REVIEW OF LITERATURE
Different studies have shown altered levels of plasma lipoproteins in the pathogenesis of atherosclerosis, particularly high levels of low density lipoprotein (LDL) and reduced levels of high density lipoprotein (HDL) appear to be strong risk factors for the development of atherosclerosis.

High blood pressure is an important risk factor for the development of atherosclerosis, namely, ischaemic heart disease and cardiovascular disease. The risk increases progressively with increasing blood pressure.

In the Framingham study (1976), the incidence of ischaemic heart disease in the middle aged men with blood pressure exceeding 160/95 was more than five times than in the normotensive men (BP 140/90 or less). Conversely, the risk of atherosclerosis appears to diminish by therapeutic reduction of blood pressure. Special urgency for the reduction of blood pressure is important when hyperlipidemias, diabetes or other risk factors are concomitantly present.

Atherosclerosis is a disease of large and medium sized muscular arteries and has a basic lesion - atheroma or fibro-fatty plaque consisting of an accumulation of lipid filled smooth muscle cells and macrophage (Foam cells) and fibrous tissue in focal area of the intima.
The fatty streak is usually sessile and causes little obstruction and no symptoms. The lesion is universal appearing in various segment in arterial tree at different ages beginning at the aorta in early infancy. In all children, regardless of race, sex or environment, fatty streaks are invariably present in the aorta by the age of ten and increases to occupy as much as 30 to 50 percent of the aortic surface by the age of 25 years but they donot appear to extend further with ageing.

Fatty streaks may be the earliest lesion of atherosclerosis. In the coronary arteries, the extent of fatty streaks may be a better indicator of the development of clinically significant raised lesion later in life. In the coronary vessels, atherosclerosis is nearly always found in the epicardial portion of the vessels, while the intramural coronary arteries are spared. Coronary atherosclerosis is often diffuse. Typical atheromatous plaque also develop in the saphanous vein aorta coronary by-pass grafts also.

Works of the previous decades have revealed a complex set of events that control plasma lipoprotein level.

Some specific protein have been implicated in the regulation of lipoprotein synthesis. Besides this, many factors such as age, sex, cigarette smoking, obesity, hypertension, dietary habit and life style exert their influence on lipoprotein level and development of atherosclerosis in their own way. Many of the risk factors are reversible but influence of age, sex & genetic factors are irreversible.
Hypertension enhances atherosclerