CYPY.

When cholesterol uptake occurs without a parallel uptake of apolipoproteins, cholesterol ester selective uptake is suggested. Marie Claude

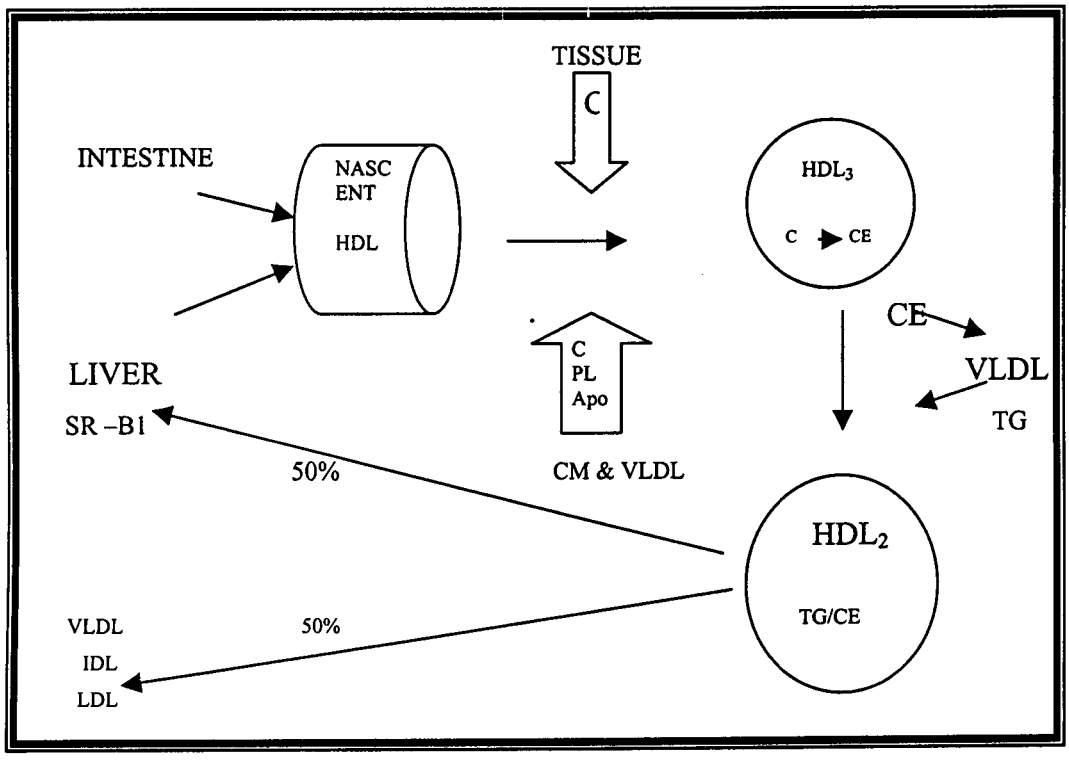
Charest *et al.,* reports that LDL – cholesterol ester selective uptake pathway plays an important physiological role in Hep G_2 cell cholesterol homoeostasis. [20]

High Density Lipoprotein [HDL] Pathway: Nascent HDL particles are produced by the liver and intestine. They composed of phospholipids, mainly apo A-I and apo A-II. Nascent HDL is disc shaped, but undergoes rapid transformation which involves esterification of cholesterol by the action of Lecithin cholesterol acyl transferase [LCAT].[11] The transfer of cholesterol from the extra hepatic cell across the cell membrane to the nascent HDL particle in the plasma during reverse cholesterol transport occurs by virtue of Adenosine-triphosphate - binding cassette protein or ABC 1 transporter protein. This is one of a family of proteins that mediate the transport of molecules across cell membrane. Unesterified cholesterol is removed from peripheral cells, through the transfer of cholesterol across the cell membrane by ABC1 transporters, is picked up by nascent HDL particle. A free fatty acid from lecithin is transferred to unesterified cholesterol in nascent HDL by action of LCAT and Apo A-1 and spherical HDL is formed. [10]. Cholesteryl esters thus formed increase the volume of HDL core, so that HDL particle within the peripheral circulation is spherical rather than discoid. Both nascent disc shaped HDL and young spherical, relatively cholesterol poor HDL particle falls within the denser part of the HDL density range (d= 1.125 – 1.21 gm/d L) and are referred to as HDL_3. Formation of cholesterol esters increases the capacity of the surface of the HDL particles for the free cholesterol which is acquired from the cell membrane possibly after interaction with a specific HDL receptor. As the volume of core increases it allows other

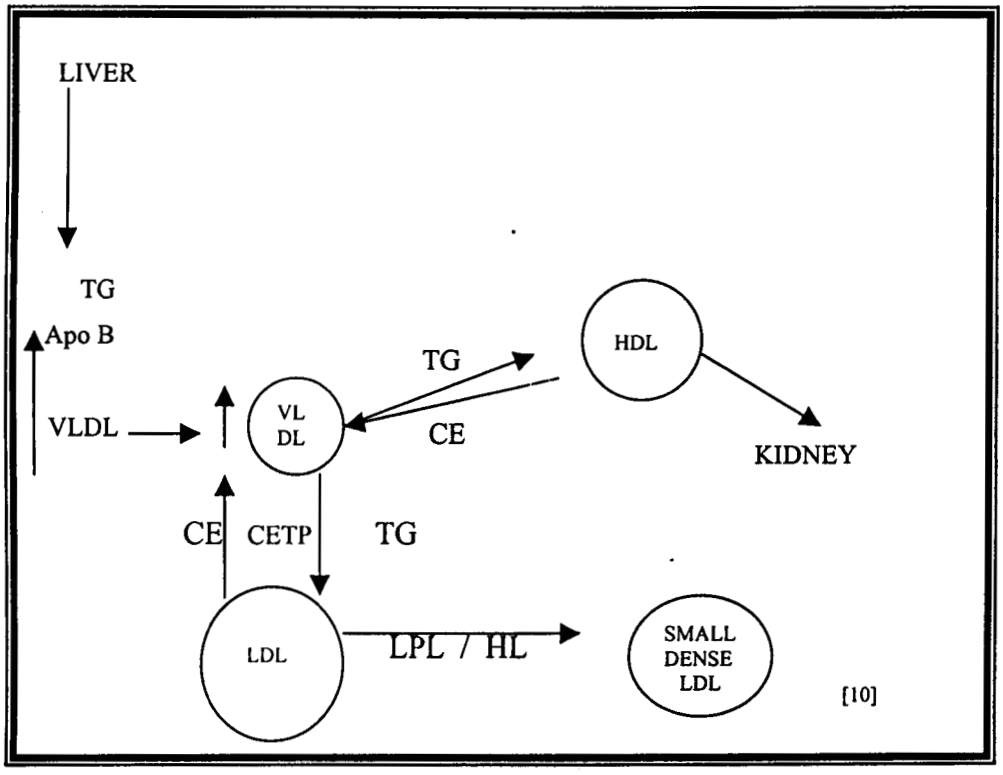
apolipoproteins (C-II, C-III & E) and phospholipids to be accommodated in the surface layer; these are acquired by transfer from the triglyceride rich lipoproteins- chylomicrons and VLDL- and results in larger, lipid enriched particles of lower density (d= 1.063 – 1.125), referred as HDL_2. HDL_2 particles can act as donors of cholesteryl esters to chylomicrons and VLDL receptors. This transfer is effected by the action of cholesteryl ester transfer protein (CETP). [11]. Yunchi Fusigawa *et al.,* reports that, cholesteryl ester fatty acid composition of lipoproteins varied widely among diet groups, with the more polyunsaturated cholesteryl ester of poly group being associated with a higher rate of cholesteryl ester transfer to endogenous acceptor apo- B containing lipoproteins. [21]. CETP contains binding sites for cholesteryl ester and triglycerides and probably acts by a carrier mediated mechanism. CETP mediates catabolism of HDL cholesteryl esters, with secondary decrease in HDL size and protein content. CETP plays a central role in reverse cholesterol transport, i.e. centripetal movement of cholesterol from periphery back to liver. CETP gene expression is up regulated in response to increased response to increased dietary cholesterol or endogenous hypercholesterolemia. Although CETP reduced HDL levels, its role in reverse cholesterol transport suggests a dominant anti atherogenic action *in vivo.* [22]

The cholesterol ester in the mature HDL particle may be selectively taken up by the liver through interaction of HDL with HDL receptor, also called as SR-B1 receptor, [10] (Scavenger receptor class B type 1). SR – B1 is a fatty acylated glycoprotein that mediate selective lipid uptake from HDL to cells. [23]. About 50% of cholesterol ester in mature HDL will be delivered to liver through the HDL – receptor. Other 50% are transferred by CETP from HDL to apo- B

containing lipoproteins – VLDL, IDL and LDL. HDL receptor interacts with HDL on the surface of steroidogenic tissues, such as Liver, Adrenal and ovary. The cholesterol esters in the core of the HDL are selectively taken up by these cells and, HDL (depleted in cholesteryl ester) is released into plasma to recycle and pick up more cholesterol. After hydrolysis of cholesteryl esters, the liberated cholesterol can be used to form steroid hormone or bile acid synthesis. [10]



Chung *et al.,* has showed that endogenous CETP and LCAT in plasma lipoprotein from individuals, together with lipoprotein lipase in plasma lipoprotein lipase, resulted in an alteration in LDL density and this effect become more pronounced as the triglyceride content of the plasma is increased, resulting in production of *small dense LDL.*



Ambrosch *et al.,* has proved that increasing CETP activity was associated with decreasing LDL particle size. Variation in CETP gene determines CETP activity. As CETP promotes transfer of cholesteryl ester from HDL to triglyceride rich lipoproteins in exchange of triglycerides, CETP is considered as a candidate for determining LDL size heterogeneity. Guerin *et al.,* found that capacity of LDL particle to receive cholesteryl ester from HDL was highly correlated to LDL-triglyceride content. Chapman *et al.,* suggested that the reason for small, dense LDL particle in hyperlipidemic state is due to high production of VLDL, which results in an increase in triglyceride pool, thus re-directing Cholesteryl ester from HDL to VLDL and the preferential transfer of Cholesteryl ester from HDL to VLDL rather than LDL. The LDL particle then became triglyceride enriched, and

the subsequent action of hepatic lipase on the triglyceride rich LDL particle converting them to small, dense particle. [24] The cholesteryl ester in the core of HDL may be exchanged by CETP for triglycerides in VLDL producing triglyceride enriched, but cholesteryl ester depleted HDL. Such HDL appears to be catabolised more rapidly by kidney. Thus, HDL level is reduced. [10]

A number of life style factors as well as various pharmaceutical agents affect serum HDL cholesterol levels. Cigarette smoking, obesity, sedentary life style as well as beta blockers and androgenic steroids are all associated with lower HDL cholesterol concentration.[25]

Increased visceral fat, substitution of carbohydrates for saturated fat in diet, reduces the HDL cholesterol levels while moderate alcohol consumption increases HDL cholesterol.[26]

There are four potential mechanisms by which HDL cholesterol may be cardio protective:

1. Reverse cholesterol transport,
2. Inhibition of LDL oxidation,
3. Reduction in the adhesion proteins such as vascular cell adhesion molecules E selectin, and
4. Increased fibrinolysis [10]

Lp(a): Lp(a) was first described in 1963 by Kare Bergh.[14] It was considered to be an autosomal dominant trait and represents quantitative rather than qualitative marker, the concentration of which can vary enormously between different individual. It has been recognized as an independent risk factor for coronary

artery disease and Lp(a) level above 30 mg/ d L form a threshold above which the risk of premature CAD increases rapidly. [27]. Stable adult level of Lp(a) is reached early in infancy and its atherogenesity is ten times more than LDL cholesterol.[28]

Lp(a) is a spherical particle of 250 Angstroms diameter that floats in a density range of 1.05-1.18/ml. The lipid composition of Lp(a) closely resembles that of LDL. The protein moiety consist primarily of two distinct proteins, namely the apoB-100 and a unique carbohydrate rich protein – apo(a). These two proteins are linked together by one or more disulphide bonds within the lipoproteins. Apo(a) can be separated from Lp(a) by reduction of disulphide bonds linking it to apoB-100 and the residual lipoprotein particle is similar to LDL in many of its physico-chemical and immunochemical properties. Further more this particle has similar affinity for LDL receptor in cultured fibroblasts while un-reduced Lp(a) has much reduced affinity and capacity for binding and degradation via this receptor. The modification effected by attachment of apo(a) to apoB- 100 via disulphide bond confers distinct physico immuno-chemical characteristics upon Lp(a).[14].

Lp(a) can not be measured by nuclear magnetic resonance, so an immuno-chemical assay must be used.

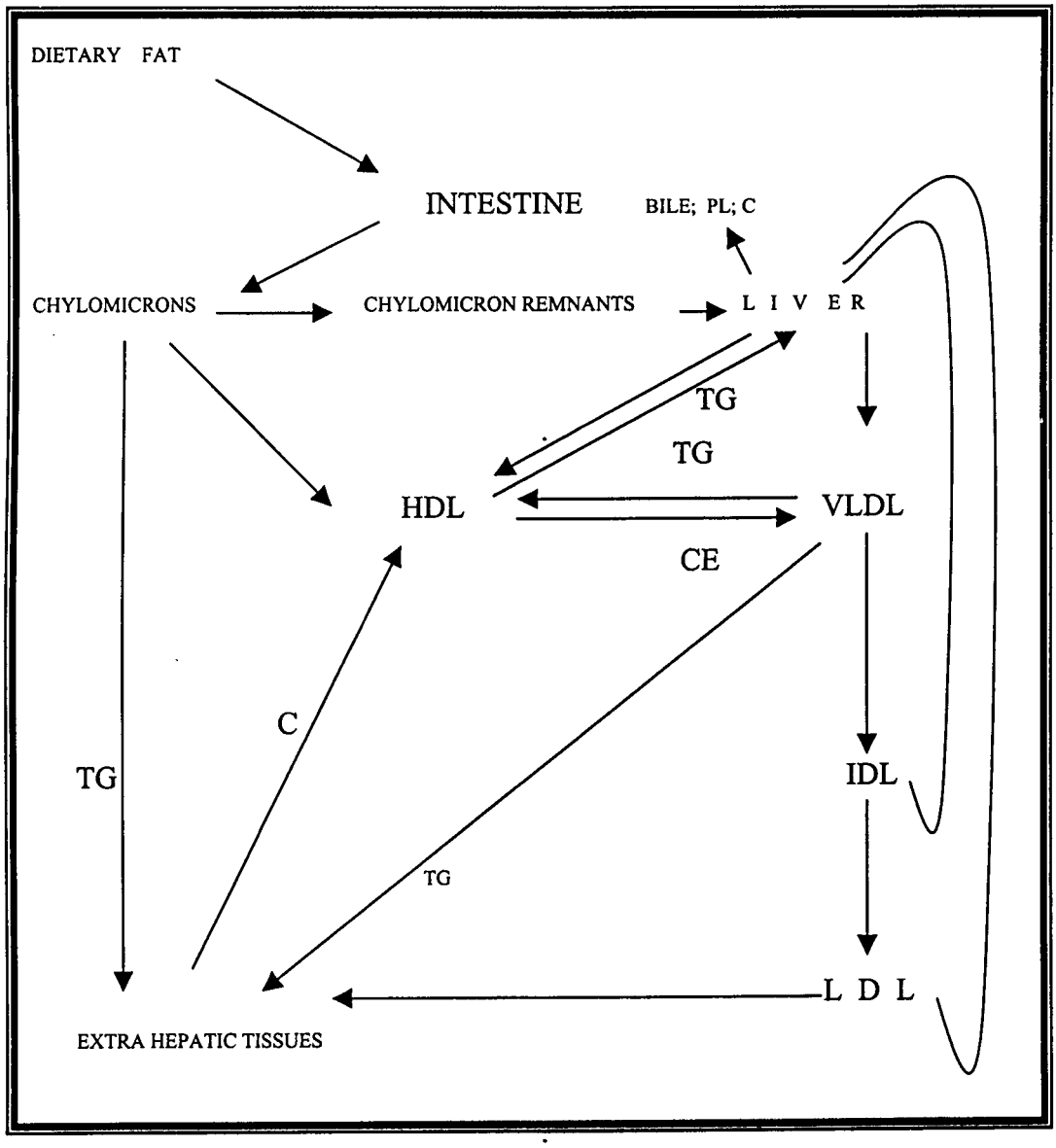
Lp(a) competes with plasminogen binding sites on the endothelial cell surface through its apo(a) component, decreasing the conversion of plasminogen to plasmin. Consequently, fibrinolysis decreases, thereby promoting thrombosis. Lp(a) can be also oxidized in the vascular wall. Lp(a) can also promote

thrombosis by stimulating plasminogen activator inhibitor 1 synthesis.[10]. Apart from above effects it is postulated that Lp(a) can promote smooth muscle cell growth, and endothelial damage. [28]

The Liver appear to be the obvious source of Lp (a). This is supported from the fact that individuals with chronic hepatic disease such as cirrhosis have low levels of Lp(a) and that apoB-100, is predominantly of hepatic origin. The concentration of Lp(a) is minimally influenced by metabolic, endocrine, and anthropometric variables. Though Lp (a) levels are largely determined by a major autosomal genetic locus (presumably apo (a) gene itself) a number of other gene loci and/or environmental factors may be influential in determining the Lp(a) levels.[27]

Lipoprotein X [LpX] : This lipoprotein does not occur in normal people, but is seen in the plasma of subjects with cholestasis and also in persons with familial lecithin cholesterol acyl transferase deficiency. It is composed of phospholipids, free cholesterol and protein; the major portion being albumin, but small amounts of apo-C and D are also present. Unlike other lipoproteins it migrates to the cathode on agar gel electrophoresis[11].. LpX has a lamellar structure and on electron microscopy appears as rouleaux of stacked disc like vesicles. LpX comprises of 6% protein, of which half or more is albumin enclosed within the vesicles. Its presence in blood is largely due to the reflux of biliary phospholipids into circulation, which attract cholesterol out of the cell membranes. LpX is catabolised by the reticuloendothelial system including kupffer cell. It may interfere with hepatic uptake of chylomicron remnants. [29]

The major pathways of lipoprotein metabolism are tightly interrelated and interdependent.



2.9 Hyperlipoproteinemias

Mutation in transporter molecules or receptors can lead to an accumulation of cholesterol and breakdown in the normal process of reverse cholesterol transport and cholesterol metabolism. The most common abnormalities lead to accumulation of excessive lipids in plasma. These diseases are collectively known as the hyperlipidemias, or more properly, the hyperlipoproteinemias.

Four types of lipoprotein abnormalities are frequently found in the population:

1. Increased low-density lipoprotein cholesterol level
2. Decreased high-density lipoprotein level, usually accompanied by increased triglyceride or VLDL levels.
3. Increased concentration of chylomicron remnants or intermediate density lipoprotein, and
4. Presence of increased concentration of abnormal lipoprotein called LP(a).

1. Increased LDL cholesterol levels:

The genetic disorder may involve LDL receptor or apoB. Increased mutation in LDL receptor gene has characterised to produce high levels of LDL cholesterol in Familial hypercholesterolemia. The genetic disorder may involve LDL receptor or apoB. They have a frequency 1 in 500 in general population and heterozygosity for a mutant allele approximately doubles the LDL cholesterol levels. ApoB gene abnormalities can be of 2 types. In *familial defective apoB-*

100, a mutation causing amino acid 3500 to change from arginin to glutamine resulting in LDL particles that fail to bind receptors and accumulate in the plasma. In some population the frequency of the mutation is as high as 1 in 500. Heterozygosity for one mutant allele increases the LDL cholesterol levels by 50%. Second type of apoB gene mutation causes the disorder *hypobetalipoproteinemia.* Many different mutations have been described with an aggregate frequency of 1 in 1000. The common feature of these mutations is that they all fail to allow translation of full length apoB polypeptide, that may act either to decrease VLDL production or to increase LDL catabolism. In either case, affected heterozygote has about 50-70% reduction of LDL cholesterol levels. [30]

An increase in LDL often occurs secondary to overproduction of VLDL, resulting in expression of 2 related disorders, *familial combined hyperlipidemia* and *familial hyperapobetalipoproteinemia.* Polygenic hypercholesterolemia results from the small contribution of a number of genes and is usually associated with moderately elevated total cholesterol and LDL cholesterol levels. [10]

2. Decreased HDL cholesterol levels and increased triglyceride levels:

Relatively rare defects in apo A-I gene can affect HDL cholesterol levels. So also, rare genetic defect in LPL, apo C-II genes and over expression of ApoC-III gene can result in hypertriglyceridemia. There is an inverse relation between HDL and triglyceride levels.

There are at least two mechanisms known to link triglyceride and HDL metabolism:

a). In the hydrolysis of VLDL triglycerides by LPL excess, surface components including apo-C are transferred to HDL. This can result in increase in HDL cholesterol level.

b). VLDL triglyceride can exchange with cholesteryl ester from HDL in presence of CETP causing decreased HDL cholesterol levels.

3. Increased Chylomicron remnants and IDL cholesterol levels:

Defects in apoE allele, results in defective clearance and subsequent accumulation of chylomicron remnants and IDL

4. Increased LP (a) levels:

Lp (a) may be atherogenic by interfering with both LDL and plasminogen metabolism [30]

It is estimated that over 60% of variability in serum lipids are genetically determined. Most of them being due to polygenic influences. Interaction between the later and the environmental factors is probably the commonest cause of hyperlipidemia in the general population. [31]

Hyperlipidemia frequently co-exists with other diseases. The co-existent disease may occur as:

a. Complication of existing hyperlipidemia – as in case of Coronary artery disease (CAD) in familial hypercholesterolemia or acute pancreatitis in hypertriglyceridemia.

b. Another primary disease affecting the lipoprotein metabolism – can give rise to secondary hyperlipidemia.

c. Hyperlipidemia may be associated with another disorder, when neither occurs as a complication of the other, such as hypertriglyceridemia and gout [29]

World health organisation's classification of lipoprotein phenotypes provides a useful means of indicating which lipoprotein are present in excess in individual patients but has a major limitation that it does not differentiate between primary and secondary forms of hyperlipidemia. This differentiation depends on the demonstration of presence or absence of an underlying causes and the result of family studies. [31].

Table 1: WHO classification of hyperlipidemias:

WHO type	Major lipid Abnormalities	Minor lipid Abnormalities	Electrophoretic change (compared to normal)	Lipoprotein abnormality
I	↑Triglycerides	↑Cholesterol	Staining at origin	Chylomicron present
IIa	↑Cholesterol		↑β band	↑LDL
IIb	↑Cholesterol ↑Triglycerides		↑β and pre β band	↑LD &↑VLDL
III	↑Cholesterol ↑Triglycerides	↑Cholesterol	Broad β band	IDL present in detectable amount
IV	↑Triglycerides	↑ Cholesterol	↑ pre β band	↑VLDL
V	↑Triglycerides		↑ pre β band & staining at origin	↑ VLDL & ↑ CMs [11]

Primary hyperlipidemias characterised by severe hypertriglyceridemia predisposes to acute pancreatitis, where as those disorders characterized by

hypercholesterolemia, apart from hyperalphalipoproteinemia, are associated with increased risk of premature vascular diseases.

Frequently, primary hyperlipidemia is polygenically determined and therefore rather ill defined, but several monogenic or dominantly inherited disorders have been described, as well as some, which are recessively inherited. The chief clinical consequence of type I and V phenotype is acute pancreatitis, while phenotype II, III and IV are associated with peripheral vascular diseases.[31].

Extra-vascular manifestations of hyperlipidemias are:

1. Xanthelasma (periorbital) – seen in any form of hypercholesterolemia; may occur in subjects with normal lipid levels; common in hyperlipidemias due to cholestasis

2. Arcus Juvenilis- Arcus senilis can occur with increasing age in subjects with normal lipid levels. If present in a subject of less than 45 years, probably denotes presence of significant underlying hyperlipidemia

4. Tendon xanthoma- Typical of autosomal dominant familial hypercholesterolemia. Also found in the very rare conditions of β – sitosterolemia and cerebrotendinous xanthomatosis

5. Tuberous & tuberoeruptive xanthomas- seen in Remnant hyperlipoproteinemia and in homozygous familial hypercholesterolemia

6. Planar xanthomas- seen in Remnant hyperlipidemia

7. Eruptive xanthoma – seen in Chylomicronemia [11]

Type I hyperliporoteinemia: This is an autosomal dominant condition and this phenotype includes:

1. LPL deficiency / familial hyperchylomicronemia,

2. Apolipoprotein C-II deficiency / C-II anapolipoproteinemia / ApoC-II deficiency

3. Familial chylomicronemia due to circulating inhibitor of LPL

Familial hyperchylomicronemia:

Holt *et al.*, reported first the familial occurrence of this syndrome. This group is characterized by massive hyperchylomicronemia, when the patient is on normal diet and disappears completely in a few days on fat free diet. On a normal diet alpha and beta lipoproteins are low. A defect in the removal of chylomicron (fat induced) and of other triglyceride rich (carbohydrate induced) is present. Reduced Post heparin lipase activity is noted. Clinically manifested by attacks of abdominal pain, hepatosplenomegaly, eruptive xanthoma and lactascence of plasma. Heterozygotes may show slight hyperlipemia and reduced post heparin lipase activity. Deficiency of LPL is the basic defect in type I hyperliporoteinemia. This can also occur in deficiency of activator of LPL – apo CII –which is called as fat induced hypertriglyceridemia.

Although heterozygotes do not usually displays the gross phenotypic features such as chylomicronemia, xanthomata or episodes of abdominal pain, they have only half normal LPL activity which might not suffice to keep plasma triglyceride concentration within the normal limit when stress is placed on the plasma lipid transport system. Responds readily to antioxidant therapy.

Apo C-II deficiency:

Apo C-II is a necessary co-factor for the activation of LPL, the enzyme that hydrolyses triglycerides in plasma and transfer the fatty acid to tissues. Beckenridge et al., (1978) reported the first case of complete deficiency of apo C-II. Clinically and bio-chemically this disorder closely resembles LPL deficiency or type I hyperlipoproteinemia and is referred as hyperliporoteinemia type IB. Xanthoma and hepatosplenomegaly are less common than LPL deficiency. Apo CII deficiency is inherited as an autosomal recessive trait. Heterozygotes have no abnormality of plasma lipid and lipoproteins in spite of reduced plasma apo CII.

Familial chylomicronemia due to circulating inhibitor of LPL:

Brunzell *et al.,* described type I hyperliproteinemia with very low levels of post heparin lipase activity and circulating inhibitor of LPL, with much higher levels of LPL in adipose tissues and normal or increased levels of ApoC-II. There appeared to have an inhibitor to LPL activity, which have been present in post heparin of normals. The inhibitor was non- dialyzable, heat stable and sensitive to repeated freezing and thawing; it appeared to be present in non- lipoprotein fraction of the plasma. Dietary fat restriction reduced triglyceride levels and prevented recent attacks of pancreatitis.

Type II hyperlipoproteinemia:

Type II hyperlipoproteinemia is classified to type IIa and IIb depending on the absence or presence of hypertriglyceridemia respectively.

Familial hypercholesterolemia: FH/LDL receptor disorder:

Familial hypercholesterolemia is an autosomal disorder characterized by elevation of serum cholesterol bound to LDL. Mutation in LDL receptor gene on chromosome 19 causes this disorder.

Heterozygotes develop tendinous xanthoma, corneal arcus, and CAD, the last usually becomes evident in the fourth or fifth decade. Homozygous develop these features at an accelerated rate in addition to planar xanthoma, which may be evident at birth in the web between first two digits. Biochemical changes are increase in serum cholesterol and LDL cholesterol (in mg/d L) of 250- 450 and 200-400 in heterozygous, greater than 500 and 400 in affected homozygous, 150-250 and 75 -175 in unaffected homozygous. Individuals with CAD may have significantly higher mean Lp(a). Defects with LDL receptor affect proper internalisation resulting in withdrawal of regulation of cholesterol biosynthesis by HMG CoA reductase, and clearance of LDL cholesterol. The frequency of homogygotes in population is 1 in million and heterozygotes not less than 1 in 500[31][32]. Diagnosis of heterozygous familial hypercholesterolemia at birth is best achieved by measuring LDL cholesterol in cord blood.

Eric J Sijbrands *et al.,* reports that mortality in familial hypercholesterolemia increased after 1915 reached its maximum between 1935

and 1964 and reduced thereafter. The variation in mortality points to strong interaction with environmental factors. [33]

Hyper beta lipoproteinemia / Hyper low density lipoproteinemia / familial hypercholesterolemic xanthomatosis:

Characterised by increased LDL – apoB of more than 120 mg/d L in plasma despite a normal concentration of LDL cholesterol.[31]. On a normal diet, the blood shows an increase in beta lipoprotein, resulting in increased serum cholesterol, while the phospholipids and triglycerides remaining constant. Clinical features are xanthoma tuberosum and tendinosum, corneal arcus, and atheromatosis. This phenotype is the commonest type of hyperlipoproteinemia. Individuals who are not affected by the LDL receptor defect, but who demonstrate a type II phenotype will likely and eventually be shown to have an abnormality that interferes with regulation of activity of HMG CoA. Most of the biochemical variants can be considered as dominant traits, though their prevalence makes appearance of homozygotes or genetic compounds relatively frequent. The latter individuals generally are more severely affected than heterozygotes and their presence in families may suggest autosomal recessive inheritance. [32]

Familial Defective Apo B100:

Characterised by a single base substitution in two codon for Arginine 3500 in the apoB gene. This gives rise to a form of apo B100 which impairs the ability of LDL to bind to the LDL receptor. Affected individual have a moderate hypercholesterolemia with a raised LDL.

Polygenic Hypercholesterolemia:

Summated effect of many different genes and environmental factors determine the distribution of cholesterol levels in the population. Clustering in of several genes which tends to induce moderate elevation of the plasma cholesterol should theoretically result in polygenic hypercholesterolemia.

Familial Combined Hyperlipidemia :

This condition was first described by **Goldstein** *et al.,* Over all 50% relatives of affected subjects were hyperlipidemic - one third hypercholesterolemic (type IIa), one third hypertriglyceridemic (type IV or V) and one third with both abnormalities (type IIb). This disease is familial and relatively common occurring in up to 0.5% of general population. This is characterised by increased synthesis of the apo B-100, with high rate of turn over of both VLDL and LDL apoB. VLDL triglyceride synthesis is increased but to a lesser extent than in familial hypertriglyceridemia, where as VLDL apoB synthesis increased to a more marked extent than in the later disorder. Conversion of IDL is increased, LDL cholesterol : apoB ratio is decreased and HDL cholesterol concentration tends to be low. No distinctive clinical features. – Diagnosis depends on demonstrating multiple phenotypes within the family. In practice individuals with type b phenotype, who does not have tendon xanthoma are often presumed to have familial hypercholesterolemia. [31].

Type III Hyperlipoproteinemia / Dysbetalipoproteinemia:

This condition is autosomal recessive with pseudo-dominance due to high gene frequency. In normal individual, chylomicron remnants and VLDL remnants are rapidly removed from the circulation by the receptor mediated endocytosis in liver. In type III hyperlipoproteinemia, increased plasma cholesterol and triglycerides are the consequence of impaired clearance of the chylomicron remnants and VLDL remnants because of a defect in apoE. Accumulation of the remnants can result in xanthomatosis and premature peripheral vascular diseases. Type III hyperlipoproteinemia can be either due to primary heritable defects in lipoprotein metabolism or to other conditions such as hypothyroidism, systemic lupus erythamatosis or diabetic acidosis. Most patients are homozygous for E2 isoform. Only rarely this disorder takes place in homozygous phenotypes. E2 isoform shows defective binding or the remnants to hepatic lipoprotein receptors and delayed clearance from the plasma. Additional genetic and / or environmental factors must be required for the disorder, because only 1-4 % of E2 / E2 homozygotes develop familial dysbetalipoproteinema. As this disorder involves exogenous cholesterol transport system, the degree of hypercholesterolemia is sensitive to the level of cholesterol in the diet. Even on normal diet, the patient may show increased plasma cholesterol and the presence of an abnormal lipoprotein called beta –VLDL. VLDL in general is increased, while LDL is reduced. Carbohydrates induce or exacerbate the hyperlipidemia, resulting in marked variability in plasma levels of lipoproteins. Often tuberous and planar and sometimes tendon xanthomas occur as well as precocious atherosclerosis and abnormal glucose tolerance. [32]. Clinical features include

corneal arcus, xanthoma, tuberoeruptive and palmar striae. Both serum triglycerides and cholesterol are increased. On ultracentrifugation the density <1.006 fraction contains cholesterol rich remnants with beta mobility on lipoprotein electrophoresis. LDL cholesterol is reduced. Management involves remedying of obvious precipitating factors like hypothyroidism, diabetes mellitus and iatrogenic influences. [31] The abnormal pattern of apoE by isoelctric focusing, specifically, the absence of apoE3 is the most characteristic feature of type III hyperlipoproteinemia. ApoE isolated from patients suffering from type III hyperlipoproteinemia had a decreased fractional catabolic rate. A variety of factors modulates or exacerbates type III hyperlipoproteinemia. In women it occurs mostly after menopause and such patients are sensitive to oestrogen therapy. Hypothyroidism exacerbates type III hyperlipoproteinemia and thyroid hormone is known to enhance receptor mediated lipoprotein metabolism. Obesity, diabetes mellitus and age are associated with increased hepatic synthesis of VLDL and / or cholesterol. [32].

Type IV Hyperlipoproteinemia/Carbohydrate Induced Hyperlipoproteinemia:

An autosomal dominant condition, in which plasma triglycerides are persistently increased, while plasma cholesterol are usually within normal limit. Precocious atherosclerosis, abnormal glucose tolerance and atheroeruptive xanthoma may occur. This disorder is heterogenous and phenotype is strongly influenced by the environmental factor, particularly carbohydrate and ethanol consumption. This disorder is caused by uremia, hypopitutarism, contraceptive steroids and glycogen storage disease. [32]. Affected subjects have larger than normal VLDL particles, with increased triglyceride: apoB-100 ratio and

accompanied by a decrease in HDL – cholesterol. VLDL triglyceride synthesis is increased than VLDL apoB synthesis. Fractional catabolic rate of both VLDL components is reduced. Free fatty acid flux into triglyceride is increased, especially when they are placed on high carbohydrate intake and the resultant increase in VLDL synthesis is accompanied by a decrease in the proportion of VLDL converted to LDL This maintains plasma LDL cholesterol levels within the normal range. Glucose intolerance and Hyperuricemia are common accompaniments. Management involves adherence to a modified fat diet designed to achieve ideal body weight, avoidance of sugars and alcohol, and encouragement of physical activity. [31]

Type V Hyperlipoproteinemia:

Autosomal dominant disorder characterised by increased amount of chylomicron and VLDL, and decreased LDL and HDL. Many conditions can cause this phenotype including Insulin dependant diabetes mellitus, contraceptive steroids, and alcoholism and glycogen storage disease. [32] Liability to develop acute pancreatitis and subjects with this type may develop xanthoma, glucose intolerance, Hyperuricemia, and peripheral neuropathy. VLDL apoB synthesis is increased and more marked decrease in fractional catabolic rate. [31].

Secondary Hyperlipidemia:

Secondary hyperlipidemia is common and occurs frequently in disorders such as obesity, alcoholism, diabetes mellitus, hypothyroidism, liver and renal diseases, and as a side effect of drug therapy, particularly for hypertension. Secondary hyperlipidemia is important because:

1. The primary disease presenting as hyperlipidemia may be an important diagnosis on its own right.

2. Secondary hyperlipidemia may be a cause of morbidity, e.g.: CAD in diabetes and renal diseases.

3. Disordered lipoprotein metabolism may accelerate the progress of primary disease as has been suggested in renal and liver diseases.

The impact of secondary hyperlipidemia depends on the milieu in which they occur and will be more extreme in people already genetically and nutritionally predisposed to hyperlipoproteinemia. Secondary hyperlipoproteinemias are not only associated with increased levels of circulating lipoproteins, but also with changes in their composition, altering both chemical and physical properties. Even in the absence of elevated lipid levels, qualitative changes in lipoprotein may render them more atherogenic.

The causes of secondary hyperlipoproteinemia are given below:

Endocrine	:	Diabetes mellitus
		Thyroid diseases.
		Pituitary diseases
		Pregnancy
Nutritional	:	Obesity
		Alcohol
		Anorexia nervosa
Renal diseases	:	Nephrotic syndrome.
		Chronic renal failure

Hepatic diseases	:	Cholestasis
		Hepatocellular disorders.
		Cholelithiasis
		Hepatoma
		Porphyria
Immunoglobulin excess	:	Myeloma
		Macroglobulinemia
		Systemic lupus erythamatosis
Hyperuricemia		
Drugs	:	Beta adrenoreceptor blockers.
		Thiazide diuretics
		Steroid hormones.
		Microsomal enzyme inducing agents
		Retinoic acid derivatives
Miscellaneous	:	Stress
		Intestinal malabsorption
		Glycogen storage disease.
		Lipidodystrophy
		Idiopathic hypercalceamia of infants
		Hypervitaminosis D
		Osteogenesis imperfecta.
		Sphingolipodystrophies
		Progeria
		Werners syndrome
		Cholesteryl ester storage disease.
		Carnitine acyl transferase deficiency
		Tangier disease
		Familial LCAT deficiency

The major effects of secondary hyperlipidemia on serum lipoprotein levels are given below:

Table 2: Effects of Secondary Hyperlipidemia on Serum Lipoprotein Levels

Cause	VLDL	LDL	HDL
NIDDM	Increased	Increased	Reduced
IDDM	Increased	Normal / reduced	Normal / reduced
Hypothyroidism	Increased	Increased	Reduced
Pregnancy	Increased	Increased	Increased
Obesity	Increased	Normal / increased	Reduced
Alcohol	Increased	Normal / increased	Increased
Nephrotic syndrome	Increased	Increased	Normal / reduced
Chronic renal failure	Increased	-	Reduced
Cholestasis	-	LpX increased	Reduced
Hepatocellular disease	Increased IDL	-	Reduced
Hyperuricemia	Increased	-	Reduced

Diabetes Mellitus:

Diabetes is not only a disorder of carbohydrate metabolism, but also of lipid and protein metabolism. Two major complication of diabetes, atherosclerosis and keto acidosis are disorders of lipid metabolism. The dominant hyperlipidemia in diabetes is hypertriglyceridemia. The enzyme LPL is activated by insulin. Insulin deficiency and / or insulin resistance associated with uncontrolled diabetes

may lead to elevation of triglyceride levels. In patients under reasonable glycemic control, hypertriglyceridemia is produced by overproduction of VLDL. Insulin deficiency or resistance produces increased lipolysis and the consequent overproduction of non esterified fatty acids results in the enhanced synthesis of the VLDL–triglyceride by liver. Increased secretion of VLDL from liver is facilitated by decreased insulin secretion and / or insulin resistance which will decrease the direct inhibitory effect of insulin on secretion of VLDL. [30]. Compositional changes in lipoproteins in diabetes includes glycosylation of LDL making it susceptible to oxidation, smaller and denser LDL and LDL has more affinity to arterial wall. The disordered lipoprotein metabolism associated with diabetes mellitus are:

i. Glycosylated VLDL is catabolised more slowly than normal VLDL.

ii. Glycosylated HDL may be less able to mobilize cholesterol out of the cells.

iii. Perhaps, most importantly, glycosylated LDL itself is more atherogenic than native LDL and is more easily oxidated, there by producing more atherogenic form of LDL.

Type 2 diabetes mellitus is associated with increased triglycerides, reduced HDL cholesterol, preponderance of small, dense LDL, increased Lp (a) and increased apoB fraction. The major causes of morbidity and mortality are related to the long term vascular complication in type 2 diabetes (NIDDM) - increased risk of large vessel diseases such as coronary artery disease, stroke, and

peripheral vascular diseases. In type 1 diabetes (IDDM) the major cause of morbidity and mortality is small vessel diseases (microangiopathy), which includes retinopathy and nephropathy. [34].

Two abnormalities characterises the lipoprotein metabolism in NIDDM. Fasting and post prandial concentration of triglyceride rich lipoprotein, especially the VLDL are higher and HDL are lower than the non diabetic people. Insulin resistant state impairs the normal suppression of fatty acid release from adipose tissue in the postprandial state. Consequently, the flux of free fatty acids to liver increases and over production of VLDL from these substrates is effected.

In non – diabetic person insulin maintains the balance between triglyceride rich lipoprotein derived from intestine and liver. In NIDDM this regulation fails and inappropriate production of VLDL results.

Hydrolysis of core triglyceride by hepatic lipase produces small, dense, HDL particles that have high fractional catabolic rate.

Small and dense LDL particles predominate.

Lipoprotein lipase activity is reduced.

Hypertriglyceridemia contribute to low HDL concentration via:

a. Reduced LPL activity and impaired lipolysis results in reduction of surface remnants available for incorporation into HDL particle.

b. Increased amount of triglyceride rich lipoprotein and their prolonged residence time in circulation increases the exchange of cholesteryl

ester from HDL to triglyceride rich lipoprotein and triglyceride to HDL, mediated by CETP. Thus HDL becomes enriched by triglyceride and triglyceride enriched HDL have faster catabolic rate that tends to reduction in number of circulating HDL particles.

HDL particles are smaller due to higher activity of hepatic lipase. Small dense HDL_2 predominate at the expense of larger, cholesteryl enriched HDL_3. [36].

Reaven and others have developed the concept of metabolic syndrome - also called as Syndrome –X, pleurimetabolic syndrome, Reavens syndrome,[34] - based on the insulin resistance that includes hypertriglyceridemia, decreased HDL, hypertension and Hyperuricemia, [36] raised concentration of glucose and high insulin levels in plasma[37].

Kay Tea Khaw *et al.,* reports that glycosylated hemoglobin (Hb A1c) was continuously related to subsequent all causes, cardiovascular and ischeamic heart disease (IHD) mortality through the whole population distribution, with lowest rate in those with HbA1c concentration below 5%. An increase of 1% in HbA1c was associated with 28% increase in the risk of death independent of age, blood pressure, body mass index, and cigarette smoking. [38].

Patients with type 2 diabetes have a two fold to three fold increased incidence of disease related atheroma and those who presents in their 40^s and 50^s have a two fold increased total mortality. R.C Turner *et al.,* reported an increased CAD risk of 11% for each increment of 1% HbA1c. [37]

Proteinuria in diabetes nephropathy may indicate a generalized increase in vascular permeability and thus macromolecules such as LDL may enter the arterial sub intima at increased rate.

Thyroid Diseases:

Hypothyroidism is characterised by low HDL cholesterol and less frequently increased triglyceride levels. There is decreased receptor mediated LDL catabolism and triglyceride catabolism and LPL activity may be also reduced. Sub clinical hypothyroidism does slight effect on LDL. There is a tendency for decreased LDL and HDL level in hyperthyroidism.

Renal Diseases:

Proteinuria in patients with relatively normal creatinine clearance produces hypercholesterolemia due to an increase in LDL. Severity of hypercholesterolemia is often proportional to reduction in serum albumin. Hypertriglyceridemia is presented in patients with chronic renal failure and is due to deficiency of LPL activity. Hypercholesterolemia is usually not associated with increased levels of LDL from VLDL and intravenous infusion of albumin reduces the LDL level. This suggests a direct hepatic secretion of LDL without a precursor VLDL and this may be important. Dr.C.Short reports that patients with Nephrotic syndrome have a high serum concentration of Lp(a). Serum HDL and apoA-1 levels are either normal or decreased. Even when total HDL is normal, HDL_2 fraction is increased. Synthesis of apoA-1 is increased, and the increased catabolism might contain normal or low circulating level. There is increased loss through kidney, related to selectively and extent of glomerular leak.

In chronic renal failure without proteinuria serum triglyceride are raised both in VLDL and LDL and remnant particles persist in circulation. This may be due to decreased activity of LPL and hepatic lipase. Hemodialysis exacerbates hypertriglyceridemia due to depletion of LPL from frequent use of heparin and apo CII from circulation. Chronic ambulatory dialysis leads to absorption of considerable amount of glucose producing obesity and hypertriglyceridemia. Serum HDL is reduced. Pre beta HDL is increased and this may be accumulated due to decreased LCAT activity. [29]

Renal diseases were observed in which there was an abnormal accumulation of lipids – lipoprotein thrombi - in glomerular capillary membrane. This has been described as lipoprotein glomerulopathy. This disorder is characterised by proteinuria, normal LCAT activity, type III hyperlipoproteinemia like lipid profile and significantly higher levels of plasma apoE. [39].

Obesity:

Obesity will exacerbate any primary hyperlipoproteinemia. Its dominant effect is to produce hypertriglyceridemia, particularly type III hyperlipoproteinemia, but in susceptible individuals hypercholesterolemia due to increased LDL will be exacerbated. Android obesity is more likely to provoke hypertriglyceridemia, than gynoid obesity assessed by determining waist: hip ratio. The cause of hypertriglyceridemia is increased VLDL production, resulting from increased release of non-esterified fatty acids from adipose tissue. Increased VLDL secretion is often matched by an enhanced catabolism due to increased LPL activity, so that hypertriglyceridemia does not invariably ensue.

Hypertriglyceridemia is more likely if there is pre-existing defect in triglyceride metabolism. There is an increased synthesis of cholesterol and serum HDL cholesterol tends to be decreased. Though triglycerides and cholesterol decreases during weight reduction, even after substantial weight reduction, HDL is not increased. [30].

A Gerald Shaper reports that "the body mass index (BMI) associated with a lowest incidence of CAD, stroke, and diabetes is not known. The risk of cardio vascular mortality, heart attack, and diabetes increased progressively from a BMI of less than 20, the lowest risk was in the range of 20-24, the levels of wide range of cardiovascular risk factor increased progressively from an index of less than 20 and a healthy BMI in middle aged men seems to be around 22". [40]. Abdominal obesity measured by the waist: hip ratio is associated with insulin resistance. High waist and fasting triglyceride measurements is a marker for metabolic syndrome. Waist circumference associated with hyperinsulinemia and high apoB and hypertriglyceridemia is associated with dense LDL cholesterol particles. Hence the abdominal obesity has taken as a marker for the production of CAD and type 2 diabetes. [41]. Ashton *et al.*, reports that as women BMI increased from less than 20 to more than 30, the blood pressure, total cholesterol, LDL cholesterol, apoB, fasting triglycerides, fasting blood sugar, levels are increased and HDL cholesterol – apoA-1 levels are decreased. A recent study in Chinese peasants also shown that Blood pressure, total cholesterol, LDL, triglycerides and blood glucose levels are increased significantly as BMI increased from less than 17 to 24 and HDL cholesterol decreased. [42]. Obesity, defined as BMI of

more than 27, is accompanied by increased risk for coronary heart disease in men and women. [1].

Thomas D Rea *et al.,* reports that excess adiposity as measured by BMI was associated with an increased risk of recurrent coronary artery events following acute myocardial infarction, especially among those who was obese. [43]. Visceral obesity also has been shown to be associated with increased risk of cardio vascular diseases. Desirable waist: hip ratio (WHR) in men is less than 0.9 where as for middle aged and elderly women it is less than 0.8 [1] Jassim Al Suwardi *et al.,* reports that obesity is associated with structural as well as functional abnormalities of early coronary artery atherosclerosis. The mechanism by which obesity contributes to the progression of atherosclerosis is independent of traditional risk factors. Intra vascular coronary ultrasound study demonstrates that obesity is independently associated with coronary atherosclerosis in-patient with angiographically normal or mild coronary artery disease. [44]

Alcohol:

The dominant effect of alcohol is to produce hypertriglyceridemia by increasing hepatic and triglyceride synthesis. Here is an increased hepatic VLDL secretion. Fatty liver ensues, if this fails to keep pace with production of triglycerides. Type IV hyperlipoproteinemia is usually produced, but individuals with constitutional tendency to delayed triglyceride catabolism, type V hyperlipoproteinemia may occur. Ethanol inhibition of oxidation of substrates tends to divert non esterified fatty acids away from oxidative pathways into triglyceride synthesis. Triglyceride synthesis further accelerate increased release of non esterified fatty acids, particularly when ethanol is taken during fasting or

by food induced fatty acidemia., when alcohol is taken during a meal. Serum LDL cholesterol tends to be low in chronic alcoholics and HDL cholesterol tends to be raised unless liver disease develops. [29]. Eric B Rimm *et al.*, reports that alcohol intake of approximately 30 gm per day, taken as beer / wine / spirits, increased concentration of HDL cholesterol by approximately 3.99 gm/d L and apo AI by almost 8.22 gm/d L, along with moderate increase of about 5.69gm/dL of triglycerides. Alcohol induced changes in lipid and haemostatic factors reduce the risk of coronary artery disease by 27%. [45]. M.S.Van der Gaag reported that plasma cholesterol esterification was increased by 10.8% after alcohol. HDL lipids changed after alcohol consumption – HDL total cholesterol, HDL cholesteryl ester, HDL free cholesterol, HDL phospholipids and apo A1 are all increased. [46].

Drugs: A large number of drugs affect the serum lipoprotein concentration.

Table 3: Effect of Drugs on Lipoprotein Levels

DRUG	VLDL	LDL	HDL
Beta adreno receptor blockers without intrinsic sympathomimetic activity	Increased	-	Reduced
Thiazides	Increased	Increased	-
Oestrogens	Increased	- or decrease in postmenopausal women	Increased
Progesterons	-	Increased	Reduced
Androgens	Decreased	Increased	Reduced
Glucocorticoids	- or increased	Increased	Increased
Hepatic microsomal enzyme inducing agents- phenytoin, phenobarbitone, rifampicin, griesofluvin	- / may be un-sustained increase	- or decreased in post menopausal women	Increased
Retinoic acid derivatives. eg: Etretinate	Increased	-	-

Decrease in triglyceride rich lipoprotein by beta adrenoreceptor blockers is due to its direct effect in reducing LPL activity. Oestorgen raises the triglyceride level by increased hepatic VLDL production.

Liver Diseases:

Obstructive jaundice without severe Hepatocellular dysfunction is associated with hypercholesterolemia and sometimes a moderate hypertriglyceridemia. This disorder is usually associated with LpX.

Moderate hypertriglyceridemia often accompanies hepatocellular diseases. This is due to triglyceride rich lipoprotein with density between VLDL and LDL range, and having beta mobility forming broad beta band on electrophoresis. HDL also has beta mobility. The accumulation of small HDL and decrease in cholesteryl ester is secondary to LCAT deficiency. Presence of lipoprotein intermediate between VLDL and LDL is due to hepatic lipase deficiency and other defects to the remnant removal mechanism.

Hyperuricemia and Gout:

Hypertriglyceridemia and Hyperuricemia are not causally related since lowering of uric acid with allopurinol does not affect triglyceride levels and conversely, with the two exemptions of nicotinic acid and fenofibrate, lipid lowering therapy does not alter the serum urate concentration. [29].

2.10 National Cholesterol Education Program (NCEP)

NCEP Suggest two major strategies for preventing coronary artery disease by lowering blood cholesterol level:

1. Clinical or patient based approach – seeks to identification of high risk individual, who will benefit from intensive intervention effort.
2. Population or public health approach. – Attempt to lower the blood cholesterol levels in the population by effecting changes in dietary habits and physical activity.

A large body of epidemiologic evidence supports a direct relationship between the level of serum LDL cholesterol (or total cholesterol) and rates of CAD. High blood cholesterol is a powerful risk factor for CAD. In many animal species, both spontaneous and diet induced hypercholesterolemia cause a form of atherosclerosis. This lesion regress when the serum cholesterol level is lowered by diet or drugs, suggesting that atherosclerosis may be reversible under certain circumstances. A large number of trials have shown that cholesterol and LDL lowering significantly reduced the incidence of CAD in primary prevention (lipid research clinic, coronary primary prevention trials, Helsinki heart study) as well as recurred CAD events and in secondary prevention (coronary drug trial). Angiographic studies have further shown that, intensive cholesterol lowering often to LDL cholesterol of 100 mg/d L or below retards the rate of progression or regress the atherosclerosis.

Clinical trails of relatively short term duration indicate that a 2% reduction in CAD rates results from each 1% reduction in serum cholesterol. And the reduction achievable with long term cholesterol lowering may be, perhaps, 3% for each 1% reduction in cholesterol. The demonstration that cholesterol lowering

prevents CAD is the cornerstone of both the public health and clinical approach to the controlling of high blood cholesterol. Lower cholesterol level is responsible for the fewer incidences of CAD in Japan, as well as seventh day Adventists group in United States. [1]. In the projected leading causes of morbidity and mortality in 2020 Car J Pepine reports that ischemic heart disease will rank first world wise - being first in developed countries and third in developing countries.[47]

A review of a large number of prospective cohort studies reported that the lowest total mortality apparently occurred in men having total cholesterol level below 200 mg/dL specifically in the range of 160-199 mg/d L. The relation between cholesterol levels and mortality appeared J-shaped. Higher total mortality occurred with high level of cholesterol, but a relatively higher mortality was also noted in men with very low total cholesterol levels. The lowest category of cholesterol analysed was that below 160mg/d L.

CAD is the leading cause of death for both men and women in United States, and accounts for 500,000 deaths per year. The total burden costs United States from $50 to $100 billion per year, including $20 to $40 billion for direct medical care costs. These amounts do not take into account of psychological costs. [1].

Eastern stroke and coronary heart disease collaborative research group reports a positive association of cholesterol with non –hemorrhagic stroke and negative association with hemorrhagic stroke in eastern Asia. [48].

Studies have shown that CAD prevalence in Indian urban population increased from 3.5% in 1960^s to 9.5% in 1990^s. In rural areas it increased from 2%

in 1970[s] to 4% at present. Several studies have clearly shown that CAD is a significant problem in India.[49]. Cardiovascular disease in 1998 accounted for 30.2% of all cause mortality in India compared to 25.5% in 1990. CAD mortality in India accounted for 16.9% CAD death worldwide. CAD prevalence shows that the problem is increasing in India, more in urban than in rural areas. Gopinath *et al.,* reported that a mean cholesterol in normal subjects of 199mg/d L and 169 mg/d L in urban and rural subjects. Rajeev Gupta reports a mean of 191 ± 53 mg/d L in a cohort of 210 adult men of higher class in Jaipur. The increase in total cholesterol levels in urban India is in contrast to the falling mean population cholesterol in USA. [2]. Aleyamma Joseph, V.Ramankutty and C.R.Soman reports the alarming situation of mean levels of lipids in a community study of an urban settlement in Thiruvananthapuram. Mean value of serum lipids of this study was given below:

Table 4: Mean Value of Serum Lipids in a study of an urban community settlement in Thiruvananthapuram

Lipid	All	Men	Women	Difference
Total cholesterol	223.7 ± 45.3	223.7 ± 44.9	223.7± 45.8	0
HDL cholesterol	54.1 ± 13.2	50±11.2	57.2± 13.8	-7.2
LDL cholesterol	145.9±41	146.3±38.7	145.7±42.8	0.6
VLDL cholesterol	23.9±16.2	28.4± 19.8	20.6± 12	7.8
Triglyceride	117.8±78.2	137.7±9.5	103.2±59.5	34.5 [6]

Indians around the globe have the highest rate of morbidity and mortality from CAD, despite the fact that nearly half of them are life long vegetarians.

Incidence of CAD rates are same among vegetarians and non – vegetarians, Hindus and Muslims, and in men and women. More over, prematurity is an important feature of CAD among Indians. The increasing incidence of CAD in India is due to combination of genetic predisposition (elevated levels of Lp(a)) and environmental factors or life style changes. The mean cholesterol level in kerala (219 mg/d L) is substantially higher than the rest of India-(New Delhi -190 mg/d L). Kerala has almost the double the rate of adults with high cholesterol (more than 240mg/d L) compared to US (Kerala 32% and US 18%). The HDL cholesterol level in Indians is 5-10 mg /d L lower than US. [5].

Executive summary of the adult treatment panel III (ATP III) [50] of National cholesterol eradication program have classified the LDL cholesterol, HDL cholesterol total cholesterol and triglyceride levels as follows:

LDL cholesterol:	Below 100 mg/d L	- Optimal
	100 – 129 mg/d L	- Near optimal / above optimal
	130 – 159 mg/ d L	- Border line high
	160 – 189 mg/d L	-High
	More than 190 mg/d L	-Very high
HDL Cholesterol:	Less than 40 mg/d L	- Low
	More than 60 mg/d L	- High

Total Cholesterol:	Less than 200mg/dL	-Desirable
	200- 239 mg/d L	-Border line high
	More than 240 mg/dL	-High
Triglyceride:	Less than 150 mg/d L	-Normal
	150 -199 mg/d L	-Border line high
	200- 499 mg/d L	- High
	More than 500 mg/d L	-Very high

ATP III identifies the sum of LDL + VLDL cholesterol (termed as non – HDL cholesterol) as a secondary target of therapy in persons with triglyceride level of 200 mg/d L or more. The goal for non – HDL cholesterol in person with high serum triglycerides can be set at 30 mg/d L higher than that of LDL cholesterol (130 mg/d L.), i.e. 160mg/d L, on the premise that a VLDL cholesterol level of 30mg/d L or less is normal.

ATP III recommends following LDL Cholesterol and non HDL cholesterol for three risk categories:

Table 5: Risk Categories (ATP III)

Risk Category	LDL goal (mg/d L)	Non-HDL cholesterol (mg/d L)
CHD & CHD equivalents (10 year risk for CAD more than 20%)	Less than 100	Less than 130
Multiple (2+) risk factors (10 year risk for CAD 20% or less)	Less than 130	Less than 160
0-1 Risk factors	Less than 160	Less than 190

ATP III does not specify a goal for HDL raising, Although clinical trial results suggests that HDL will reduce risk, the evidence is insufficient to specify a goal of therapy. Further more, currently available drugs do not robustly raise HDL cholesterol. Never the less a low HDL should receive clinical attention and management according to the following sequence. In all persons with low HDL cholesterol, the primary target of therapy is LDL cholesterol and after the LDL goal is achieved, emphasis should be given to weight reduction and increased physical activity. (When metabolic syndrome is present). When low HDL is associated with high triglycerides (200-499 mg/d L) the second priority goes to achieving non – HDL cholesterol. Currently available drugs that affect lipoprotein metabolism and their major characteristics as given in ATP III is given below:

Table 6: Effects of Currently Available Drugs on Lipoprotein Levels

Drug class Agents and daily doses	Lipid / Lipoprotein Effects	Side effects	Contra indications	Clinical trial results
HMG CoA reductase inhibitors (statins)	LDL ↓ 18-55% HDL ↑ 5-15% TG ↓ 7-30%	Myopathy Increased liver enzymes	Absolute: Active / chronic liver disease Relative: Concomitant use of some drugs	Reduced major coronary events, CAD deaths, need for coronary procedures, stroke, and total mortality
Bile acid sequestrants	LDL ↓ 15-30% HDL ↑ 3-5% TG No change or increase	Gastrointestinal distress. Constipation Decreased absorption of other drugs	Absolute: Dysbetalipoprotei nemia TG- > 400 mg / d L Relative: TG >200 mg/d L	Reduced major coronary events and CAD deaths
Nicotinic acid	LDL ↓ 5-25% HDL ↑15-35% TG ↓20-50%	Flushing Hyperglycemia Hyperuricemia Upper GI distress Hepatotoxicity	Absolute: Chronic liver disease. Severe Gout. Relative: Diabetes. Hyperuricemia Peptic ulcer disease	Reduced major coronary events, and possibly total mortality
Fibric acids	LDL ↓5-20% HDL ↑10-20% TG ↓20-50%	Dyspepsia Gallstones Myopathy Unexplained non-CAD deaths	Absolute: Severe renal disease Svere hepatic disease	Reduced major coronay events [51]

The changes in lipoprotein in the major studies - Scandinavian simvastatin survival study (4S), Cholesterol and recruitment events (CARE), Long term intervention with pravastatin in ischemic heart disease (LIPID), West of Scotland coronary prevention study (WOSCOPS), Air force/Texas coronary atherosclerosis prevention study (AFCAPS / TexCAPS) are given below:

Landmark trial	4S	CARE	LIPID	WOSCOPS	AFCAPS/ TexCAPS
Number of subjects	4,444	4,159	9,014	6,595	6,605
Mode of therapy	Simva Statin	Prava statin	Prava statin	Prava statin	Lova statin
Duration of study (years)	5.4	5	6.1	5	5.2
Baseline mean total cholesterol (mg/d L)	270	209	218	272	221
Total cholesterol reduction (%)	25	20	19	20	18
LDL reduction (%)	35	28	27	26	25
HDL increase (%)	8	5	4	5	6
Triglyceride reduction (%)	10	14	13	12	15

[5]

The changes produced by the currently available drugs are given below:

	Statins	Nicotinic acid	Fibrates	Bile acid Sequestrants
Examples	**Atorvastatin Pravastatin Simvastatin**		**Gemfibrozil**	**Colestipol Cholestyramine**
LDL cholesterol ↓	20-25%	10-25%	10-15%(may in Patients with TG	15-30%
HDL cholesterol ↓	5-20%	10-35%	5-15%	3-5%
Triglycerides ↓	5-30%	20-50%	20-50%	NONE [51]

It was reported by Ian P Hargreaves that statin lowers the concentration of endogenous coenzymeQ_{10}. A major function of Q_{10} is to serve as essential electron carrier in the mitochondrial respiratory chain, in which it plays an essential part in oxidative phosphorylation with concomitant production of ATP. A reduction in this enzyme produces severe deficits in mitochondrial energy metabolism, which in some instances presents as myopathy with exercise intolerance, and recurrent episodes of rhabdomyolysis and myoglobulinuria. Gemfibrozil was also found to decrease coenzyme Q_{10}.[52].

2.11 Review of Homoeopathy:

As a system of medicine Homoeopathy is based on the doctrine of the law of similars – "similia similibus curentur". Any agent capable of producing sickness in the healthy individual is capable of curing it in diseased individual, but

on the condition resembling its effects under experiment. Homoeopathy considers symptom complex or syndrome or disease as the result of reaction of defense mechanism (vitality) of the body to counter specific and non- specific causes. So also, its removal takes place as the resultant effect of counter action of defense mechanism to the particular drug. The actions of the drugs are studied by noting the changes produced in the spirit – psyche - physical levels during the proving on healthy individual. More over homoeopathic medicines are prepared by the process of dilution and succession - called as potentisation. The sources of homoeopathic medicines include plants, animals, minerals, secretions and excretions from healthy organs, products of diseases and various energies (like magnet, electricity etc.). By the process of potentisation the toxicity of the medicines is made null or negligible depending upon the potency. Homoeopathic medicines are given singly and in minimum doses.

Homoeopathy considers human existence in the three dimensional constitution:

1. Psychosomatic constitution - the actual emotional and physical constitution.

2. Developmental constitution – the phases through which the person passes to reach the actual psychosomatic constitution.

3. Environmental constitution – The changes effected in relation to the environmental factors of the individual. [53]

On this background each individual have a unique constitutional entity. This unique feature of the individual is attributed to the "miasmatic" influence of the individual. The term miasm is the general terminology applied to the cause of diseases before the germ theory of disease was established. Accordingly, three basic miasms are defined - psora (the deficiency miasm or hypo active miasm), sycosis (the proliferative or hyperactive or depositive miasm) and syphilis (the degenerative or destructive miasm). Combination of these miasm in different proportions predisposes the individual to different disease process and pathological changes. The development of hyperlipoproteinemia is predominantly due to sycotic miasm on a psoric background. The psoric influence produces the "deficiency of either the synthesis or/and activity of enzymes / hormones / receptors" involved in lipoprotein metabolism, while sycotic miasm induces "hyper-action" that will increase the production of the lipoproteins leading to hyperlipoproteinemia. The influence of sycotic miasm manifests its "depositive" action by deposition of cholesterol particles on the intima of vessels leading to the development of atherosclerosis. A well developed thrombus can interfere with blood supply to end organ leading to development of infarction / gangrene / necrosis. This effect is produced under the influence of the "syphilitic miasm". As the existence of the human being is based on the psychosomatic, developmental and environmental constitution, the characteristics of which are determined by the miasmatic influence, the study of disease and drug action is also based on this background.

While proving the drugs the nature of miasm and its expression in psychosomatic, developmental and environmental constitution are noted as the

characteristic along with other common features and same methodology is accepted in the study of disease also. The medicine is selected on the background of similarity of the above features.

Homoeopathic method of treatment is found to be effective in non-communicable, chronic, systemic and metabolic disorders apart from acute diseases. The drug indicated in different clinical condition under various circumstances is mentioned in different books on materia medica and repertories. This includes some of the clinical condition that can produce secondary hyperlipoproteinemia like Glycosuria, hypertension, thyroid enlargement (Goiter), inflammation of kidney, albuminuria, casts in urine, Gout, jaundice, obesity and other clinical conditions that have been produced as a complication of hyperlipoproteinemia like peripheral vascular disease (Gangrene), claudication, angina pectoris, and cerebrovascular accidents (apoplexy).

Some of the commonly used homoeopathic medicines for the above conditions are given below:[54]

Arteriosclerosis: Adrenalinum, Ammonium iodatum, Ammonium vanadinicum, Amyl nitrosum, Antimonium arsenicosum, Argentum nitricum, Arnica, Arsenicum album, Aurum metallicum, Aurum bromatum, Aurum iodatum, Aurum mur natronatrum, Baryta carbonicum, Baryta iodatum, Batryta muriaticum, Bellis perrennis, Benzoic acid, Cactus grandifolis, Calculus renalis, Calcarea carbonica Hahnemanni, Calcarea arsenicosa, Calcarea flourica naturalis, Carduus marianus, Chininum sulph, Chloralum hydratum, Conium maculatum, Crataegus oxycantha et monogyna,Cuprum metallicum, Ergotinum, Flouricum

acidum, Formica rufa, Formicum acidum, Fucus vesiculosis, Glonoinum, Hedra helix, Hypericum perforatum, Iodium purum, Kali iodatum, Kali Salicylicum, Kreosotum, Lachesis mutus, Lithium carbonicum, Magnesia flourata, Manganum aceticum, Naja tripudians, Natrum iodatum, Nitric acid, Phosphorus, Plumbum metallicum, Plumbum iodatum, Polygonum aviculare, Radium bromatum, Rauwolfia serpentine, Secale cornutum, Silicea, Solidago viraurea, Strontium carbonicum, Strontium iodatum, Strophantus, Sumbucus moschatus, Tabacum,Thalaspi bursa pastoris, Thyroidinum, Vanadium metallicum,Viscum album, Zincum phosphoricum.

Atheroma: Aurum muriaticum; Belladonna; Bromium;Calcarea carbonica Hahnemanni, Calcarea fluoratum, Capsicum,Graphites naturalis, Kali iodatum, Lachesis mutus, Lactic acid, Lycopodium clavatum, Phosphorus, Plumbum metallicum, Silicea, Sulphur.

Stroke, Apoplexy: Aconitum napellus, Agaricus muscarius, Alcoholus, Anacardium orientale, Antimonium crudum, Antimonium tartaricum, Arnica Montana, Arsenicum album, Arsenic sulphuratum flavum, Asarum europaeum, Asterias rubens, Aurum metallicum, Baptisia tinctora, Baryta carbonicum, Bellaonna, Bromium,Bryonia alba, Bufo rana, Cactus grandiflorus., Cadmium bromatum, Cadmium sulphuratum, Calcarea carba Hahnemanni, Camphora, Carboneum hydrogenisatum, Carboneum sulphuratum, Carbo vegetables, Causticum Hahnemanni, Chenopodium anthelminthicum, China officianalis, Chininum arsenicosum, Chloralum hydratum, Cocculus indica, Coffea cruda, Conium maculatum, Crocus sativus,Crotalus horridus, Cuprum metallicum,

Cuprum aceticum, Digitalis purpurea, Erigeron canadensis, Ferrum metallicum, Flouricum acidum, Formica rufa, Gastein aqua, Gelsemium sempervirens, Glonoinum, Gaurea trichillodes, Helleborus niger, Hepar sulphuris calcareum, Hydrocyanic acidum, Hyoscyamus niger, Ignatia amara, , Iodium, Ipecacuanha, Juniperus virginiana, Kali bromatum, Kali muriaticum, Kreosotum, Lachesis mutus, Laurocerasus, Lithium bromatum, Loleum temulentum, Lycopodium clavatum, Mercurius solubilis, Millefolium, Morphinum aceticum, Natrum muriaticum, Natrum nitricum, Natrum nitrosum, Nux moschata, Nux vomica,Oenanthe crocata, Oleander, Opium, Oxallicum acidum, Phosphoricum acidum, Plumbum metallicum, Pulsatilla Nigricans, Ranunculus glacialis, Rhus toxicodendron, Sabadilla officianalis, Sambucus nigra, Sarasaparilla officianalis, Secale cornutum, Sepia, Silicea, Sinapis nigra, SolanumArrebenta, Strammonium, Strontium carbonicum, Sulphur, Tabacum, Thuja occidentalis, Veratrum album, Veratrum viride, Viola odorata, Vipera berus.

Adipose tissue increased: Agaricus muscarius, Ambra grisea, Ammonium bromatum, Ammonium muriaticum, Anagallis arvensis, Antimonium tartaricum, Arnica montana, Arsenicum album, Asafoetida, Aurum metallicum, Baryta carbonicum, Belladonna, Borax veneta, Bryonia alba , Calcarea carba Hahnemanni, Calcarea arsenicosa, Camphor, Cantharis vesicatoria, Capsicum annum, Chamomilla romana, China officianalis, Clematis erecta, Coccus cacti , Colocynthis, Conium maculatum, Crocus sativa, Cuprum metallicum, Digitalis purpurea, Euphorbium officianarum, Ferrum metallicum, Graphites naturalis, Guaicum officianale, Helleborus niger, Hyoscyamus niger, Iodium, Ipecacuanha, Kali carbonicum, Lac vaccinum defloratum, Lachesis mutus, Laurocerasus,

Lycopodium clavatum, Magnesia carbonica, Mercurius, Muriaticum acidum, Natrum carbonicum, Nux moschata, Opium, Phytolacca decandra, Platinum metallicum, Plumbum metallicum, Pulsatilla nigricans, Rheum Palmatum, Sabadilla officianalis, Sarasparilla officianalis, Senega, Sepia, Silicea, Spigelia anthelmia, Spongia tosta, Strammonium, Sulphur, Thuja occidentalis, Veratrum album, Viola odorata.

Gouty constitution: Apis mellifica, Asparagus officianalis, Benzoicum acidum, Calcarea carba Hahnemanni, Calcarea phosphoricum, Capsicum annum, Chamomilla romana,Colchicum autemnale, Crotalus horridus, Guaicum officianale, Ledum palustre, Lithium carbonicum, Lycopodium clavatum, Magnesia carbonica, Menianthus trifoliata, Sabina, Urtica urens.

Hypertension: Adonis vernalis. Adrenalinum, Agaricus muscarius, Amyl nitrosum, Aranea diadema, Argentum nitricum, Arsenicum album, Asarum europaeum, Asterias rubens, Aurum metallicum, Aurum iodatum, Aurum muriaticum, Aurum muriaticum natro natrum, Bayta carb, Barium muriaticum, Calcarea renalis, Calcarea carbonicum Hahnemanni, Calcarea flouratum, Calcarea phosphoricum, Causticum Hahnemanni, Chininum sulphuricum, Coffea cruda, Conium maculatum, Cortisonum, Crataegus oxycantha et monogyna, Cuprum metallicum, Cuprum aceticum, Digitalis purpures, Flouricum acidum, Glonoinum, Gratiola officialis, Ignatia amara, Iodum, Iris versicolor, Kalium arsenicosum, Kalium muriaticum, Lahesis mutus, Latrodectus mactans, Lycopodium clavatum, Lycopus virginicus, Magnesium carbonicum, Naja tripudiens, Natrum muriaticum, Nitric acid, Nux vomica, Phosphoricum acidum, Phosphorus,

Picricum acidum, Pitutarium posterium,Plumbum metallicum, Psorinum, Pulsatilla nigricans, Radium bromatum, Reserpinum, Rauwolfia serpentina, Rhus toxicodendron, Sanguinaria canadensis, Scopolia carniolica,Secale cornutum, Sepia, Silicea, Squilla maritima, Strontium carbonicum, Stropahntus hispidus, Sulphur, Sumbulus moschatus, Tabacum, Thlaspi bursa pastoris, Thuja occidentalis, Valarianum, Vanadium, Veratrum album, Veratrum viride, Viscum album.

Jaundice: Aconitum napellus, Aesculus hippocastanum, Agaricus muscarius, Agnus castus, Aloe socotrina, Alumina, Ambra grisea, Ammonium muriaticum, Antimonium crudum, Antimonium tartaricum, Argentum nitricum, Arnica montana, Arsenicum album, Arsenicum iodatum, Arsenic sulph flavatum, Asafoetida, Astacus fluvitalis, Aurum metalicum, Aurum mur natronatrum, Belladonna, Berberis vulgaris, Bryonia alba, Bufo rana, Calcarea carbonicum Hahnemanni, Calcarea phosphoricum, Calcarea sulphuratum, Calendula officianalis, Cannabis sativa, Cantharis, Carboneum sulph, Carbo vegetabilis, Carduus marianus, Cascarilla, Causticum Hahnemanni, Oenanthe crocata, Cedron, Chamomilla romana, Chelidonium majus, Chenopodium anthelminthicum, China officianalis, Chininum Arsenicosum, Chionanthus verginica, Cholesterinum, Cina, Coca, Coccus cacti, Conium maculatum, Cornus circibata, Cornus florida, Crocus sativis, Crotalus horridus, Cuprum met, Digitalis purpurea, Dolichos pruriens, Dulcamera, Elaterium officinarum, Euphorbium officinarum, Eupatorium perfoliatum, Ferrum metallicum, Ferrum arsenicosum, Ferrum iodatum, Gelsemium sempervirens, Graphites naturalis, Helleborus niger, Hepar sulphuris calcareum, Hydrastis canadensis, Ignatia amara,

Iodium, Iris versicolor, Juglens cinerea, Kali arsenicosum, kali bichromicum, kali phosphoricum, Lachesis mutus, Laurocerasus, Leptandra virginica, Lycopodium clavatum, Magnesium muriaticum, Manganum aceticum, Medorrhinum, Mercurius, Mercurius corrosives, Mercurius dulcis, Mercurius sulph, Myrica cerifera, Natrum arsenicosum, Natrum carbonicum, Natrum muriaticum, Natrum phosphoricum, Natrum sulphuricum, Nitric acid, Nux vomica, Oleander, Opium, ostrya virginica, Petroleum, Phosphoric acid, Phosphorus, Picric acid, Plumbum metallicum, Podophyllum peltatum, Ptelia trifoliata, Pulsatilla nigricans, Rananculus bulbosus, Rheum palmatum, Rhus toxicodendron, Rumex crispus, Ruta graveolens, Sabadilla officianalis, Sanguinaria Canadensis, Secale cornutum, Sepia, Silicea, Spigelia anthelmia, Stillingia silvatica, Sulphur, Sulphuric acid, Tabacum, Taraxacum officianale, Tarentula hispanica, Thuja occidentalis, Thyroidinum, Veratrum album, Vipera berus, Yucca filamentosa..

Urine - Albuminous – Proteinuria - chronic: Atropinum purum aut sulphuricum, Cedron, Glonoinum, Petroleum, Plumbum metallicum.

Urine – Granular casts : Cantharis, Carbolicum acidum, Carcinosinum Burnett, Coccus cacti, Mercurius corrosivus, Natrum hypochlorosum, Petroleum, Phosphorus, Plumbum metallicum.

Urine – Casts – Hyaline: Carbolicum acidum, Brachyglottis repens,Medorrhinum, Petroleum, Phosphorus, Plumbum metallicum.

Urine – Sugar: Aceticum acidum, Adrenalinum, Alfalfa, Allium sativum, Alumina, Ammonium carb, Antimonium tartaricum, Argentum metallicum,

Argentum nitricum, Aristolochia milhomens, Arnica Montana, Arsenicum album, Arsenicum bromatum, Arsenicum iodatum, Aurum metallicum, Baryta carbonica, Belladonna, Benzoicum acidum, Acid boricum, Bovista lycoperdon, Bryonia album, Calcarea carbonicum, Calcarea phosphoricum, Camphor, Capsicum annum, Carbolicum acidum, Oenanthe crocata, Chamomilla romana, Chelidonium majus, Chimaphilla umbellata, China offianalis, Chininum arsenicosum, Chionanthus verginica, Coca, Codeinum purum aut phosphoricum, Coffea cruda, Colchicum autumnale, Conium maculatum, Convallaria majalis, Crotalus horridus, Cuprum metallicum, Cuprum arsenicosum, Curare, Elaps corallinus, Eupatorium perfoliatum, Fel tauri, Ferrum iodatum, Ferrum metallicum, Flouricum acidum, Glonoinum, Glycerinum, Grindelia robusta, Helleborus niger, Helonias diocia, Hepar sulphuris calcareum, Iodum, Iris versicolor, Kalium arsenicosum, Kalium bromatum, Kalium chlorosum, Kalium nitricum, Kalium phosphoricum, Kreasotum, Lac defloratum, Lac caninum, Lecithinum, Lithium carbonicum, Lycopodium clavatum, Lycopus virginicus, Lyssinum, Magnesium sulphurica, Medorrhinum, Moschus, Morphinum, Muriaticum acidum, Murex purpureus, Natrum muriaticum, Natrum phosphoricum, Natrum sulphuricum, Nitric acid, Nux vomica, Opium, Petroleum, Phosphoricum acidum, Phaseolus nanus,Phosphorus, Picricum acidum, Plumbum metallicum, Podophyllum peltatum, Ratanhia peruviana, Rhus aromaticus, Salicylicum acidum,Secale cornutum. Sepia, Silicea, Squilla maritima, Sulphur, Sulphuricum acidum, Taraxacum officianale, Tarentula hispanica, Terebinthiniae oleum, Thuja occidentalis, Thyroidinum, Tuberculinum bovinum Kent, Uranium nitricum, Urea, Vanadium, Zincum metallicum, Zizia aurea.

Homoeopathic medicines are prepared in three different methods of potentisation - decimal, centesimal and 50 millesimal scale. Of these centesimal scale medicines are most widely used. These medicines are available in 30, 200, 1000 (1m), 10m, 50m and cm potencies. The potency of medicine is selected on the basis of the susceptibility of patient, which is modified by seat of disease, nature and intensity of the disease, stage and duration of disease, and the previous methods of treatment. The nature of different medicinal substances, the corporeal constitution of the patient, and the magnitude of the disease guide the repetition of medicine.

Though the drugs for various causative and modifying factors of above clinical conditions are explained in detail, no biochemical correlations of them are yet mentioned. It is to be particularly noted that the drugs for cholesterol metabolism disorders were not yet been known to be mentioned in the Homoeopathic literature. At the same time, many of the patients with above clinical conditions were found to be responding well to the Homoeopathic method of treatment. On this background this study was conducted.

Chapter - 3

MATERIALS AND METHODS

This study was conducted in patients attending Unit 1 of the Organon and Homeopathic philosophy department at Government Homoeopathic Medical College, Calicut. The study started from 27.9.01 and results obtained up to 16.10.2003 were taken for statistical analysis.

Patients with obesity, Xanthoma, hypertension, liver diseases, and those presented with known history of hyperlipidemia and coronary artery diseases were screened for the selection. A total number of 148 patients were screened for lipid profile and 86 patients continued for different duration for the treatment.

The cases were taken and analyzed according to Homoeopathic philosophy and prescriptions were made on the salient features expressed on the psychosomatic, developmental and environmental constitution. The Lipid profile was estimated after 12 hours fasting and other necessary investigations were also conducted. Total cholesterol, triglycerides and high-density lipoproteins were estimated by enzymatic method. Very low-density lipoprotein and low-density lipoprotein were calculated from the above values. The results were correlated with clinical history and medicine with suitable potency was selected. The selected potencies were either of 30, 200, 1m, 10m, 50m, or cm potencies. One medicated globule of number 30 size is divided into two doses after mixing with sugar of milk and is administered orally at 12 hours interval. On an average 30^{th} potency was repeated at fifth or seventh day, 200^{th} potency was repeated biweekly, 1m potency was repeated at monthly interval, 10m potency was

repeated at three or four months interval, 50m potency was repeated at six monthly interval and cm potency was repeated at yearly interval depending on the improvement. The cases were evaluated monthly according to the clinical features and value of lipid profile. The same medicine was repeated in the order of increasing potency. In cases where vide fluctuations in lipid profiles were resulted, a new medicine under the follow up group of first prescription or an anti-miasmatic medicine was administered.

Reagents from Autospan, Reckon diagnostics private limited and Merck Labkit were used for the estimation of Cholesterol, HDL and Triglyceride and readings were made by photoelectric calorimeter from Erma Inc, using filter with wavelength 530nm.

3.1 Estimation of Cholesterol:

a) Principle: Cholesterol and its esters are released from lipoproteins by detergents. Cholesterol esterase hydrolyse the esters and hydrogen peroxide is formed in the subsequent enzymatic oxidation of cholesterol by cholesterol – oxidase according to the following equation:

Cholesterol esters $\xrightarrow{\text{cholesterol esterase}}$ Cholesterol + fatty acids

Cholesterol + H_2O $\xrightarrow{\text{Cholesterol oxidase}}$ Cholestenone + H_2O_2

H_2O_2 + 4-Aminophenazone +Phenol $\xrightarrow{\text{Peroxidase}}$ Quinoneimine + H_2O

The quantity of this red dye quinoneimine is proportional to the cholesterol concentration. Absorbance of quinoneimine measured is proportional to the cholesterol concentration in the specimen.

Reagent 1 (cholesterol reagent) of Autospan contains cholesterol oxidase, cholesterol esterase, peroxidase and 4-Aminophenazine and reagent 2 (cholesterol diluent) contains buffer, phenol, surfactant, preservatives and stabilisers. The regents of other firms also have similar combination.

Working reagent is prepared by dissolving the cholesterol reagent with cholesterol diluent and is labeled as "working reagent", which is stable for 90 days when stored at 2-8°C.

The reagents and samples are brought to room temperature before use.

b) Procedure:

Pipette in to tubes marked	Blank	Standard	Test
Serum	--	--	10µl
Cholesterol standard	--	10µl	--
Working cholesterol reagent	1000µl	1000µl	1000µl

Mixed well. Incubated for 10 minutes at 37°C and absorbance is measured against blank

c) Calculation:

$$\text{Cholesterol concentration (mg/d L)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times 200$$

3.2 Estimation of Triglyceride:

a) Principle: The triglycerides are enzymatically hydrolysed to glycerol and free fatty acids. The glycerol liberated reacts with Glycerol kinase and glycerol-3-phosphate oxidase yielding H_2O_2. The H_2O_2 concentration is determined through the Trinder's reaction.

$$\text{Triglyceride} + H_2O \xrightarrow{\text{Lipoprotein lipase}} \text{Glycerol} + \text{fatty acids}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{Glycerol kinase}} \text{Glycerol-3-Phosphate} + \text{ADP}$$

$$\text{Glycerol-3-Phosphate} + O_2 \xrightarrow{\text{Glycerol-3-phosphate oxidase}} \text{Dihydroxyacetone-P} + H_2O_2$$

$$2H_2O_2 + \text{Chromogen} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine} + H_2O$$

Absorbance of the Quinoneimine is directly proportional to triglyceride concentration. Triglyceride mono reagent of Autospan (Liquid gold) contains 4-chlorophenol, magnesium ion, ATP, lipase, peroxidase, glycerol kinase, sodium azide, 4-aminoantipyrine, glycerol 3 phosphate oxidase and detergents. Mono-reagent is ready for use.

Reagent 1 of Labkit gold buffer Ph 7.5, p-chlorophenol and reagent 2 contain lipoprotein lipase, glycerol kinase, and glycerol – p- oxidase, peroxidase, 4-aminophenazone and ATP. Working reagent is prepared by dissolving contents of reagent 2 to the contents of buffer reagent 1. The working reagent is stable for 4 weeks at 2-8° C.

b) Procedure:

Pipette in to tubes marked	Blank	Standard	Test
Serum	--	--	10µl
Triglyceride standard	--	10µl	--
Triglyceride reagent	1000µl	1000µl	1000µl

Mixed well. Incubated for 10 minutes at 37^0C and absorbance is measured against blank

Calculation:

$$\text{Triglyceride concentration (mg/d L)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times 200$$

3.3 Estimation of HDL:

a) Principle: Low density lipoprotein (LDL & VLDL) are specifically precipitated by phosphostungstic acid and magnesium ions (Labkit) and can then be removed by centrifugation. High density lipoprotein (HDL) remain supernatant. HDL cholesterol is estimated in the supernatant, using cholesterol working reagent, by a series of enzymatic reactions which are initiated by the oxidation of cholesterol into Cholestenone by cholesterol oxidase, accompanied by the formation of hydrogen peroxide. In a second reaction catalyzed by peroxidase, 4-aminoantipyrine and phenol react with hydrogen peroxide to form red coloured quinoneimine. Absorbance is directly proportional to HDL concentrations. The precipitating reagent of Autospan contains PEG 6000, stabilizer, and preservative.

b(i) Procedure for Autospan:

Step 1:

Serum	0.3 ml
Precipitating reagent	0.3ml

Mixed well and kept for 10 minutes and then centrifuged for 15 minutes at 2000 rotation per minute. The supernatant solution obtained is used for the step 2.

Step 2:

Pipette into tubes marked	Blank	Standard	Test
Supernatant from step -1	--	--	100µl
HDL cholesterol standard	--	100µl	--
Cholesterol working reagent	1000µl	1000µl	1000µl

Mixed well and incubated for 10 minutes at 37°C and absorbance measured against blank

Calculation:

$$\text{HDL - Cholesterol concentration (mg/d L)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times 50 \times 2$$

b(ii) Procedure for Merck –Labkit

Step -1:

Serum	500µl
Precipitating reagent	50µl

Mixed well and kept for 10 minutes at room temperature and then centrifuged for 20 minutes at 4000 rotation per minute. The supernatant solution is used for the second step.

Step - 2:

Pipette into tubes marked	Blank	Standard	Test
Supernatant from step -1	--	--	20µl
HDL cholesterol standard	--	20µl	--
Cholesterol working reagent	1000µl	1000µl	1000µl

Mixed well and incubated for 5 minutes at 37^0C. Then absorbance is measured against blank.

$$\text{HDL - Cholesterol concentration (mg/d L)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times 50$$

3.4 Estimation of VLDL: Calculated from the formula,

$$\text{VLDL} = \frac{\text{Triglyceride}}{5}$$

3.5 Estimation of LDL:

LDL cholesterol was estimated by the Friedewald calculation from fasting measurements of Total Cholesterol, HDL – cholesterol and Triglyceride.

$$\text{LDL} = \text{TC} - (\text{HDL} + \text{VLDL})$$

Those patients under allopathic medicine for hyperlipidemia were advised to continue the drugs and to gradually stop them. Some of the patients under

allopathic medicines reported after stopping them. In such patients the lipid profile value after one month of stopping the medicine are taken for tabulation.

Along with the lipid profile other investigations like fasting blood sugar and urine sugar in diabetic group, serum bilirubin in patients with liver disease, blood urea and serum creatinine in hypertensive patients, ECG and routine blood & urine were made as and when necessary.

The various standard characteristics of the data were obtained for the total sample and sub- samples. Further data were tested for significance in mean value by using student t-test and using necessary statistical tools for testing them.

Statistical analysis was done using SPSS (statistical package for social science) for MS windows release 6.0.

The percentage of developing cardio vascular risk in 10 years in patients of primary and hypertensive groups are calculated using online LDL cholesterol goal calculator, provided by Medical college of Wisconsin, General Internal Medicine 9200 W. Wisconsin Ave. Milwaukee, WI. 53226.

3.6 Medicines

The characteristic features of the medicines used in this study are given below:

Iodum: Dr.Hahnemann proved this medicine.

- This medicine is useful in indurations, effusions, tumours and goiter.

- ❖ Emaciation of single parts.
- ❖ Increased appetite – eats voraciously, yet emaciates.
- ❖ Suited to persons of dark hair and complexion, dark, yellow, tawny, skin.
- ❖ Excitable and restless, moving about one place to another, impulsive.
- ❖ Atrophy of nerve, brain tissue, and other tissue, hydrocephalus, pleuritic effusions, tubercular affections, rheumatism, heart affection, indurations or atrophy of testes or ovaries or uterus, affection of salivary gland and pancreas, hypertrophy of heart.
- ❖ Complaints are aggravated by warmth, movement, wet weather, touch, pressure, and ameliorated by sitting up and eating.

Lachesis mutus:

This medicine is prepared from the venom of surukuku. Proved by Dr.Hering and is introduced in 1828. The characteristic features of this remedy include:

- ❖ Suited to melancholic and choleric constitution.
- ❖ Aggravation of the complaints on going to sleep and waking from sleep; from alcohol; spring; summer; extremes of temperature; sun rays; change of weather.
- ❖ Excessive sensitiveness of the surface with intolerance to touch or a constrictive sensation.
- ❖ Left sided complaints or complaints proceeds from left to right

- ❖ Complaints are relieved by onset of any discharges; by eating, especially fruit; warmth; and night hours.
- ❖ Hemorrhagic diathesis, with degeneration of blood.
- ❖ Desires oysters, wine, and coffee.
- ❖ Palpitation with numbness down the chest with difficulty in breathing, irregularities of heartbeats and constrictive pain in the region of the chest.
- ❖ Lachesis mutus is useful in heamorrhagic disorders, cardiovascular disorders, central nervous system diseases, renal disorders, genito – urinary diseases, inflammations, tumours and malignancies

Luesinum:

This medicine was proved by Swan and is characterised by:

- ❖ Aggravation at night. Complaints begins with twilight and end with daylight.
- ❖ Ulceration, with foetid discharges.
- ❖ Abscesses with foul secretions. Succession of abscesses
- ❖ Useful in ptosis, diplopia, opthalmia neonatorum, tendency for decay of teeth at the edge of gum, copper coloured eruptions, glandular affections, impaired nutrition leading to emaciation, obstinate constipation, affection of bones.
- ❖ Pain aggravates and ameliorates gradually.

- ❖ Obsession to wash hand often.
- ❖ Craving for alcohol.
- ❖ Sleep, anxious, distressed, and often wakeful, and violently restless.
- ❖ Complaints are aggravated by touch, movement, raising arm laterally, warm weather, damp weather and thunder-storm, sea side, winter and relieved by cold water.

Lycopodium clavatum:

This medicine was introduced into homoeopathic practice by Dr.Hahnemann in 1826. This medicine was prepared from the spores of ever green club moss. The characteristic features of this medicine include:

- ❖ Used for cardialgia, and flatulent colic of children and young girls, diseases of children, nephritic colic and calculi, rheumatism, epilepsy, pulmonary diseases, enlargement of heart.
- ❖ It has action primarily on digestive organs and adjoining glands, on liver, and large intestine.
- ❖ Aversion to bread, and from eating bread and food made of fermented and fermentable dough.
- ❖ Deficiency of vital heat.
- ❖ Suited to persons of keen intellect, but feeble muscular development, upper part of body emaciated and lower semi-dropsical, lean, and

predisposed to lung and hepatic conditions, lithic acid diathesis and haughty disposition.

- Complaints are right sided or extends from right to left.
- Complaints are aggravated from 4 – 8 pm; cold food and drinks, touch, pressure, afternoon, after eating, warm room, and ameliorated from uncovering; loosening the garments, in open air.
- Fan like movement of alae nasi occurring in cerebral, pulmonary, and abdominal complaints, rapid and not synchronous with respiration.
- Spasmodic movements of facial muscles; tongue; nodding and side-to-side movement of head.
- Half opened condition of the eyes during sleep.
- Extreme despondency and melancholy.
- Excessive appetite easily satisfied. Fullness of abdomen with flatulence.
- Suddenness of the complaints.
- Restlessness relieved by movement.
- Right foot hot and, left foot cold.
- Burning pain relieved by heat; Burning like hot coals in between scapulae; burning and stinging in breast.
- Dryness of the mucus membrane and skin.

- ❖ Fearful, apprehensive, sadness, inclination to weep, peevish, forgetful, avaricious, and is a remedy for misers.

- ❖ Excessive sensitiveness cannot bear any strong smells, noises.

Medorrhinum:

Medorrhinum is one of the most important nosode and is characterized by:

- ❖ Aggravation of the complaints from sunrise to sunset; early morning hours, rest, warmth and relieved by lying on face or mouth, damp weather and 3-4 am.

- ❖ Intense nervous sensibility – starting at slightest sound.

- ❖ Desire to be fanned.

- ❖ Tremor, spasm.

- ❖ Obstinate rheumatism and sequelae of acute rheumatism.

- ❖ Convulsions coming in early morning during menstrual period. Clonic spasms, the legs suddenly shot up from the bed.

- ❖ Polypi having their origin in chronic suppurative discharges.

- ❖ Albuminuria, when the urine contains some mucus as well.

- ❖ Asthma aggravated at 2-4 am

- ❖ Useful in ovarian tumours, oophritis, salpingitis, metritis, parametritis, endometritis, peritonitis.

Natrum muriaticum:

This medicine is prepared from common salt. This medicine was first proved by Dr.Hahnemann and followed by others. The characteristic features of this medicine include:

- ❖ Suited to cachetic persons, old people, teething children, anemia, chlorotic persons with catarrhal troubles, tuberculous, scrofulous and emaciated people.

- ❖ Chilly patient, suffers from cold extremities, and have a sensation of cold along the spine, with strong desire for common salt.

- ❖ Salty taste and pathological secretions are corrosive.

- ❖ Tearful mood, wants to be alone (to cry), consolation irritates, very much inclined to weep, and be excited. Melancholic.

- ❖ Constipation, with sensation of contraction of rectum during stool, hard feaces at first, evacuated with the greatest exertion, which causes tearing in anus, bleeding, and soreness, afterwards thin stool also passed.

- ❖ The unclean complexion of earthy hue, "dirty face" in spite of any amount of washing.

- ❖ Skin is greasy from excess of sebaceous secretions.

- ❖ Complaints from loss of vital fluids.

- Headache associated with errors of refraction and consequent eyestrain; headache aggravated at 10-11 am, mental exertion, of school going girls.

- Mapped tongue with red islands or clean shining tongue, sensation of hair on the tongue.

- Children – late learning to talk.

- Dryness of mouth and throat and other mucus membrane.

- Corresponds to anemia, delayed menarche from anemia.

- Low backache relieved by lying on back and pressure.

- Heart – fluttering palpitation with faint feeling aggravation on lying down. Sensation of constriction in different parts of the body.

- Sinking sensation in abdomen.

- Ravenous appetite. Eats well but emaciates.

- Desires bitter things, bear, farinaceous food, sour things, salt, oysters, fish, milk.

- Aversion to bread, meat, coffee, tobacco.

- Eruptions – herpes, eczema, warts, corns.

- Generally complaints are aggravated by sea side, heat of stove or sun, summer, moving, mental exertion, talking, reading, writing, after sleep,

morning, 10-11 am, 3-4 pm, bread, acid food, fat wine and ameliorated by lying down, empty stomach.

- Complaints are usually produced after disappointment, fright, fits of passion, loss of fluids, injury to head, bread, fat, wine, acid food, salt.

Spongia tosta:

The horney skeleton of the spongia geratinosum consists of silicaceous or calcareous material and spongy portion consist of elastic and compressible and traversed many lacunae, with circular openings and surface. The whole body including skeleton are used for the preparation of medicine. This medicine was proved by Dr.Hahnemann. The virtues of spongia have been attributed to iodum contained in it, though it contained many other elements. The characteristic feature of this medicine includes.

- Acts on respiratory system; lymphatic glands; thyroid; testes; blood, heart and veins.

- Best suited to blue eye, fair-haired person, scrofulous constitution, women with lax fiber and children.

- Dryness of mucus membrane of tongue, pharynx, larynx, and trachea.

- Cough dry, intensly hacking, crowing "dry as a bone" or sounds like a saw being driven through a pine board. Cough excited by talking, in dry cold winds, and relieved by eating and drinking, and by warm food.

- Laryngismus and croup, with difficulty to expectorate sputum and relieved by swallowing it.

- Larynx sensitive, complaints aggravated after sleep, rouses up from sleep as if from a great fright.

- Congestion of the chest coming on moving with sudden weakness as if he would fall.

- Rawness of chest.

- Hypertrophy of heart especially right side.

- Croupous deposits on valves of heart. Useful in rheumatic heart disease, valvular heart disease. Pain and anxiety in the region of heart. Palpitation with suffocation.

- Orchitis with heaviness and squeezing pain in cord and testicles.

- Timidity, fear, terror are the leading mental symptoms and excitement and thinking produces aggravation of symptoms.

- Complaints are aggravated from thinking of the complaints; touch; pressure; movement; talking; spring; lying with head low; before midnight; warm room; and dry cold weather.

- Amelioration of complaints on descending, rest, warm food or drink.

Staphysagaria:

This medicine is prepared from the seeds of Delphinium Staphysagaria and was first proved by Dr.Hahnemann.

- This medicine is especially suited to effects of anger, especially if the indignation cannot have its natural expression. Irritated by trifles. The consequences of the anger are usually reflected through the colic.
- Useful in physical and sexual disturbances, provokes excesses and irregular sexual appetite, a tendency to masturbate and to the bad effects of that habit.
- Complaints are aggravated by the sexual contact.
- Useful in diseases of generative organs of both sexes.
- Sinking sensation in abdomen.
- Craving for tobacco and cough excited by tobacco smoke.
- Inability to sweat.
- Eruptions moist and foul smelling, ; warts, ulcers are painful and sensitive.
- Exostoses and gouty nodes on fingers and toes.
- Complaints are aggravated by pressure, movement, swallowing, drinking cold water, anger, emotions, sexual contact, after urination, and relieved by rest.

Sulphur:

Dr. Hahnemann introduced the virtue of this medicine into the homoeopathic practice.

- Sulphur has been used as the most powerful specific against itch, and it caused an intolerably disagreeable, tingling, itching, gnawing, as the parts scratched and commencing to burn, burning continues after scratching.

- Dr.Hahnemann found in sulphur the counterpart of peculiar constitutional dyscrasia, which he called – psora and considered it as chief antipsoric remedy.

- When apparently well-selected remedies fail to act, sulphur will either clear up the case or open the way for further action of other remedies.

- Irregular distribution of the circulation- flushes of heat, rush of blood to head, chest, heart, plethora from suddenly suppressed eruptions, piles, discharges, heat and burning of all parts or coldness, sweating of many parts.

- Redness of orifices and parts near the orifices of the body, with soreness and hypersensitiveness, making the excretion painful.

- Faint sinking, all gone sensation at 11 am.

- Complaints are aggravated at 11 am, from warmth of bed, night, pressure, rest, standing, stooping, ascending, milk, and relieved by lying on right side, movement.

- Increased appetite.
- Large head, disproportionate to body.
- Lymphatic glands enlarged.
- Mainly a left sided remedy and affections are more on left side.
- This medicine acts on gastro – intestinal system, respiratory system, eye, useful in absorption of effusions, pleurisy, hydrocephalous and on synovial membrane.
- Skin affection alternate with asthma.
- Periodical headache.
- The characteristics of this medicine includes:
    - Aversion to be washed.
    - Relapsing complaints – patient feels better when disease returns again and again.
    - Congestion to single parts.
    - Chronic alcoholism.
    - Sensation of burning.
    - Hot head, and cold feet. Hot flushes during the day, with weak faint spells, passing off with a little moisture.

- Diarrhoea – painless, after midnight, drawing out of bed in morning.
- Constipation – stool hard, dry, knotty, as if burnt, large, painful, child afraid to have stool on account of pain.
- Boils coming in crops.
- Skin affections that have been treated by medicated soaps and washes.
- Happy dreams, wakes up singing.
- Everything looks pretty, which patients takes a fancy to, even rags seems to be fanciful
- Ailments from abuse of metals.
- Offensive odour despite frequent washing.
- Poor breakfast eaters.
- Sulphur is an great resorbant and frequently needed after acute illnesses which do not entirely clear up.
- Suited to lean, stoop shouldered person, who walk and talk stooped, standing is the most uncomfortable position.
- Persons of nervous temperament, quick motioned, quick tempered, plethoric, skin excessively sensitive to atmospheric changes.
- Dirty, filthy people, with greasy skin and long, straight, matted hair, prone to skin affection.

Thuja Occidentalis:

This medicine is prepared from the leaves and twigs of the plant. Introduced into practice by Dr.Hahnemann in 1819. The therapeutic property of Thuja was first made known by Dr.Hahnemann. This is the Dr.Hahnemann's typical antisycotic and in it he found the antidote to the sycotic miasm.

- ❖ All morbid manifestations are excessive, but appear quietly, so that the beginning of the diseased state is scarcely known.
- ❖ It is well known for anti-vaccine effect.
- ❖ Left sided complaints – left sided inguinal hernia, left ovarian neuralgia.
- ❖ Chilly patient.
- ❖ Useful in neuralgia, morbid skin disorders, indigestion, constipation, warts, and new growths.
- ❖ Sensation as if "some one else was thinking by her side."
- ❖ Sensation as if "lead were compressing over eyes"; eye lids as heavy as lead.
- ❖ Can not sleep after 3 am. Dreams much of falling.
- ❖ Left ovarian neuralgia, more before and during menses.
- ❖ Frequent micturition and accompanying pain is the key note of the Thuja. Urgency and frequency of micturition, chronic incontinence of urine from paralysis of the sphincter vesicae.

- Skin mottled and discoloured, brown or red mottled spots, discolouration of the back of hands and feet.

- Pain keep extending from original sites, pain relieved by wrapping up.

- Suited to hydrogenoid constitution, strumous, and sycotic pains, lax muscles, light hair, children, lymphatic temperament.

- Complaints are aggravated by touch, closing eyes, over lifting, movement, 3am, night, cold water, and relieved by pressure.

- Useful in complaints caused by meat.

Chapter - 4

OBSERVATION AND DISCUSSION

Eighty-six patients (58% of the total screened patients) continued under treatment.

Of these, 29 patients had primary hyperlipoproteinemia, 22 patients were diabetic, 20 were hypertensive, 14 had coronary artery disease and one patient suffered from hepatitis.

Among the CAD group 7 patients reported after stopping allopathic medication and 7 were under allopathic treatment for either diabetes or hypertension or hypercholesterolemia. -- Among the group under allopathic treatment 2 patients were under antidiabetics and anti hypertensive treatment, 2 were under anti- hypertensive drugs and these medicines were reduced gradually. 5 patients were taking statins - 3 patients stopped gradually, 1 patient stopped statin suddenly and 1 patient continued taking statins. In those patients who have stopped the statins suddenly, the lipid profile values after one month of discontinuation of medicine were taken for tabulation

The distribution of age, sex, religion, lipid profile and medicines given to the patients of primary hyperlipoproteinemia (primary), Diabetes mellitus group (DM), Hypertensive group(HTN), coronary artery disease group (CAD), hepatitis are given in table 7-11 respectively.

Table-7: Distribution of Age, sex, religion, WHR, BMI and lipid profile before and after treatment in Primary hyperlipoproteinemia group:

No:	Age	S	R	T	W:H	BMI	TC		LDL		HDL		TG		VLDL		NHC		Medicines given			
							B	A	B	A	B	A	B	A	B	A	B	A	1st	2nd	3rd	4th
1	50	M	H	IIA	0.96	20.8	307.6	225.0	255.6	154.5	36.3	42.5	78.5	140.0	15.7	28	271.3	182.5	Thu			
2	49	F	C	IIA	0.93	19.9	250.0	200.0	184.8	146.7	44.0	30	106.0	116.6	21.2	23.3	206.0	170.0	Sul	Thu		
3	52	M	I	IIA	0.92	21.5	333.0	283.3	259.4	228.3	65.0	35	53.0	100	10.6	20	270.0	248.3	Lyc	Thu		
4	52	F	H	IIA	0.92	33.6	300.0	242.8	210.0	170.1	65.8	43.4	120.0	146.1	24	29.2	234.0	199.2	Sul	Thu		
5	50	F	I	IIA	1.01	20.6	233.0	200.0	165.0	154.2	44.0	29.1	120.0	83.3	24.0	16.7	189.0	170.9	Sul	A.A	Thu	
6	49	F	I	IIA	0.90	26.4	250.0	208.0	173.4	149.2	52.6	38.8	120.0	100.0	24.0	20.0	197.4	169.2	Lyc	NM	Thu	
7	44	M	H	IIA	1.07	20.4	283.0	214.4	214.3	143.7	42.1	38.2	133.0	161.9	26.6	32.3	240.9	176.0	NM	Thu		
8	52	M	I	IIA	1.02	23.1	266.0	.228.5	192.6	180.6	44.0	32.6	147.0	76.9	29.4	15.3	222.0	195.9	Lyc	Sul	Thu	
9	27	F	I	IIA	1.04	31.4	233.3	200	160.4	142.5	57.1	39.1	77.7	92.3	15.5	18.4	175.9	160.5	NM	Thu		
10	25	M	I	IIA	0.91	19.2	236	200	161.4	132.3	48	47.7	133.0	100.0	26.6	20.0	188	152.3	NM			
11	50	F	I	IIA	0.96	31.2	233.3	192.3	156.4	137.4	47.5	25	146.4	149.9	29.3	29.9	185.7	167.3	Lyc	Thu		
12	35	M	I	IIA	0.94	20.2	333.3	176.9	249.4	119.9	62.5	45.4	106.6	58.3	21.3	11.6	270.2	131.0	NM			
13	41	F	I	IIA	1.02	25.4	360.0	350.0	293.0	283.4	40.0	33.7	133.3	166.6	26.6	33.7	319.6	317.1	NM	Thu		
14	35	F	I	IIA	0.98	24.5	340.0	261.5	268.4	222.2	52.3	30.5	93.7	44.4	18.7	8.8	287.1	231	Lyc	Thu		

Continued...

15	53	M	H	IIA	0.93	19.6	260	243.0	190.9	185.8	40.4	32.6	143.7	123.0	28.7	24.6	219.6	210.4	Lac			
16	51	M	I	IIA	0.97	28.2	200.0	242.8	141.2	189.4	41.0	45.6	89.0	40.0	17.8	8.0	159	197.4	NM	Thu		
17	44	F	I	IIA	0.88	31.6	250.0	228.5	156.9	139.0	77.5	79.5	57.0	50.0	15.6	10.0	172.5	149.2	NM	Thu		
18	33	M	I	IIA	1.01	26.0	292.3	215.0	238.5	161.9	37.5	27.7	81.8	127	16.3	25.4	254.8	187.5	Thu			
19	43	F	I	IIA	0.93	23.6	266.6	216.6	192.9	155.7	55.0	44.1	93.7	84.2	18.7	16.8	211.6	172.5	Thu			
20	36	F	I	IIA	0.85	20.8	250.0	222.2	191.0	163.0	41.6	55.0	86.9	21.0	17.3	4.2	208.3	167.2	Spo			
21	40	F	H	IIA	0.93	30.4	283.3	233.3	213.3	183.8	50	37.5	100.0	60.0	20.0	12.0	223.3	195.8	Lac			
22	41	M	C	IIA	0.93	24.0	250.0	230.7	184.0	154.1	50.0	50.0	77.7	133.3	15.5	26.6	199.5	180.7	Sul			
23	40	M	I	IIA	1.01	24.2	230.7	160.0	178.2	114.3	30.7	27.7	109.0	90.0	21.9	18.0	199.9	132.3	Sul			
24	46	M	I	IIB	1.02	25.8	266.0	237.0	176.0	167.0	42.0	30.0	240.0	200.0	48.0	40.0	224.0	207.0	Lyc	Thu		
25	40	M	H	IIB	0.74	16.9	233.0	200.0	127.4	125.9	71.0	43.4	173.0	153.8	34.6	30.7	162.0	156.6	NM	Thu		
26	43	M	I	IIB	0.98	21.5	266.0	257.1	173.4	166.6	52.6	54.5	200.0	180.0	40.0	36.0	213.4	202.6	Sul	Thu	Lue	
27	48	M	C	IIB	0.97	19.4	290.0	228.5	209.3	161.5	47.5	35.0	166.0	160.0	33.2	32.0	242.5	193.5	Lyc			
28	49	M	I	IIB	1.02	18.8	320.0	250.0	220.5	205.4	59.5	30.5	200.0	70.5	40.0	14.1	260.5	219.5	NM	Thu		
29	46	F	I	IIB	1.02	30.8	250.0	200.0	192.8	154.3	22.2	30.4	175.0	76.9	35.0	15.3	227.8	169.6	Thu			

[A=After treatment; B=Before treatment; BMI= Body Mass Index; C=Christian; F= Female; H=Hindu; I=Muslim;, HDL= High density lipoprotein cholesterol; LDL=Low density lipoprotein cholesterol; M=Male; NHC= Non HDL cholesterol; R = Religion; S = Sex; T = Type of hyperlipoproteinemia; TC= Total Cholesterol; TG=Triglycerides; VLDL = Very low density lipoprotein cholesterol; WHR = waist: Hip ratio]

[Medicines: Lac = Lachesis mutus; Lue =Luesinum Lyc= Lycopodium Clavatum; NM= Natrum muriaticum; Spo=Spongia Tosta; Sul= Sulphur ; Thu =Thuja occidentalis;]

Table -8: Distribution of Age, sex, religion, WHR, BMI, lipid profile and FBS before and after treatment in Diabetic mellitus group:

No:	Age	S	R	W:H	BMI	TC		LDL		HDL		TG		VLDL		NHC		FBS		Medicines given			
						B	A	B	A	B	A	B	A	B	A	B	A	B	A	1st	2nd	3rd	4th
1	55	F	I	0.96	25.7	250.0	250.0	171.0	175.0	63.0	55.0	80.0	100.0	16.0	20.0	187.0	195.0	233*	175	Sul	Thu		
2	40	M	I	0.92	19.2	250.0	215.3	186.5	154.2	50.0	45.4	66.0	78.5	13.2	15.7	199.7	169.9	216	107	Sul			
3	55	F	H	0.98	19.7	266.0	215.0	176.4	145.1	71.0	50.0	93.0	99.9	18.6	19.9	195.0	165.0	216	85	Lyc			
4	54	F	I	1.02	32.5	233.0	228.5	156.5	177.4	55.3	40.9	106.0	80.0	21.2	16.0	177.7	193.4	250	213	CC	Thu		
5	49	M	H	1.10	25.3	233.0	185.7	146.0	128.5	71.0	43.4	80.0	69.2	16.0	13.8	162.0	142.3	242	156	Lyc	Thu		
6	60	F	H	0.90	18.3	216.6	208.0	137.6	136.9	68.4	61.1	53.3	50.0	10.6	10.0	148.2	146.9	175	138	Pho	Thu	NM	
7	48	M	H	1.01	19.4	233.0	166.6	153.4	121.5	53.0	22.7	133.0	112.0	26.6	22.4	180.0	143.9	238	120	Lyc	Thu		
8	55	F	I	0.90	30.8	250.0	257.1	173.5	196.2	60.5	45.6	80.0	76.9	16.0	15.3	189.5	211.5	116*	113*	Lyc	Sul	Thu	
9	48	F	I	1.02	25.6	273.0	213.3	206.3	156.7	40.5	33.3	133.3	116.6	26.2	23.3	232.5	180.0	166	190	Lyc	Thu		
10	46	F	H	0.81	15.1	290.0	192.5	194.2	121.9	82.5	59.3	66.6	55.5	13.3	11.1	207.5	133.0	150	128	Sul			
11	53	F	I	0.90	30.8	216.0	164.2	146.0	109.1	48.0	43.1	111.0	60.0	22.2	12.0	168.2	121.1	133*	200	Lyc	Thu		
12	38	M	I	0.91	20.7	280.0	200.0	215.9	107.5	21.4	32.5	213.0	300.0	42.6	60.0	258.5	167.5	192	128	Lyc			
13	47	F	C	0.97	30.9	240.0	184.0	143.3	133.2	61.9	38.8	175.0	60.0	35.0	12.0	178.3	145.2	283	113	NM	Thy	Thu	
14	60	M	H	1.02	27.5	280.0	214.2	198.7	146.2	66.6	50.0	75.0	90.0	15.0	18.0	213.7	164.2	183	242	Sul			
15	52	F	H	0.98	22.3	575.0	228.5	488.9	153.6	67.5	56.5	93.3	92.3	18.6	18.4	507.5	172.0	108	113	Sul			
16	42	M	C	0.90	18.9	200.0	185.7	140.0	108.7	35.0	47.7	125.0	150.0	25.0	30.0	165.0	138.7	208	200	Sul			
17	50	M	I	1.03	23.1	233.3	200.0	137.8	119.8	55.5	52.2	200.0	140.0	40.0	28.0	177.8	147.8	193*	144	Sul	Thu		
18	39	F	H	1.00	26.8	230.7	208.3	155.2	121.0	55.5	58.8	100.0	142.8	20.0	28.5	175.2	149.5	242*	131*	Sul	Thu		
19	55	F	I	0.98	21.8	353.8	300.0	290.6	241.0	38.8	35.0	122.2	120.0	24.4	24.0	315.0	265.0	400*	269*	Ins	Thu		
20	54	F	I	0.88	26.3	213.3	185.7	155.9	1450.7	27.5	34.0	150.0	30.0	30.0	6.0	185.8	151.7	231	150	Sta			
21	75	F	H	1.00	22.2	246.1	215.3	192.4	160.2	32.6	42.5	105.8	63.1	21.6	12.6	214.0	172.8	114*	125	Iod			
22	54	F	H	0.92	24.2	233.0	184.6	172.4	137.6	50.0	36.1	53.0	54.5	10.6	10.9	183.0	148.5	142	81	Sul	Thu		

[**A**=After treatment; **B**=Before treatment; **BMI**= Body Mass Index; **C**=Christian; **F**= Female; **FBS** = Fasting blood sugar; **H**=Hindu; **I**=Muslim;, **HDL**= High density lipoprotein cholesterol; **LDL**=Low density lipoprotein cholesterol; **M**=Male; **NHC**= Non HDL cholesterol; **R** = Religion; **S** = Sex;

TC= Total Cholesterol; **TG**=Triglycerides; **VLDL** = Very low density lipoprotein cholesterol ; **WHR**= waist: Hip ratio ; *=Under antidiabetic medicines]

[**Medicines:**; **CC** = Calcarea carbonica –ostrearum ; **Iod** = Iodium ; **Ins** = Insulinum; **Lyc**= Lycopodium Clavatum;; **NM**= Natrum muriaticum; **Pho** =Phosphorus ; **Sta** = Staphysagaria; **Sul**= Sulphur ; **Thu** =Thuja occidentalis; **Thy**=Thyroidinum

Table -9: Distribution of Age, sex, religion, WHR, BMI, lipid profile and Blood Pressure before and after treatment in Hypertensive group are given below:

No:	Age	S	R	W:H	BMI	TC		LDL		HDL		TG		VLDL		NHC		BP		Medicines given		
						B	A	B	A	B	A	B	A	B	A	B	A	B	A	1st	2nd	3rd
1	52	F	I	1.05	28.7	250.0	215.3	181.5	165.6	55.3	43.1	66.0	33.3	13.2	6.6	194.7	172.2	136/96	150/100	NM	Thu	
2	65	M	I	1.02	19.9	216.0	223.0	134.2	146.6	73.8	63.6	40.0	64.0	8.0	12.8	142.2	159.9	160/90	160/100	Lyc	Thu	
3	52	F	H	0.98	21.1	218.0	226.6	170.2	161.7	33.0	41.6	244.0	116.6	48.8	23.7	219.0	185.0	140/90*	150/100	NM	Thu	
4	75	M	I	1.05	25.7	233.3	200.0	163.7	144.4	45.2	23.6	122.2	160.0	24.4	32.0	188.1	176.4	140/100	170/90	Lyc	Sul	Thu
5	65	F	H	0.96	24.8	233.3	233.3	149.9	171.8	53.3	33.3	155.5	141.1	31.1	28.2	181.0	200.0	150/80	130/80	NM	Thu	
6	40	M	I	1.28	19.0	260.0	230.7	170.2	122.5	20.5	52.7	346.6	277.7	69.3	55.5	239.5	178.0	210/130	220/120	NM		
7	61	M	H	1.04	28.0	280.0	171.4	207.3	134.1	45.2	28.9	137.5	42.1	27.5	8.4	234.8	142.5	140/90	120/80	Sul		
8	61	M	H	1.02	23.7	280.0	216.6	219.0	166.6	40.0	30.0	100.0	100.0	20.0	20.0	239.0	186.6	140/100	146/80	Sul	Thu	
9	57	F	H	1.07	20.5	340.0	276.9	241.7	222.5	83.3	45.9	75.0	42.8	15.0	8.5	256.7	231.0	140/80	120/90	Sul	Thu	
10	54	M	I	0.98	22.5	340.0	245.5	264.3	185.0	53.2	20.5	112.5	200.0	22.5	40.0	286.8	225.0	180/120	160/120	Thu		
11	53	F	H	0.94	26.2	300.0	266.0	214.3	201.8	53.2	36.0	162.5	141.0	32.5	28.2	246.8	230.0	188/110	220/120	NM		
12	52	F	I	1.05	24.8	262.0	222.2	193.5	150.9	43.1	47.7	127.0	118.1	25.4	23.6	236.6	174.5	126/82	120/80	Sul	Thu	
13	50	F	I	1.01	30.0	246.0	216.6	166.0	127.3	60.0	61.1	100.0	141.1	20.0	28.2	186.0	155.5	160/100	120/90	NM		
14	40	M	H	0.94	23.1	200.0	192.3	136.0	131.9	32.5	36.9	157.5	117.6	31.5	23.5	167.5	155.4	116/78*	100/70	Sul		
15	50	M	H	1.0	23.1	266.0	220.6	189.4	146.3	31.8	37.4	224.0	181.8	44.8	36.9	234.2	183.2	120/80	140/90	NM		
16	43	M	I	0.90	22.0	215.9	179.3	147.7	125.3	31.2	39.5	188.8	72.7	37.3	14.5	185.0	139.8	120/90*	120/80	NM	Thu	
17	40	M	I	1.06	30.4	270.0	200.0	181.0	138.6	41.0	30.5	238.0	154.5	48.0	30.9	229.0	169.5	150/100	120/80	Thu		
18	65	M	I	0.99	27.4	207.6	158.3	162.2	124.3	27.7	20.0	88.8	70.0	17.7	14.0	179.9	138.3	120/90	120/80	Med		
19	35	M	H	1.02	24.8	276.9	200.0	209.5	136.0	37.4	38.6	150.0	127.2	30.0	25.4	239.5	161.4	160/100	120/80	Thu		
20	47	M	H	1.0	26.1	307.6	183.3	239.4	148.3	28.2	15.0	200.0	100.0	40.0	20.0	279.4	168.3	138/100	120/90	NM		

[A=After treatment; B=Before treatment; BMI= Body Mass Index; BP= Blood pressure C=Christian; F= Female; H=Hindu; I=Muslim;, HDL= High density lipoprotein cholesterol; LDL=Low density lipoprotein cholesterol; M=Male; NHC= Non HDL cholesterol; R = Religion; S = Sex; TC= Total Cholesterol; TG=Triglycerides; VLDL = Very low density lipoprotein cholesterol ; WHR= waist: Hip ratio ; * = Under antihypertensive drugs.]

[Medicines:; Lyc= Lycopodium Clavatum; Med =Medorrhinum; NM= Natrum muriaticum; Sul= Sulphur ; Thu =Thuja occidentalis.]

Table-10 : Distribution of Age, sex, religion, WHR, BMI and lipid profile before and after treatment in CAD group are given below :

No:	Age	S	R	T	W:H	BMI	TC		LDL		HDL		TG		VLDL		NHC		Medicines given			
							B	A	B	A	B	A	B	A	B	A	B	A	1st	2nd	3rd	4th
1	70	M	I	CAD	1.02	23.2	216.0	192.8	155.8	118.0	39.0	56.8	106.0	90.0	21.2	18.0	177.0	136.0	NS	Thu		
2	72	M	H	CAD	0.90	15.9	266.0	184.6	227.4	136.8	28.0	29.5	53.0	91.6	10.6	18.3	238.0	155.1	Lyc	Thu		
3	58	F	I	CAD+ DM	1.0	19.2	316.0	271.4	211.4	206.6	94.0	56.8	53.0	40.0	10.6	8.0	222.0	214.6	Pho	Lyc	Thu	
4	67	M	I	CAD+ DM	0.98	21.0	216.0	200.0	149.8	148.7	53.0	45.0	66.0	31.5	13.2	6.3	163.0	155.0	Lyc	Thu		
5	55	F	I	CAD+HT	1.05	20.7	316.0	304.0	247.5	260.3	44.7	27.7	120.0	80.0	24.0	16.0	271.5	276.3	Sul	Thu		
6	49	M	H	CAD*	1.12	23.1	266.6	183.3	207.4	125.7	35.0	42.1	121.2	77.7	24.2	15.5	231.6	141.2	Aur	Thu		
7	58	M	I	CAD	1.0	20.4	246.1	213.3	185.5	143.4	36.3	40.0	120.0	149.9	24	29.9	209.5	179.8	Sul			
8	35	M	H	CAD	0.95	24.4	235.0	200.0	192.2	126.6	24.0	45.4	94	40	18.8	8	211.0	134.6	Thu			
9	42	M	I	CAD+ DM+HT *	1.02	25.1	183.0	192.0	127.6	142.7	39.4	33.3	80.0	80.0	16.0	16.0	143.6	158.7	Lyc	Sul	Thuj	
10	57	M	H	CAD+ DM+HT*	0.96	20.8	216.0	183.0	129.0	119.7	73.7	52.7	73.7	54.5	13.2	10.9	142.2	130.6	Lac			
11	53	M	I	CAD*	1.1	19.4	327.0	257.1	240.6	194.0	62.0	43.1	122.0	100.0	24.4	20.0	265.0	214.0	Aur	Thu		
12	47	M	I	CAD*	0.97	20.6	333.3	222.2	256.7	148.2	56.0	43.1	133.3	154.4	26.0	30.9	282.7	179.1	Sul	Thu		
13	53	M	I	CAD*	1.02	25.4	246.1	216.6	161.1	138.9	45.0	44.4	200.0	166.6	40.0	33.3	201.1	172.2	Sul	Thu		
14	52	M	I	CAD*	1.01	22.2	233.0	176.9	172.7	119.1	27.3	43.1	165.0	73.6	33.0	14.7	205.7	133.8	Sul			

[A=After treatment; B=Before treatment; BMI= Body Mass Index; C=Christian; CAD = Coronary artery disease; DM = Diabetes mellitus; F= Female; H=Hindu; I=Muslim;, HDL= High density lipoprotein cholesterol; HT= Hypertension; LDL=Low density lipoprotein cholesterol; M=Male; NHC= Non HDL cholesterol; R = Religion; S = Sex; T= Clinical conditions; TC= Total Cholesterol; TG=Triglycerides; VLDL = Very low density lipoprotein cholesterol ; WHR= waist: Hip ratio ; * = Under antilipidemic drugs.]

[Medicines:; Aur=Aurum metallicum ; Lac= Lachesis mutus; Lyc= Lycopodium Clavatum;; NS= Natrum sulphuricum; Pho =Phosphorus ; Sul= Sulphur ; Thu =Thuja occidentalis.]

Table -11: Distribution of Age, sex, religion, WHR, BMI and lipid profile before and after treatment in Liver disease group is given below:

No:	Age	S	R	W:H	BMI	TC		LDL		HDL		TG		VLDL		NHC		Medicines given			
						B	A	B	A	B	A	B	A	B	A	B	A	1st	2nd	3rd	4th
1	40	M	H	1.03	21.6	300.0	216.6	199.6	128.3	40.4	33.3	300.0	275.0	60.0	55.0	259.6	183.3	Sul			

[A=After treatment; B=Before treatment; BMI= Body Mass Index; C=Christian; F= Female; H=Hindu; I=Muslim;, HDL= High density lipoprotein cholesterol; LDL=Low density lipoprotein cholesterol; M=Male; NHC= Non HDL cholesterol; R = Religion; S = Sex; TC= Total Cholesterol; TG=Triglycerides; VLDL = Very low density lipoprotein cholesterol ; WHR= waist: Hip ratio] .

[Medicines:; Sul= Sulphur]

[As only one patient was presented with liver disease (Hepatitis) statistical analysis of this group was not done separately

Table-12: Percentage of Risk for Development of CAD in 10 years

	Primary		DM		HTN	
No:	B	A	B	A	B	A
1	12	8	3	4	2	2
2	4	2	2	2	12	12
3	6	12	4	1	5	2
4	2	2	1	2	25	20
5	2	1	4	5	5	8
6	3	1	2	1	6	3
7	6	2	4	6	20	12
8	8	8	6	6	20	16
9	1	1	3	1	5	4
10	1	0	2	1	12	16
11	1	1	8	10	5	6
12	2	1	5	2	2	2
13	2	1	2	1	5	1
14	1	1	10	8	1	1
15	8	10	6	5	10	10
16	8	10	3	1	4	1
17	1	1	6	5	4	3
18	1	1	1	1	16	12
19	1	1	11	11	5	2
20	1	1	2	1	16	4
21	1	1	30	14		
22	2	2	2	1		
23	3	2				
24	6	6				
25	1	2				
26	3	5				
27	10	6				
28	8	8				
29	8	1				

B= Before treatment; **A**= After treatment

In this study 56.97% (49) were male and 43.02 % (37) were females (Fig: 4), within a range of 25-75 years of age (Fig: 5). The mean age of the patients in different groups and total patients are 43.86 years in primary group, 51.31 years in diabetic group, 52.8 years in hypertensive group, 54.8 years in CAD group and 49.1 years in total population [figure-6.]

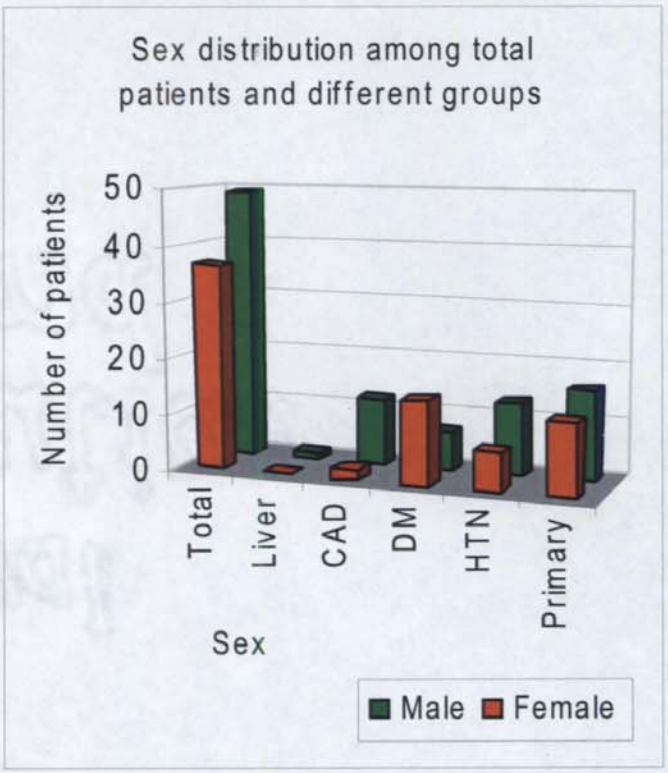


Figure: 4

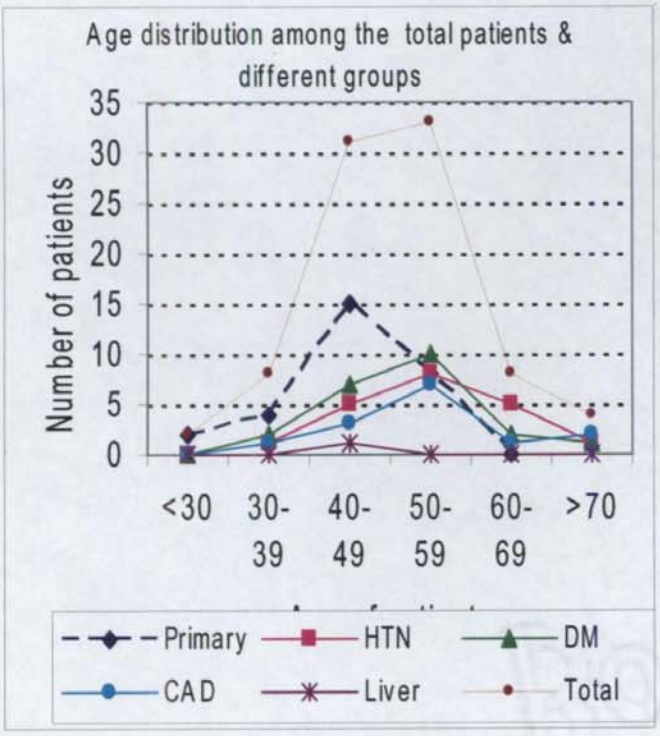


Figure: 5

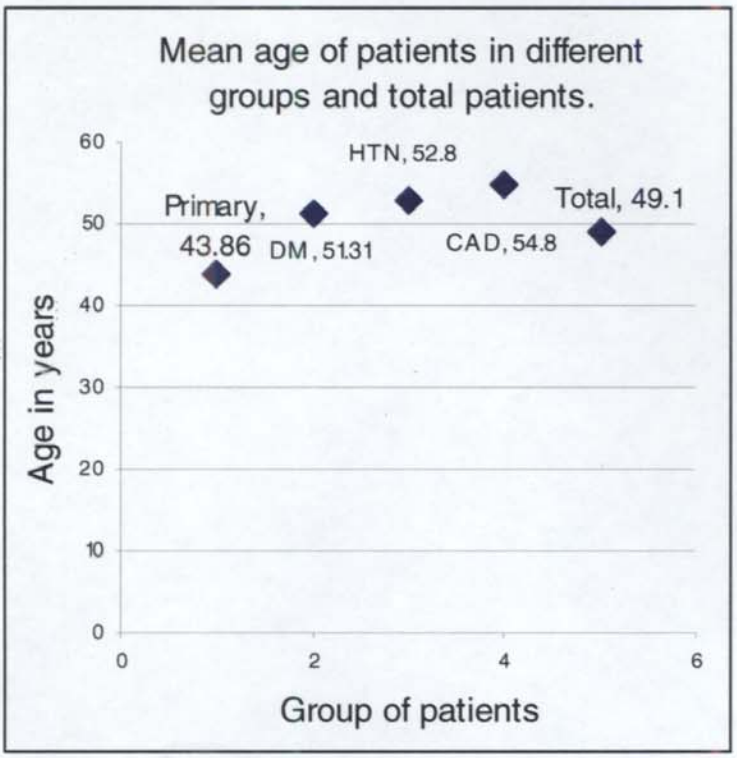


Figure: 6

In this study 58.13% (50) of the patients belonged to Muslim community, 36.04 % (31) of patients belonged to Hindu community and 5.81% (5) of patients belonged to Christian community.[Fig-7].

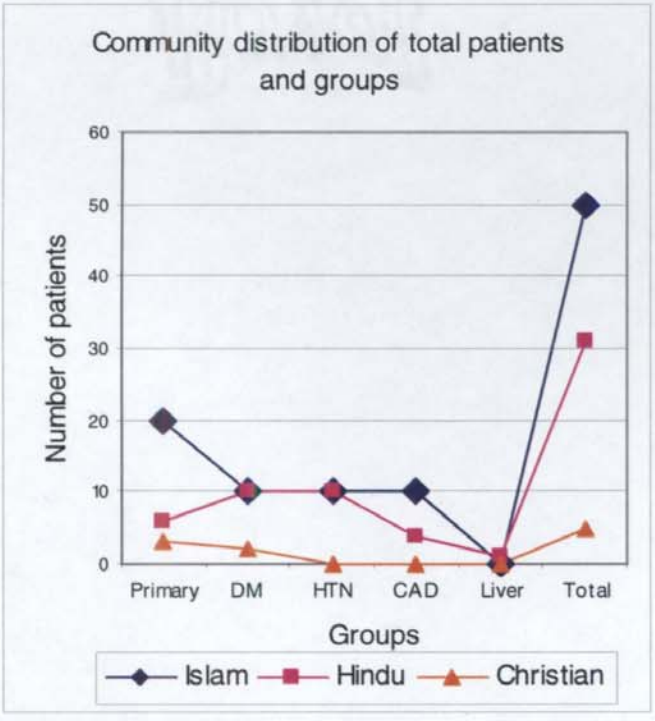


Figure: 7

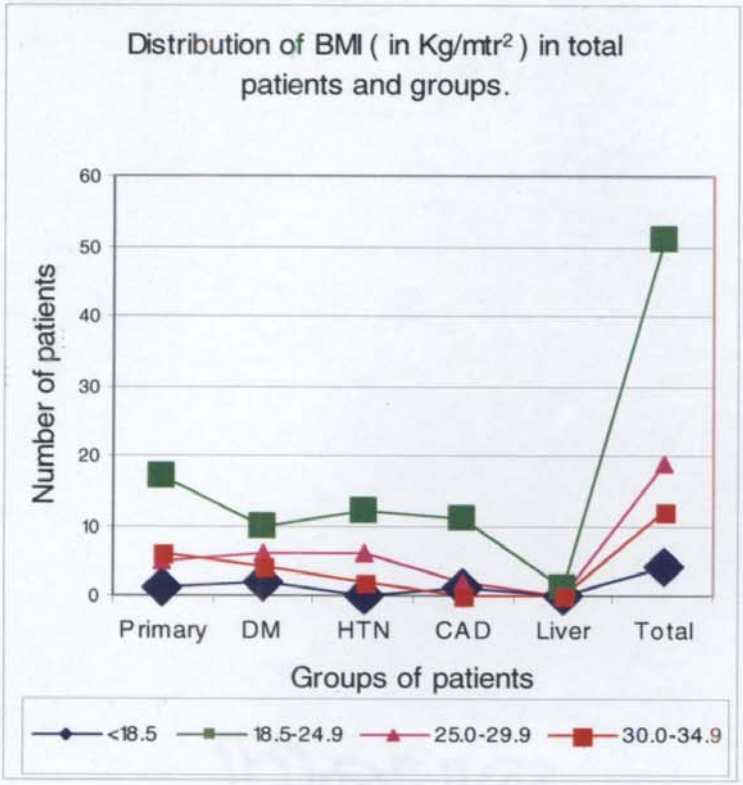


Figure: 8

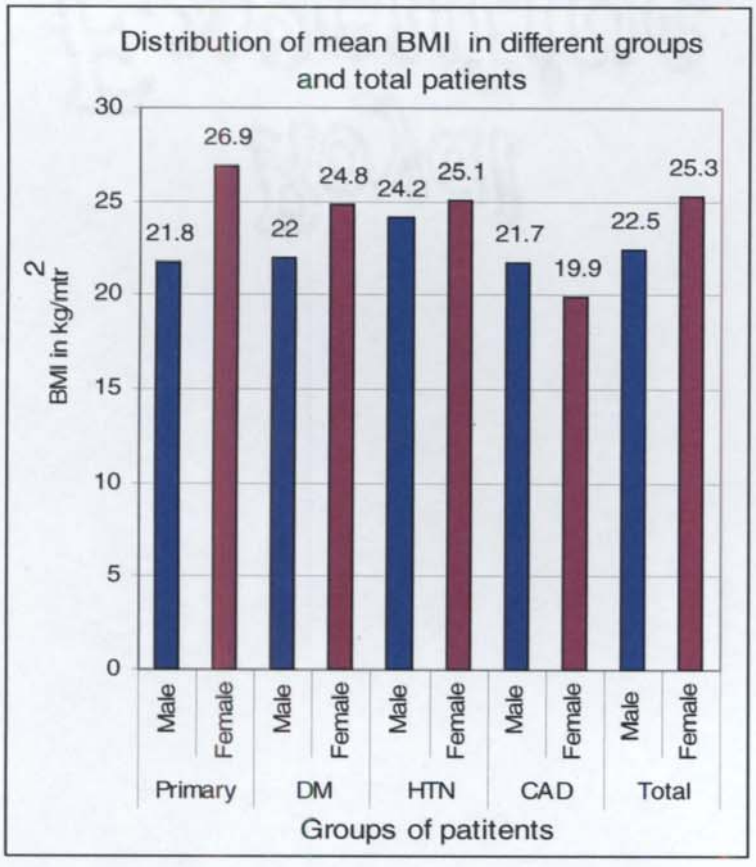


Figure: 9

The body mass index of whole patients and different groups are shown in figure -8. In this study 4.65% (4) of patients were underweight (BMI below 18.5 kg/m^2), 59.3% (51) were within normal range (BMI between 18.5 – 24.9 kg/m^2), 22.09 % (19) were overweight (BMI between 25-29.9 kg/m^2) and 13.95 % (12) had obesity of class I type (BMI between 30 -34.5 kg/m^2). The mean BMI is more in female patients, except in CAD group, than in males (Fig. 9).

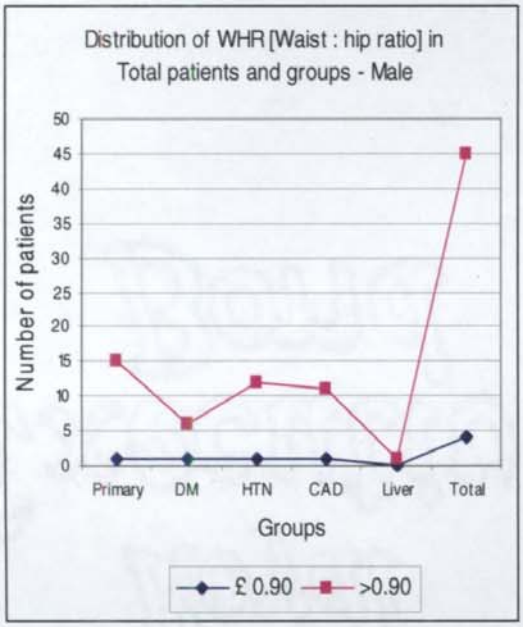


Figure: 10

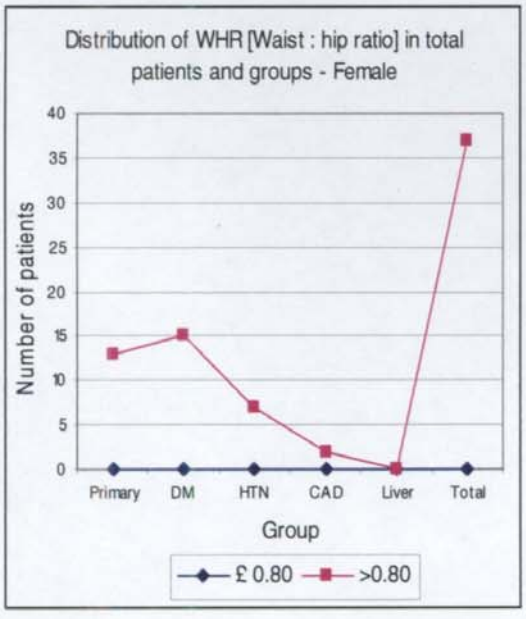


Figure: 11

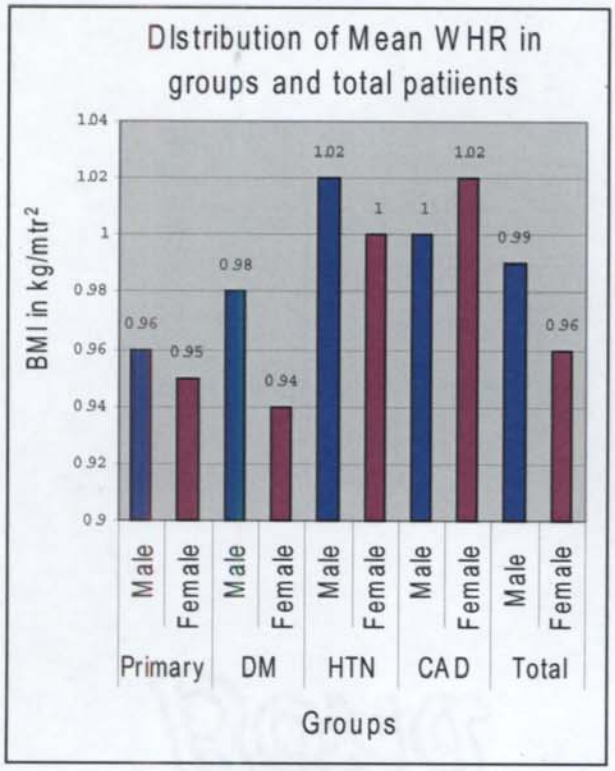


Figure: 12

Forty five male patients (91.8%) had a waist: hip ratio above 0.9 and four had W:H ratio below 0.9 (Fig-10). All females had a W:H ratio of more than 0.8. (Fig: 11). The mean WHR in both males and females are more than normal (Fig-12)

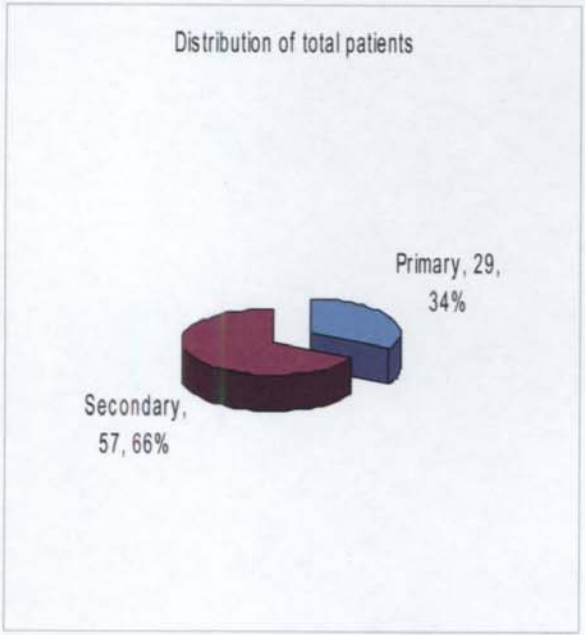


Figure: 13

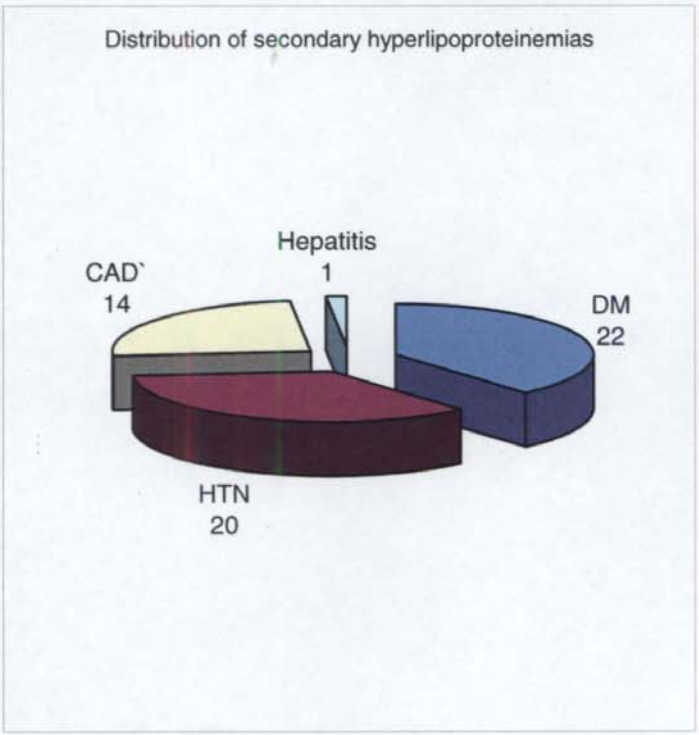


Figure: 14

Distribution of total patients and secondary hyperlipoproteinemia are given in figure 13 and 14 respectively.

In this study 33.72 %(29) of patients had primary hyperlipoproteinemia and 66.27% (57) patients had secondary hyperlipoproteinemia. Among the secondary hyperlipoproteinemia group, 22 patients were diabetic, 20 were hypertensive, 14 had coronary artery disease and one had Hepatitis.

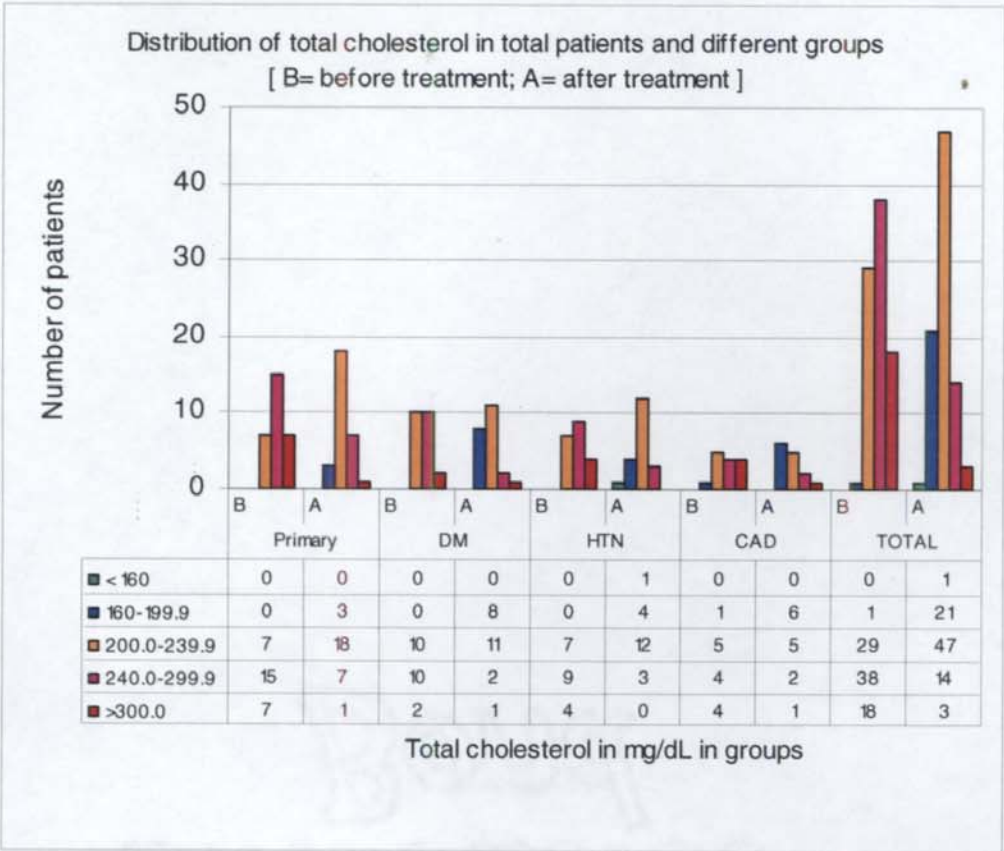


Figure: 15

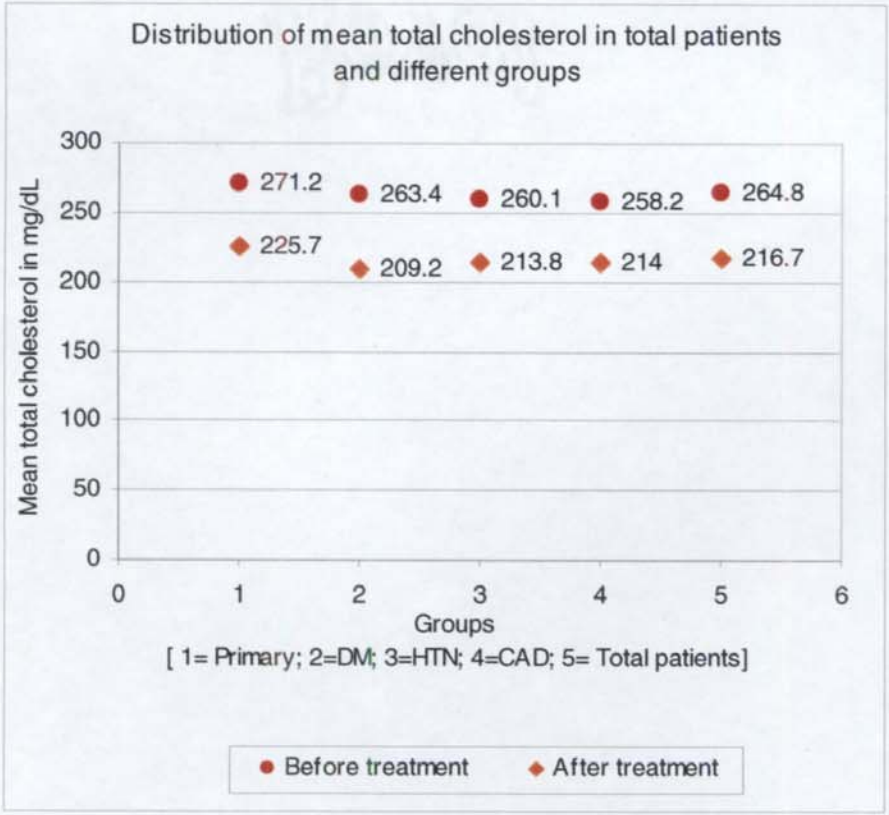


Figure: 16

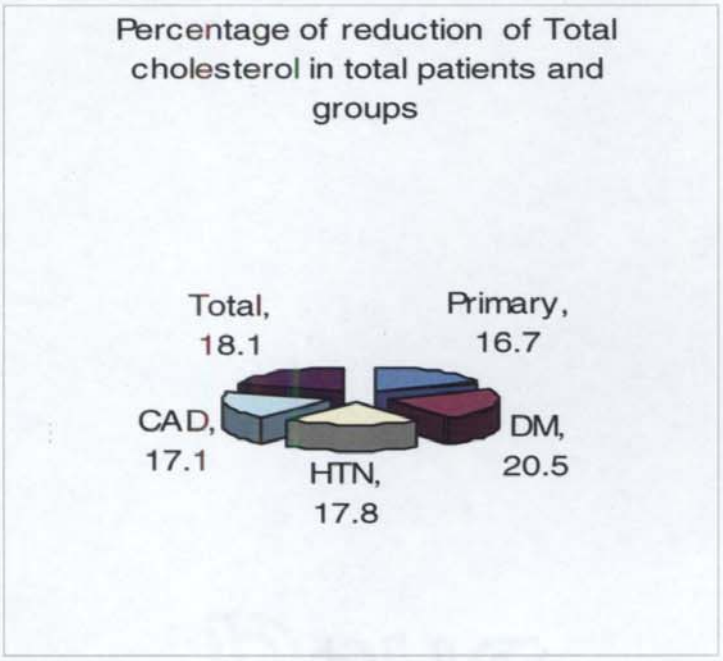


Figure: 17

Fig: 15 show the distribution of total cholesterol in the whole patients and the different groups. In this study 65.1 % of patients in the high risk group [> 240 mg/d L] were reduced to 19.7% and 33.72% patients in borderline risk group [between 200-239.9 mg/d L] were increased to 54.65%. The number of patients in desirable level (below 200mg/d L) increased from 1.16% to 25.5 %.

The mean values of total cholesterol before and after treatment and the percentage of reduction in total cholesterol after treatment are given in figure 16 and 17 respectively.

The test values for assessing the significance of reduction in total cholesterol are given in Table – 13.

Table – 13: Test values for assessing significance of reduction in total cholesterol

Variable	Group	Sample size	Description	Mean	S.D	Difference in means	S.D	S.E.of mean	t-value	p-value
TOTAL CHOLESTEROL	PRIMARY	29	Before treatment	271.25	38.99	45.48	33.66	6.25	7.27	< 0.001
			After treatment	225.7	35.44					
	DM	22	Before treatment	263.44	77.04	54.24	70.47	15.02	3.61	0.002
			After treatment	209.2	31.08					
	HTN	20	Before treatment	260.13	40.78	46.23	36.29	8.11	5.70	< 0.001
			After treatment	213.89	29.72					
	CAD	14	Before treatment	258.29	47.75	44.20	32.64	8.72	5.07	< 0.001
			After treatment	214.08	38.02					
	TOTAL	86	Before treatment	264.89	52.24	48.13	45.69	4.92	9.77	< 0.001
			After treatment	216.76	33.4					

The *p*- value of < 0.001 in total patients, primary hyperlipoproteinemia group, Hypertensive group, Coronary artery disease group and of 0.002 in Diabetic group indicates a significant reduction in total cholesterol after the treatment.

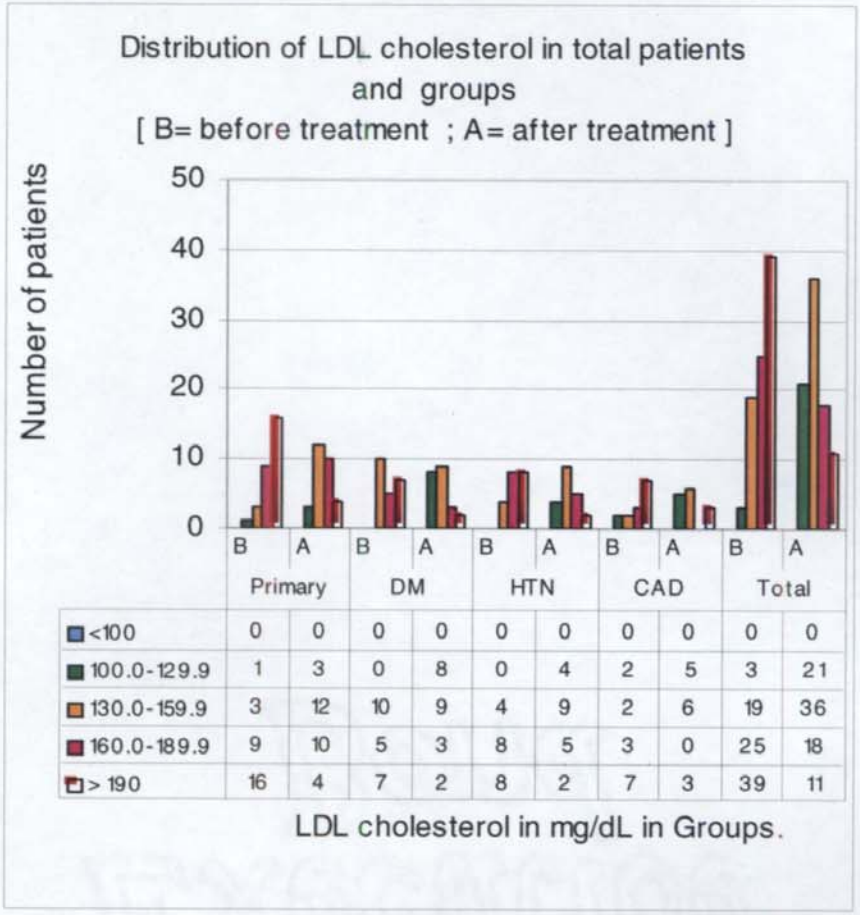


Figure: 18

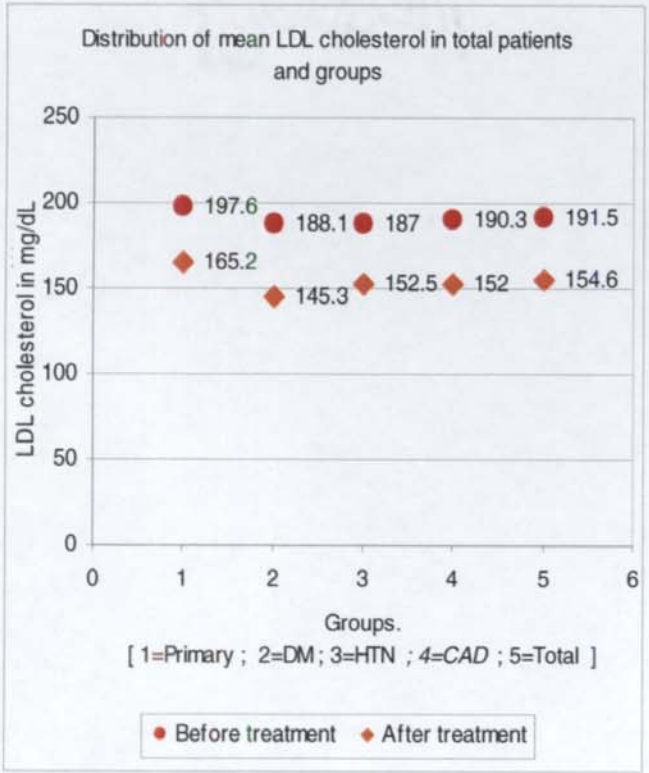


Figure: 19

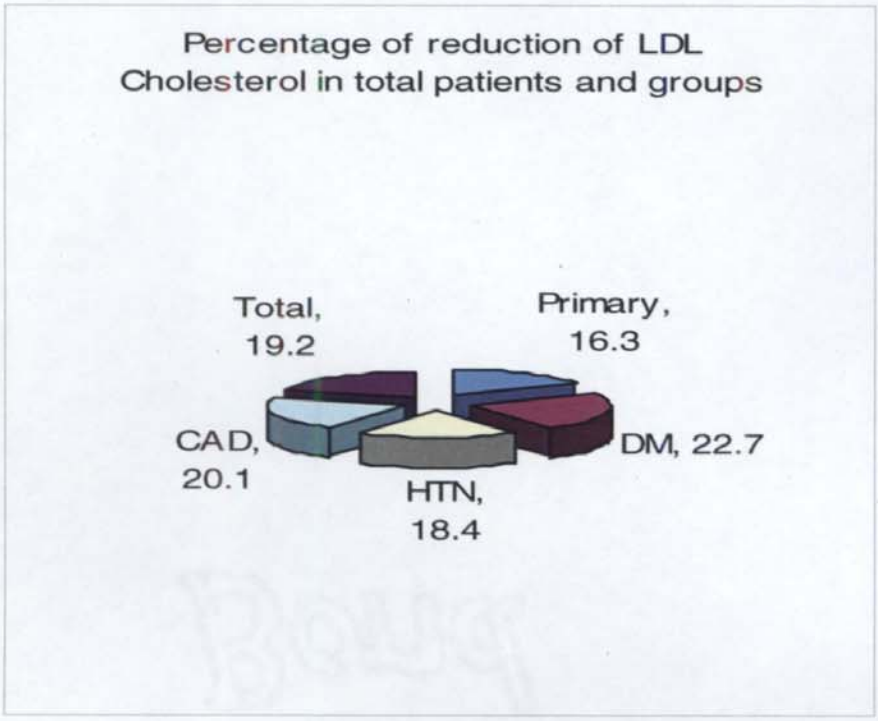


Figure: 20

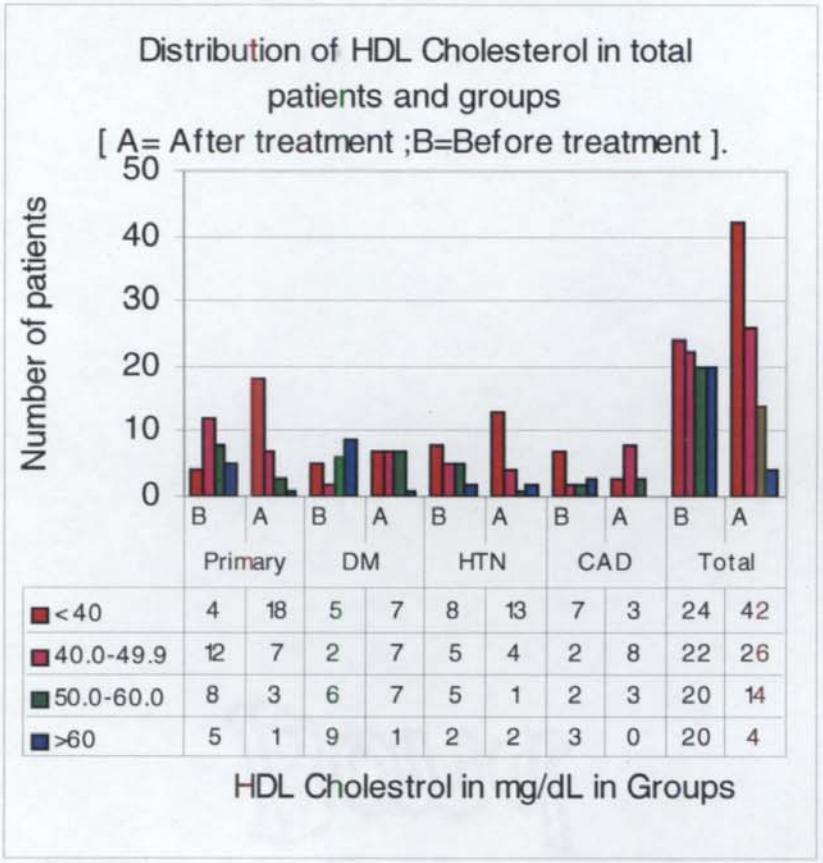
The distribution of LDL cholesterol in total patients and different groups are given in Figure-18. In this study 45.34% of patients in very high risk group (> 190 mg/d L) were reduced to 12.7%; 29.06% of the patients in high risk group (between 160-189.9 mg/d L) were reduced to20.96% and 22.09 % of patients in border line high (130-159.9 mg/d L) were increased to 41.86%. It was observed from this study that 3.48% of patients in near optimal group (100-129.9 mg/d L) were increased to 24.41%.

The mean values of LDL cholesterol in total patients and different groups before and after treatment, and the percentage of reduction of LDL cholestrol after treatment are given in figure 19 and 20 respectively. The test values for determining the significance of reduction in LDL cholesterol are given in table - 14:

Table-14: Test values for assessing significance of reduction in LDL cholesterol

Variable	Group	Sample size	Description	Mean	S.D	Difference in means	S.D	S.E.of mean	t-value	*p*-value
LDL CHOLESTEROL	PRIMARY	29	Before treatment	197.60	39.97	32.33	33.24	6.17	5.24	<0.001
			After treatment	165.26	35.52					
	DM	22	Before treatment	188.11	75.72	42.79	71.51	15.24	2.81	0.011
			After treatment	145.31	31.73					
	HTN	20	Before treatment	187.05	36.26	34.47	30.24	6.72	5.10	<0.001
			After treatment	152.07	26.82					
	CAD	14	Before treatment	190.33	42.99	38.28	38.90	10.39	3.68	0.003
			After treatment	152.05	40.83					
	TOTAL	86	Before treatment	191.56	50.40	36.93	45.77	4..93	7.48	<0.001
			After treatment	154.63	33.93					

The *p*- value of < 0.001 in total patients, Hypertensive and primary group; of 0.011 in diabetic group and of 0.003 in coronary artery disease group indicates a significant reduction in LDL cholesterol after treatment.



	Primary		DM		HTN		CAD		Total	
	B	A	B	A	B	A	B	A	B	A
<40	4	18	5	7	8	13	7	3	24	42
40.0-49.9	12	7	2	7	5	4	2	8	22	26
50.0-60.0	8	3	6	7	5	1	2	3	20	14
>60	5	1	9	1	2	2	3	0	20	4

Figure: 21

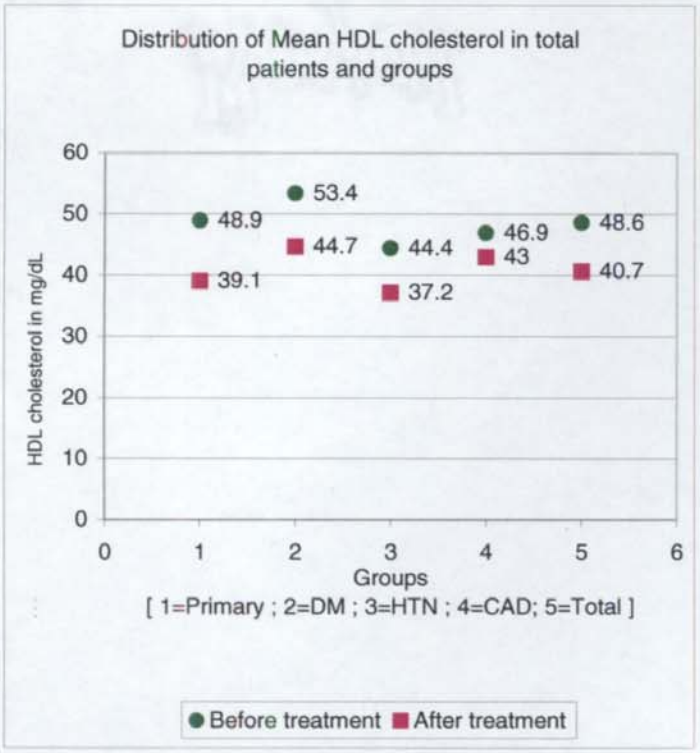


Figure: 22

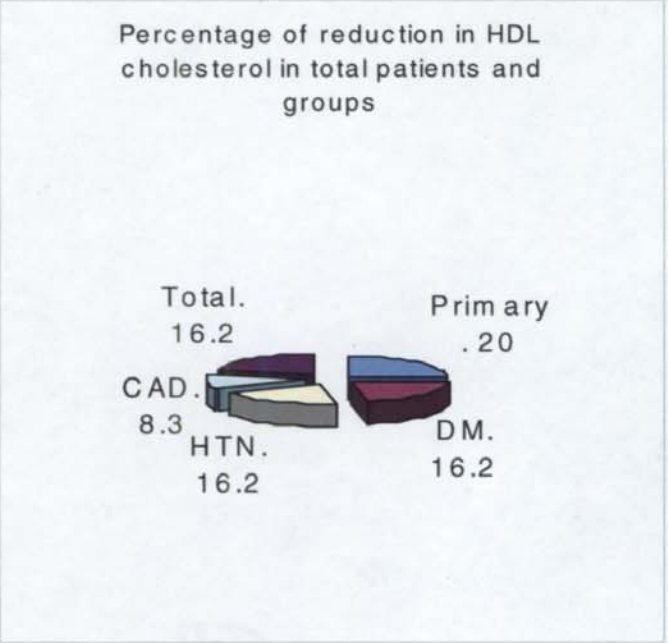


Figure: 23

The distribution of HDL cholesterol in total patients and different groups are given in Figure 21. In this study 23.25 % of the patients with HDL cholesterol of more than 60 mg/d L were reduced to 4.65%; 23.25 % of patients with HDL cholesterol between 50-59.9 mg/d L were reduced to 16.27%; 25.58% of patients with HDL cholesterol between 40-49 mg/dL increased to 30.23% and 27.98% of patients with HDL cholesterol below 40 mg/d L were increased to 48.83%.

The mean HDL cholesterol among total patients and different groups before and after treatment, and the percentage of reduction of HDL cholestrol after treatment are given in Figure 22 and 23 respectively.

The test values for reduction in HDL cholesterol in total patients and different groups are given in Table -15:

Table-15: Test values for assessing significance of reduction in HDL cholesterol

Variable	Group	Sample size	Description	Mean	S.D	Difference in means	S.D	S.E.of mean	t-value	*p*-value
HDL CHOLESTEROL	PRIMARY	29	Before treatment	48.95	12.04	9.83	11.41	2.11	4.64	<0.001
			After treatment	39.12	11.29					
	DM	22	Before treatment	53.43	15.72	8.70	12.38	2.63	3.30	0.003
			After treatment	44.72	10.12					
	HTN	20	Before treatment	44.44	15.85	7.15	15.93	3.56	2.01	0.059
			After treatment	37.29	12.95					
	CAD	14	Before treatment	46.95	19.47	3.88	16.68	4.45	0.87	0.399
			After treatment	43.07	8.80					
	TOTAL	86	Before treatment	48.62	15.31	7.92	13.57	1.46	5.41	<0.001
			After treatment	40.70	11.22					

The *p*- value of < 0.001 in primary group and total patients; of 0.399 in Coronary artery disease group; of 0.003 in Diabetic group and 0.059 in hypertensive group indicates a mixed response. Significant reduction is noted in primary group, diabetic group and in total patients while in hypertensive and coronary artery disease group the reduction is not significant.

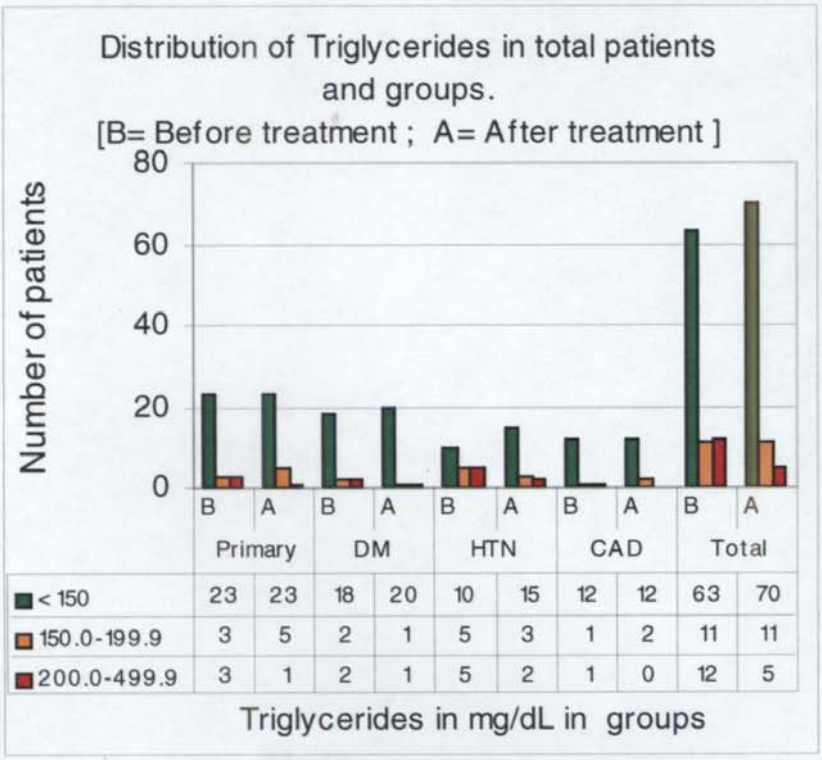


Figure: 24

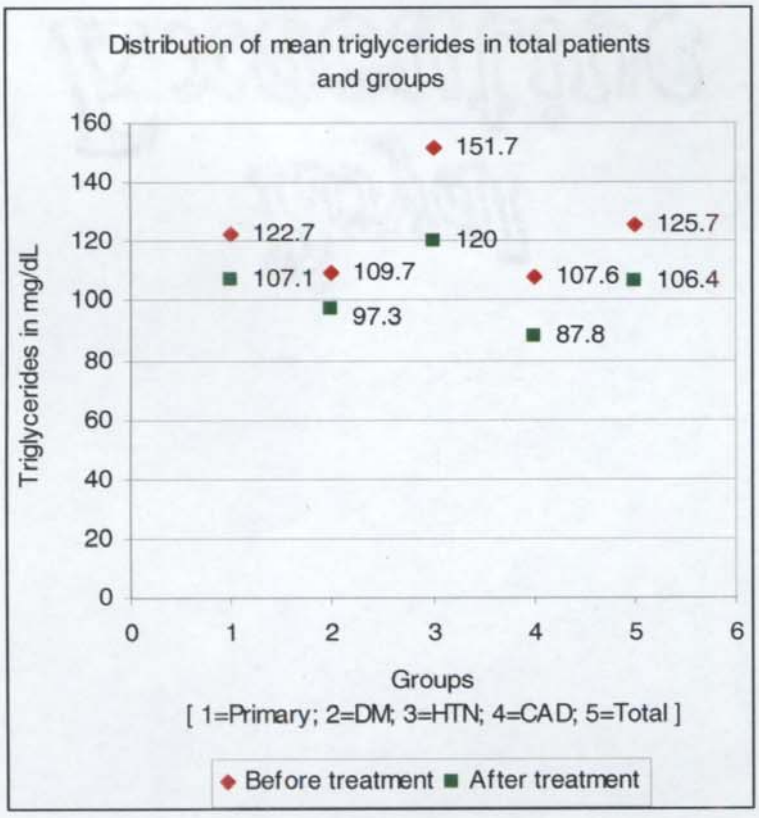


Figure: 25

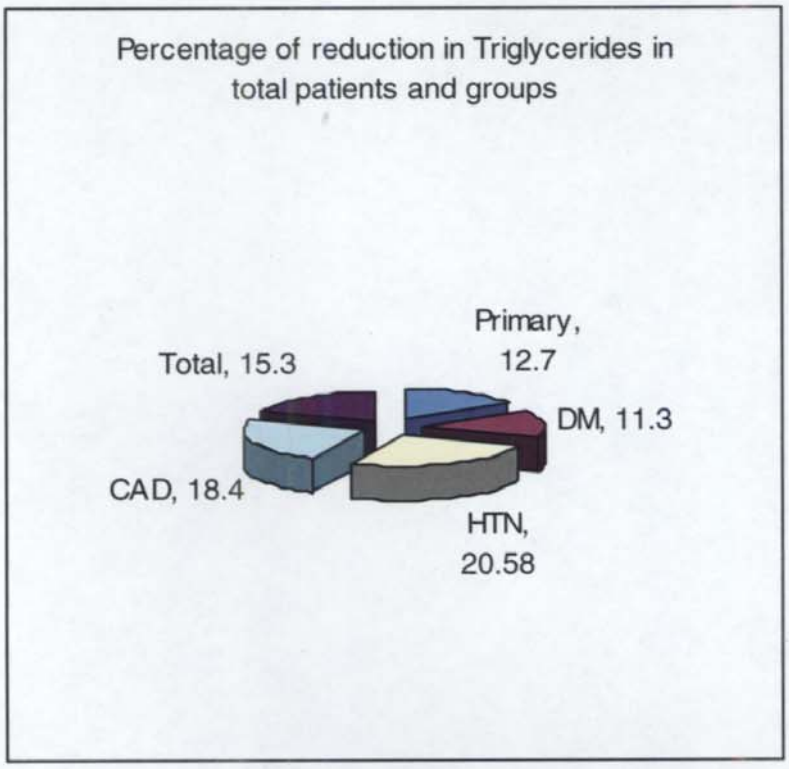


Figure: 26

In this study 13.95% of patients in high-risk group were reduced to 5.8%; 12.79% of patients in borderline high group remained the same, and 73.25% of the patients with Triglycerides below 150 mg/dL were increased to 81.39% [Fig-24].

The mean value of Triglycerides among total patients and different groups before and after treatment, and the percentage of reduction of Triglycerides after treatment are given in figure 25 and 26 respectively.

The test values for assessing the significance of reduction in Triglyceride are given in Table- 16:

Table -16:Test values for assessing the significance of reduction in Triglycerides

Variable	Group	Sample size	Description	Mean	S.D	Difference in means	S.D	S.E.of mean	t-value	*p*-value
TRIGLYCERIDES	PRIMARY	29	Before treatment	122.7	45.11	15.68	45.3	8.41	1.86	0.073
			After treatment	107.10	46.27					
	DM	22	Before treatment	109.75	44.12	12.41	46.17	9.84	1.26	0.221
			After treatment	97.33	55.68					
	HTN	20	Before treatment	151.79	73.01	31.71	55.66	12.44	2.55	0.020
			After treatment	120.08	59.60					
	CAD	14	Before treatment	107.65	42.04	19.81	34.72	9.28	2.13	0.052
			After treatment	87.84	42.91					
	TOTAL	86	Before treatment	125.79	57.03	19.35	46.19	4.98	3.89	<0.001
			After treatment	106.43	54.801					

The *p*-value of 0.073 in primary group; of 0.221 in diabetic group; of 0.052 in CAD group does not indicate any significant reduction, while *p* –value of 0.020 in hypertensive group and less than 0.001 in total patients shows significant reduction. Increase in triglycerides above normal was seen in hypertensive group alone, while in other groups triglycerides were in normal level.

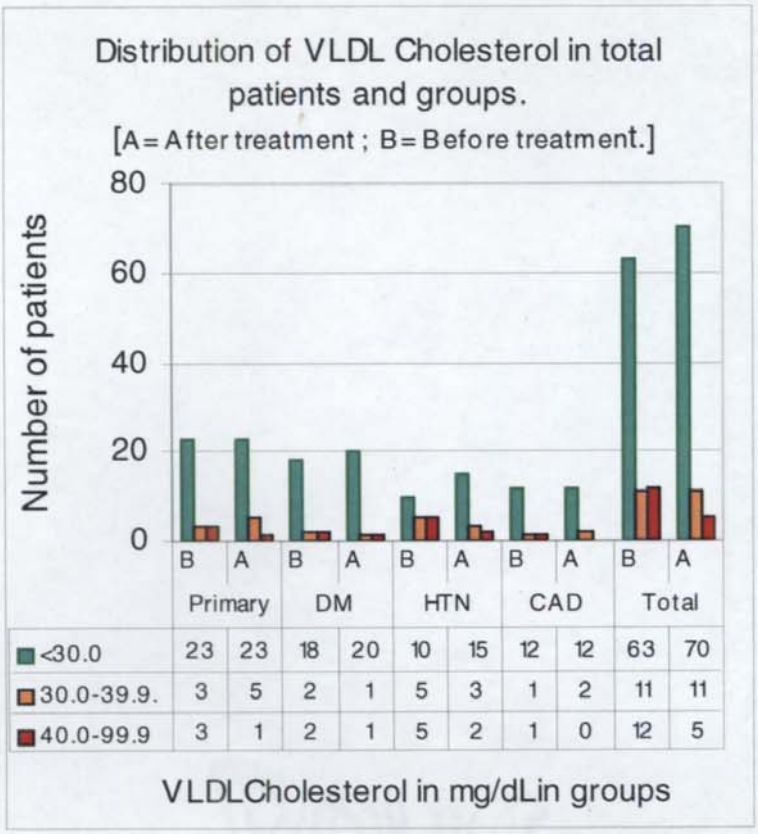


Figure: 27

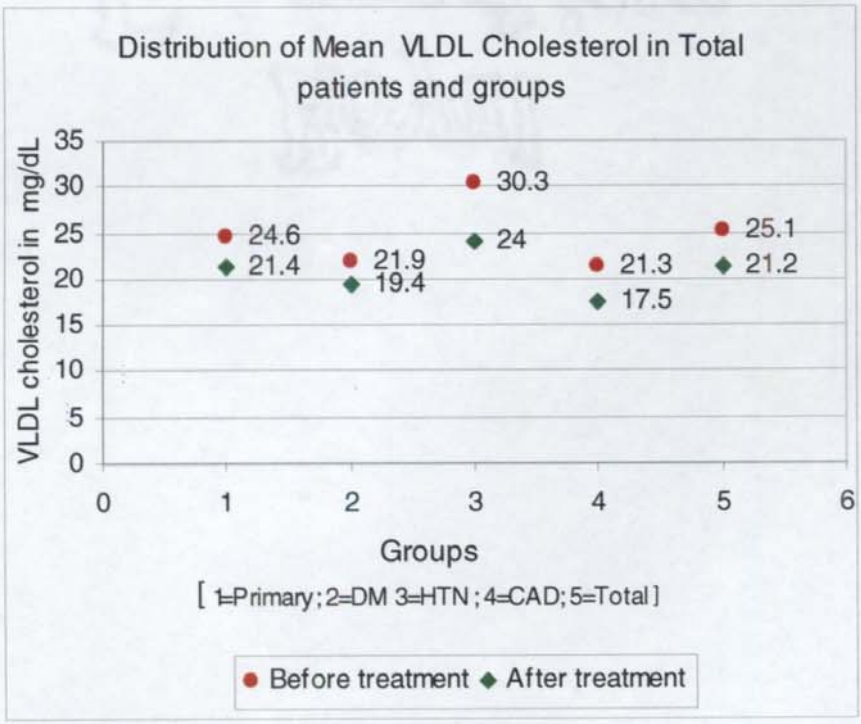


Figure: 28

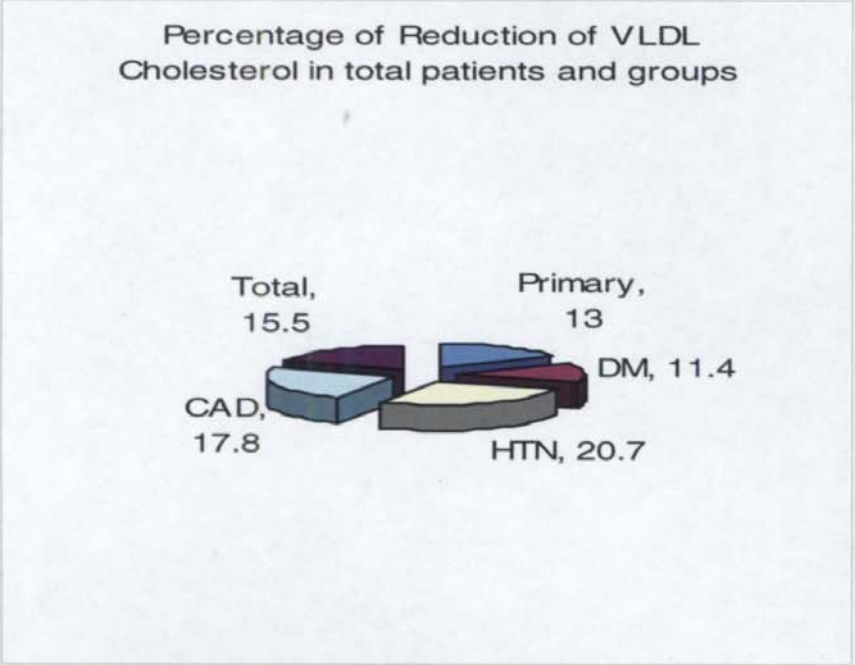


Figure: 29

The distribution of VLDL Cholesterol in total patients and groups are given in figure – 27. As in the case of Triglycerides the number of patients from high risk group were reduced and the number of patients in optimal group increased.

The mean value of VLDL cholesterol in total patients and different groups before and after treatment, and the percentage of reduction of VLDL cholestorol after treatment are given in figures 28 and 29 respectively.

The test values for assessing the significance of the reduction in VLDL cholesterol in total patients and in groups are given in Table-17:

Table -17:Test values for assessing the significance of reduction in VLDL cholesterol

Variable	Group	Sample size	Description	Mean	S.D	Difference in means	S.D	S.E.of mean	t-value	*p*-value
VLDL CHOLESTEROL	PRIMARY	29	Before treatment	24.69	8.84	3.28	9.09	1.68	1.94	0.062
			After treatment	21.41	9.27					
	DM	22	Before treatment	21.94	8.81	2.49	9.24	1.97	1.26	0.220
			After treatment	19.45	11.13					
	HTN	20	Before treatment	30.35	14.61	6.30	11.08	2.47	2.54	0.020
			After treatment	24.04	11.95					
	CAD	14	Before treatment	21.37	8.48	3.81	7.01	1.87	2.03	0.063
			After treatment	17.55	8.57					
	TOTAL	86	Before treatment	25.17	11.37	3.88	9.23	0.99	3.90	<0.001
			After treatment	21.28	10.97					

With a *p*- value of 0.062 in primary group, 0.220 in Diabetic group, 0.063 in CAD group, no significant reduction in VLDL cholesterol is noted. At the same time *p*-value of 0.020 in Hypertensive group and < 0.001 in total patients indicates a significant reduction. Increase in triglycerides above normal was seen in hypertensive group alone, while in other groups triglycerides were in normal level.

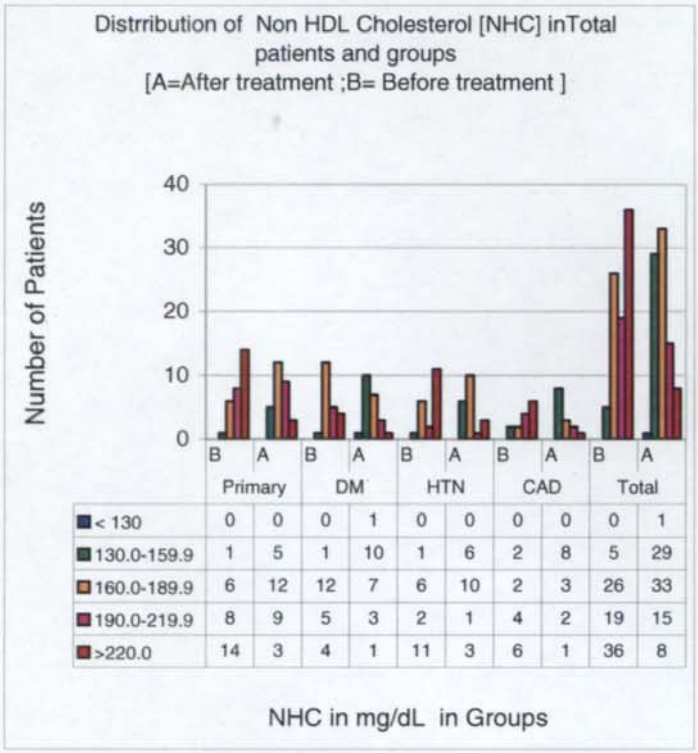


Figure: 30

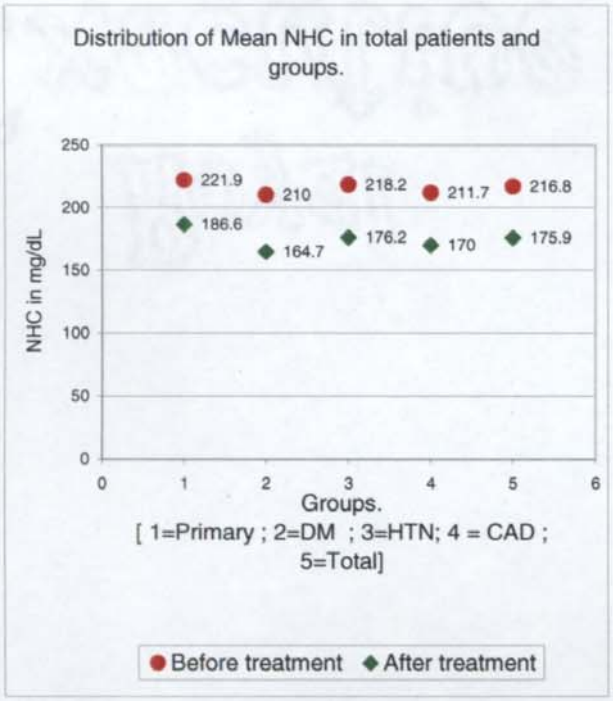


Figure: 31

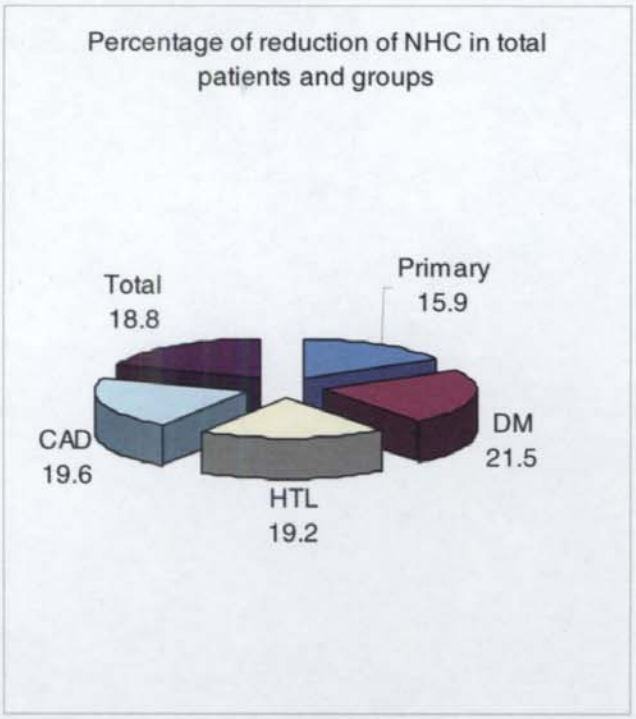


Figure: 32

The distribution of Non-HDL cholesterol in total patients and groups are given in Figure – 30. In this study 41.86% of patients with NHC above 220 mg/dL were reduced to 9.30%; 22.09% of patients having NHC between 190 -220mg/dL was reduced to 17.44%; 30.23% of patients with NHC between 160-189.9 mg/dL were increased to 38.37%; and 5.81% of patients with NHC in the range of 130-159.9 were increased to 33.72%. In this study 1.16% of patients attained NHC range below 130 mg/d L.

The mean values of NHC and in total patients and groups, before and after treatment, and the percentage of reduction of NHC after treatment are given in figures 31 and 32 respectively.

The test values for assessing the significance of reduction in NHC in total patients and groups are given in Table – 18:

Table -18: Test values for assessing the significance of reduction in NHC

Variable	Group	Sample size	Description	Mean	S.D	Difference in means	S.D	S.E.of mean	t-value	*p*-value
NON HDL CHOLESTEROL	PRIMARY	29	Before treatment	221.92	38.85	35.26	31.97	5.93	5.94	<0.001
			After treatment	186.65	36.86					
	DM	22	Before treatment	210.05	75.62	45.28	69.99	14.92	3.03	0.006
			After treatment	164.76	31.27					
	HTN	20	Before treatment	218.28	38.3	41.66	33.13	7.41	5.62	<0.001
			After treatment	176.62	27.57					
	CAD	14	Before treatment	211.70	44.59	41.63	38.24	10.22	4.07	0.001
			After treatment	170.07	41.21					
	TOTAL	86	Before treatment	216.81	50.88	40.83	45.24	4.87	8.37	<0.001
			After treatment	175.98	34.59					

With a *p*- value of < 0.001 in total patients, hypertensive group, and primary group; of 0.001 in CAD group and of 0.006 in Diabetic group indicates highly significant reduction in non HDL cholesterol.

The mean percentage of risk for developing CAD in primary and hypertensive groups, distribution of mean fasting blood sugar in diabetic group, and mean systolic and diastolic blood pressure in hypertensive group before and after treatment are given in figures 33,34 and 35 respectively.

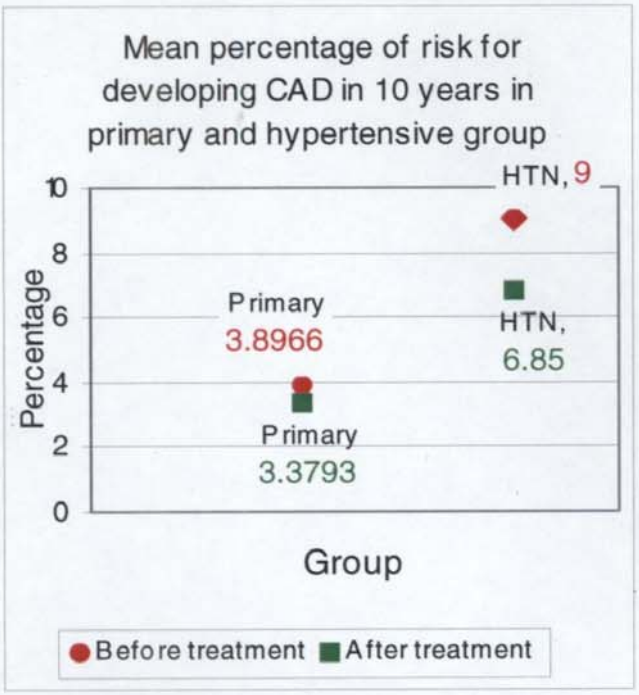


Figure: 33

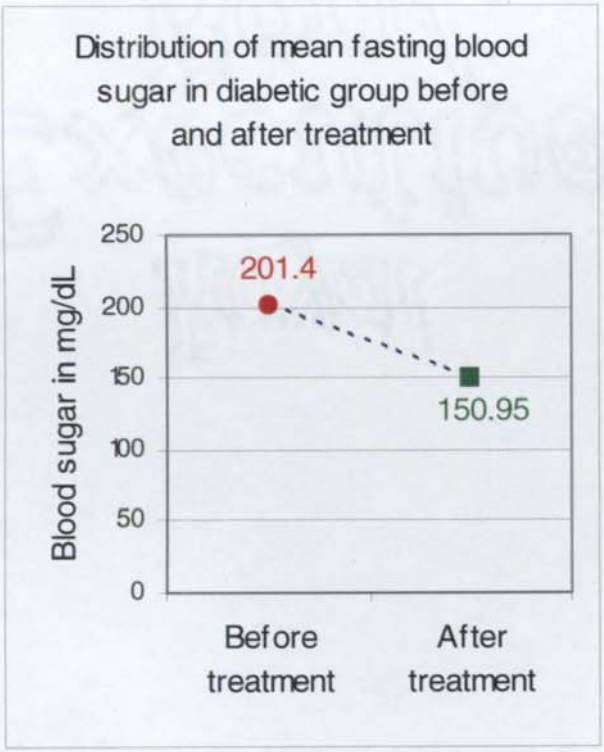


Figure: 34

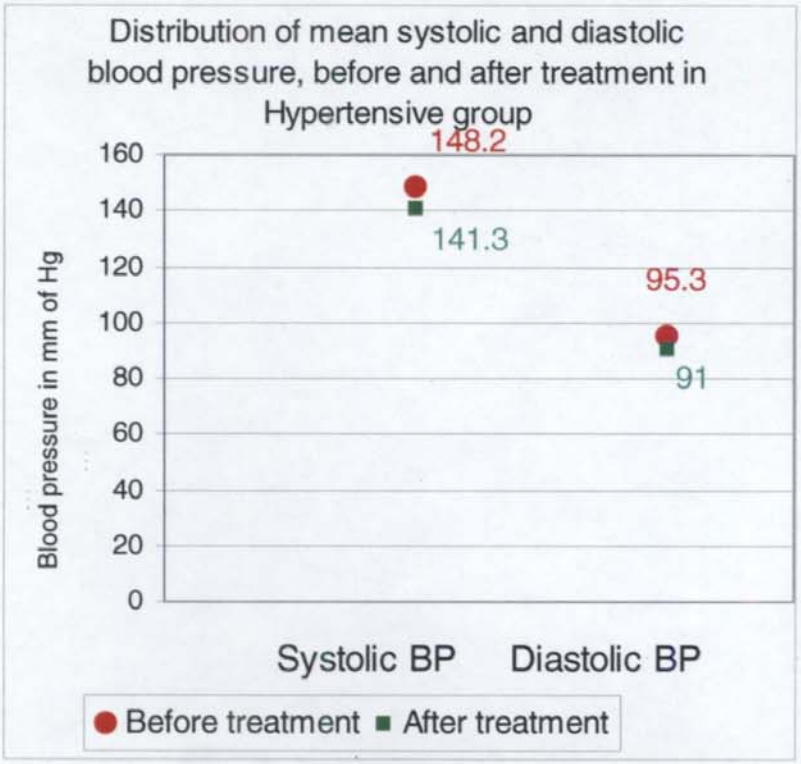


Figure: 35

The test values for assessing the significance of reduction in mean percentage of risk for developing CAD in 10 years, mean fasting blood sugar in Diabetic group, mean systolic and diastolic blood pressure in Hypertensive groups are given in table 19 and 20 respectively.

Table -19: Test values for assessing the significance of reduction in risk of developing CAD in 10 years in primary and hypertensive group and fasting blood sugar in diabetic group

Variable	Group	Sample size	Description	Mean	S.D	Difference in means	S.D	S.E.of mean	t-value	*p*-value
% OF RISK FOR DEVELOPING CAD IN 10 YEARS	PRIMARY	29	Before treatment	3.8966	3.299	0.5172	2.324	0.432	1.20	0.241
			After treatment	3.3793	3.479					
	HTN	20	Before treatment	9.000	7.004	2.1500	3.646	0.815	2.64	0.016
			After treatment	6.8500	5.958					
FASTING BLOOD SUGAR	DM	22	Before treatment	201.4091	66.331	50.4545	63.572	13.554	3.72	0.001
			After treatment	150.9545	49.671					

Table-20: Test values for assessing the significance of reduction in systolic and diastolic blood pressure in Hypertensive group

Variable	Group	Sample size	Description	Mean	S.D	Difference in means	S.D	S.E.of mean	t-value	*p*-value
SYSTOLIC BLOOD PRESSURE	HTN	20	Before treatment	148.2	25.496	6.9	23.863	5.336	1.29	0.211
			After treatment	141.3	32.446					
DIASTOLIC BLOOD PRESSURE	HTN	20	Before treatment	95.3	13.538	4.3	10.549	2.359	1.82	0.084
			After treatment	91.0	14.832					

The Homoeopathic Medicines given in this study and the number of patients responded and not – responded to the homoeopathic drugs in this study are given in Table –21 and 22 respectively.

Table -21: Homoeopathic drugs given in this study:

Group	THU			SUL			LYC			LAC			NM			MED			NS	PHO	AM	SPO	CC	STA	IOD	THY	INS	LUE
	F	S	T	F	S	T	F	S	T	F	S	T	F	S	T	F	S	T	F	F	F	F	F	F	F	S	F	T
Primary	4	14	3	6	1	-	7	-	-	2	-	-	9	1	-	-	-	-	-	-	-	1	-	-	-	-	-	1
DM	-	11	2	9	1	-	7	-	-	-	-	-	1	-	1	-	-	-	-	1	-	-	1	1	1	1	1	-
HTN	3	8	1	5	1	-	2	-	-	-	-	-	9	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
CAD	1	8	2	5	1	-	3	1	-	1	-	-	-	-	-	-	-	-	1	1	2	-	-	-	-	-	-	-
Hepatitis	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	--
Total	8	41	8	26	4	-	19	1	-	3	-	-	19	1	1	1	-	-	1	2	2	1	1	1	1	1	1	1

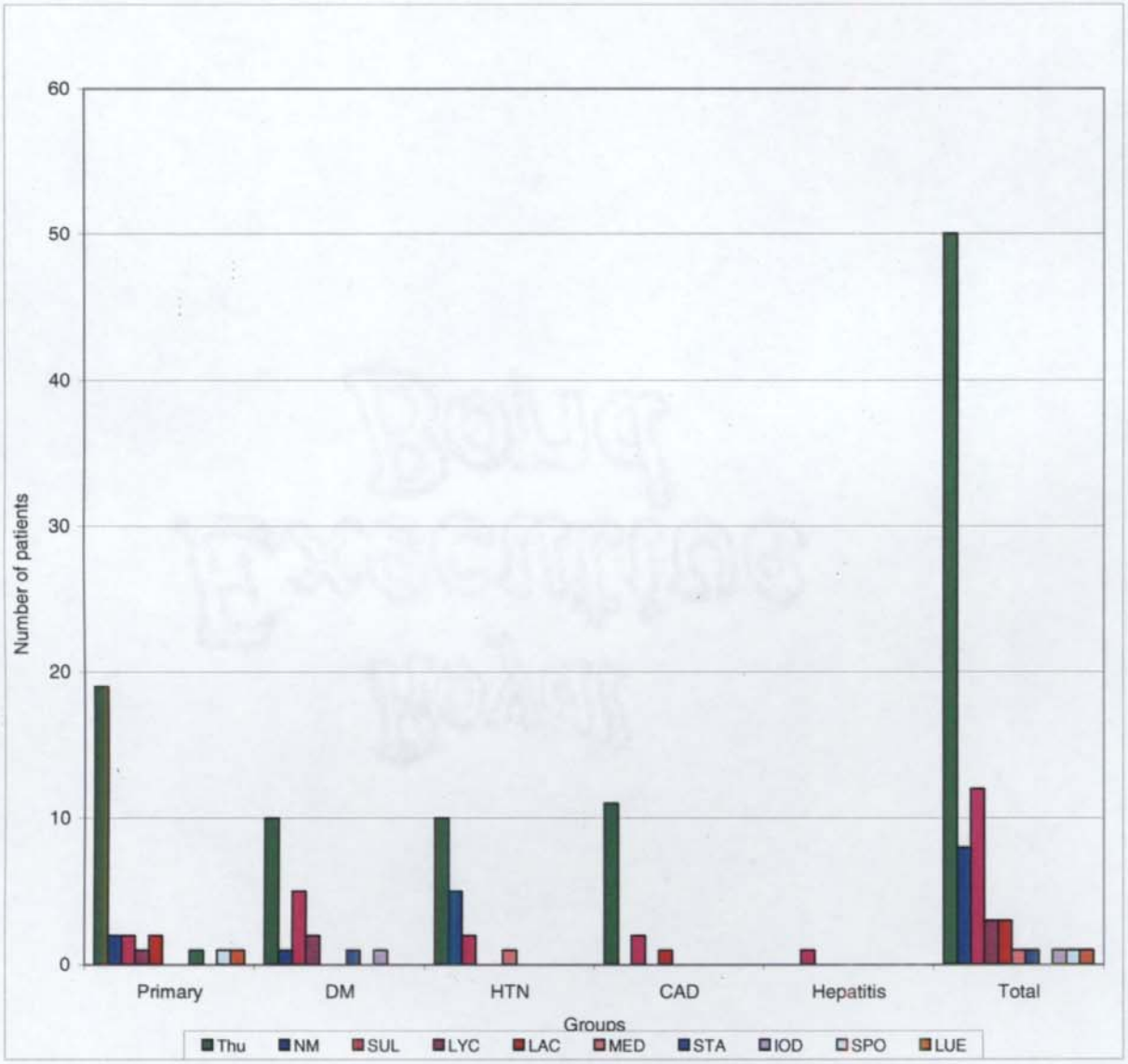
AM = Aurum metallicum **CC** = Calcarea carbonica –ostrearum ; **F**=First prescription; **Iod** = Iodium ; **Ins** = Insulinum;**Lue**=Luesinum; **Lyc**= LycopodiumClavatum; **Med** = Medorrhinum; **NM**= Natrum muriaticum; **NS** = Natrum sulphuricum; **Pho** =Phosphorus ;**S** =Second prescription; **Sta** = Staphysagaria; **Spo** = Spongia tosta;;**Sul**= Sulphur ; **T**= Third prescription; **Thu** =Thuja occidentalis; **Thy**=Thyroidinum

Table -22: Number of patients responded and not responded to homoeopathic drugs

	Thu	NM	SUL	LYC	LAC	MED	STA	IOD	SPO	LUE	TOTAL RESPONDED	NOT RESPONDED
Primary	19	2	2	1	2	-	-	-	1	1	28	1
DM	10	1	5	2	-	-	1	1	-	-	20	2
HTN	10	5	2	-	-	1	-	-	-	-	18	2
CAD	11	-	2	-	1	-	-	-	-	-	14	0
Hepatitis	-	-	1	-	-	-	-	-	-	-	1	0
Total	**50**	**8**	**12**	**3**	**3**	**1**	**1**	**1**	**1**	**1**	**81**	**5**

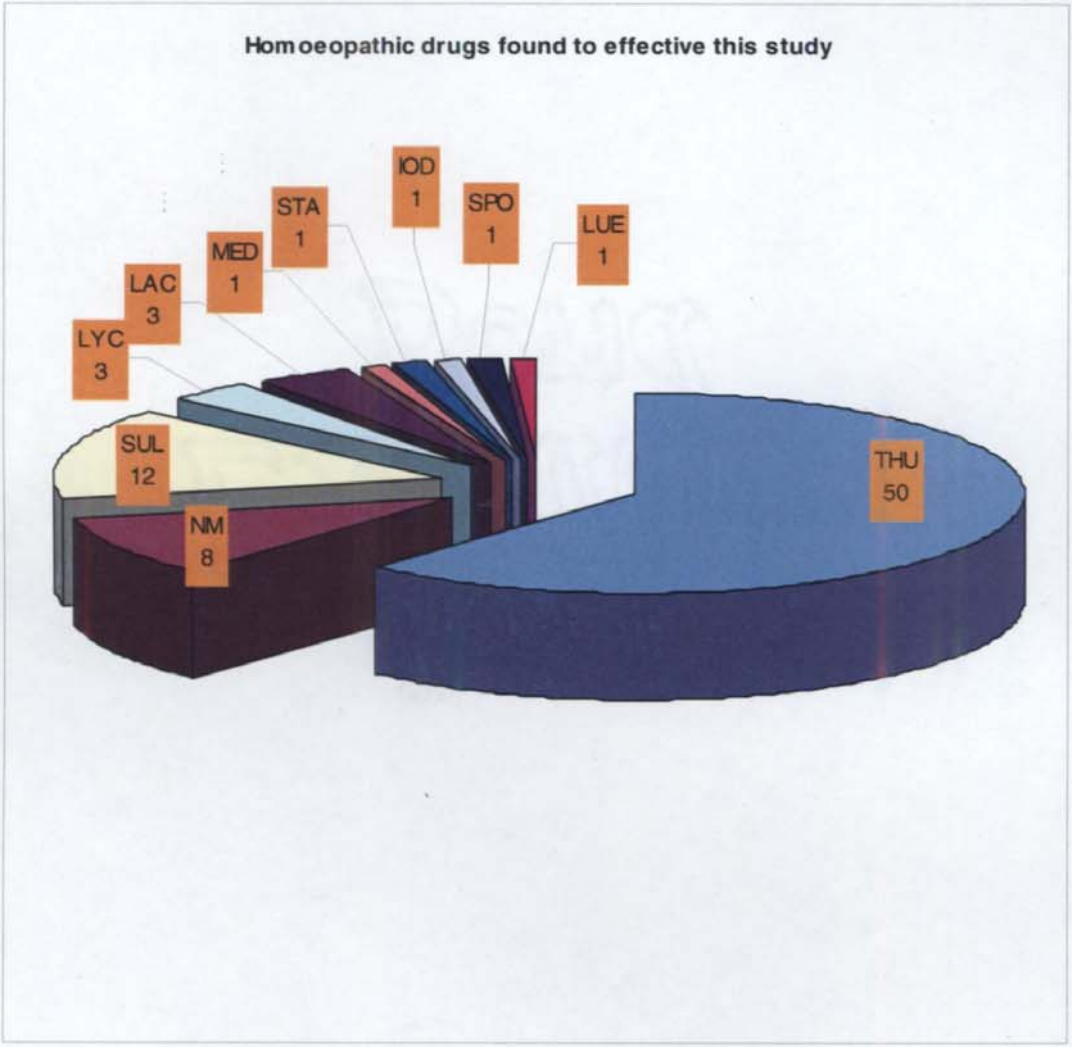
A comparative study of the drugs found to be effective in this study in different groups and total patients are given in Figure-36:

Figure: 36 Comparative study of drugs found to be effective in different groups



Homoeopathic drugs found to be effective in this study are given in Figure –37.

Figure: 37 Homoeopathic drugs found to be effective in this study



The total population of this study have shown an increased level of total cholesterol (fig-15,16), LDL cholesterol (fig – 18,19)and non – HDL cholesterol (fig-30,31) before treatment. After treatment the statistical analysis indicates a highly significant reduction in total cholesterol (fig -15,16,17 table-13), LDL cholesterol (fig 18,19,20, table-14), and non –HDL cholesterol (fig – 30,31,32, table-18). There was reduction from high risk group to low risk group and optimal levels. This reduction is seen both in total population and sub groups.

The mean distribution of triglycerides and VLDL cholesterol was within optimal level in total population and all sub groups except in hypertensive group (fig- 24, 25, 27, 28). Significant reduction of triglycerides and VLDL cholesterol were effected in hypertensive group and total population. (Fig-24, 25, 26,27,28,29 table-16, 17). Other sub groups have also shown a reduction in triglycerides and VLDL cholesterol levels, even within the normal limits.

As in the case of total cholesterol, LDL cholesterol, Non –HDL cholesterol, triglycerides, and VLDL cholesterol, a reduction was noticed in HDL cholesterol after treatment, which was not found to be uniform in sub groups (fig – 21, 22, and 23) and was not expected. Though significant reduction of HDL cholesterol was noted in primary group, diabetic group and total population, less significant reduction were noted in hypertensive and coronary artery disease group (table- 15). As all variables except HDL cholesterol have shown a uniform reduction in total population and sub groups after treatment, the non- uniform reduction effected in HDL cholesterol may not be due to the direct effect of homoeopathic drugs. Other factors like truncal obesity, lack of exercise, effects of

other drugs taken before the treatment in this study might have influenced this reduction. Sethi K K, Ashwani Mehta [51] reports a reduction of HDL cholesterol of 5- 20% by statins, 10-35% by nicotinic acid, 5-15% by Fibrates and 3-5% by Bile acid sequestrants. The reduction of HDL cholesterol in this study by homoeopathic medicines mentioned in figure - 37 has also shown a similar range of 16.2 % in total patients, 20% in primary hyperlipoproteinemia group, 8.3% in CAD group, 16.2% in Hypertensive group and diabetic group (Fig 23). How ever the mean percentage of risk for developing coronary artery disease in 10 years is found to be reduced in primary group and more significantly in hypertensive group (fig- 33, table- 19).

ATP III does not specify a goal for HDL raising, as sufficient evidence for reduction of risk from raising the HDL cholesterol is insufficient to specify a goal of therapy. More over currently available drugs does not robustly raise HDL cholesterol. In all persons with low HDL cholesterol, the primary target of therapy is LDL cholesterol. Though HDL cholesterol is found to be reduced in some groups, the uniform reduction in total cholesterol and LDL cholesterol were found to be highly significant.

ATP III defines the reduction of LDL cholesterol as primary goal of therapy. ATP III also identifies the Non - HDL cholesterol as a secondary target of therapy in persons with triglyceride levels of 200 mg/dL.

As observed in the executive summary of ATP II, clinical trails of relatively short term duration indicate that a 2% reduction in CAD rates results from each 1% reduction in serum cholesterol. And the reduction achievable with

long term cholesterol lowering may be, perhaps, 3% for each 1% reduction in cholesterol.

A comparative study of the changes in lipoprotein in the major studies - Scandinavian simvastatin survival study (4S), Cholesterol and recruitment events (CARE), Long term intervention with pravastatin in ischemic heart disease (LIPID), West of Scotland coronary prevention study(WOSCOPS), Air force / Texas coronary atherosclerosis prevention study (AFCAPS / TexCAPS) with the present minor study are given below:

Landmark trial	4S	CARE	LIPID	WOSCOPS	AFCAPS/ TexCAPS	Present study
Number of subjects	4,444	4,159	9,014	6,595	6,605	86
Mode of therapy	Simva Statin	Prava statin	Prava statin	Prava statin	Lova statin	Homoeopathic medicines
Duration of study (years)	5.4	5	6.1	5	5.2	2
Baseline mean total cholesterol (mg/d L)	270	209	218	272	221	264.89
Total cholesterol reduction (%)	25	20	19	20	18	18.1
LDL reduction (%)	35	28	27	26	25	19.2
HDL increase (%)	8	5	4	5	6	nil (↓)

It is to be noted that the major studies were conducted for more than 5 years and involves more number of cases. This minor study was of only about 2 years duration with less number of patients.

This study indicates that Homoeopathic drugs *Thuja occidentalis, Natrum muriaticum, Sulphur, Lycopodium clavatum, Lachesis mutus, Medorrhinum, Staphysagaria, Iodum, Spongia tosta, Luesinum* are effective (table-22, fig - 36,37) in the management hyperlipoproteinemia.

Statistical analysis of this study with homoeopathic drugs achieves the aim similar to that have been prescribed in ATP II and III. Further, analysis of mean fasting blood sugar in diabetic group (fig – 34, Table -19) shows significant reduction, and systolic and diastolic pressure in hypertensive group also shows a reduction after treatment (fig- 35, table- 20).

This study indicates that homoeopathic drugs are effective in reducing total cholesterol and LDL cholesterol and suggest that it can be used effectively for the primary and secondary prevention of coronary artery disease.

It may be presumed that the mode of action of Homoeopathic Medicine may be through stimulation of the LDL receptors, HTGL and LPL - as LDL cholosterol is found to be reduced significantly. Further study is required for its confirmation.

Future studies involving more number of patients and observation for longer period may give better results than this study.

CONCLUSION

This clinical study was conducted in the patients attending the unit 1 of the department of Organon and Homoeopathic philosophy, Government Homoeopathic Medical College, Calicut.

A total number of 86 patients (Primary hyperlipoproteinemia- 29; Diabetic – 22; Hypertensive – 20; Coronary artery disease -14 and Hepatitis-1) were observed for a period from 27.9.01 to 16.10.2003.

The cases were taken and evaluated according to the principles of Homoeopathic philosophy and followed up with estimation of lipid profile and other necessary investigations.

49 patients were male and 37 were female within a range of 25-75 years of age, the average age being 49.1 years. The patients belonged to Hindu, Muslim as well as Christian community.

19 patients were overweight and 12 patients had an obesity of class 1 type. Mean BMI was found to be more in females than in males, except in coronary artery disease group. All female patients and 45 male patients had a waist: hip ratio above normal.

Total population of this study group had an increased level of total cholesterol (with a mean value of 264.8 mg %), LDL cholesterol (with a mean value of 191.5 mg %), Non – HDL cholesterol (with a mean of 216.8 mg %). 24 patients had HDL below 40mg%. The triglycerides and VLDL cholesterol were

within normal limits (with a mean value of 125.7 mg% and 25.1 mg% respectively), except in hypertensive group with a mean value of 151.7 mg % and 30.3 mg %.

Significant reduction was noticed after treatment in total cholesterol with a mean level of 216.7mg% (18.1 % reduction), in LDL cholesterol with a mean level of 154.6 mg% (19.2% reduction) and in non HDL cholesterol with a mean level of 175 mg% (18.5% reduction).The triglycerides and VLDL cholesterol were also reduced with a mean level of 106.4 mg% (15.5 % reduction) and 21.2%. In hypertensive group also the triglyceride and VLDL cholesterol levels were reduced with a mean level of 120 mg% and 24 mg% respectively.

The HDL cholesterol was also reduced with a mean level of 40.7mg% (16.2 % reduction).

Statistical analysis of variables shows significant reduction in total cholesterol, LDL cholesterol, non–HDL cholesterol, Triglycerides and VLDL cholesterol in Hypertensive group while HDL reduction was found to be non uniform in different groups.

The ATP III defines the reduction of LDL cholesterol as the primary goal of therapy and identifies the non-HDL cholesterol as a secondary target of therapy in persons with triglycerides level of 200 mg%.

This study have shown highly significant reduction in LDL cholesterol with a *p*-value of less than 0.001 in primary hyperlipoproteinemia group,

hypertensive group and total patients, 0.011 in diabetic group and 0.003 in coronary artery disease group.

This study also demonstrated reduction in the percentage of risk for developing coronary artery disease in 10 years in primary hyperlipoproteinemia group and hypertensive group.

The mean fasting blood sugar level in diabetic group was also found to reduced significantly with *p*- value of 0.001.

The mean systolic and diastolic blood pressure in hypertensive group was also reduced after treatment.

This study indicates that Homoeopathic drugs *Thuja occidentalis, Natrum muriaticum, Sulphur, Lycopodium clavatum, Lachesis mutus, Medorrhinum, Staphysagaria, Iodum, Spongia tosta, Luesinum* are effective in the management hyperlipoproteinemia and can be effectively utilised in the prevention of coronary artery disease.

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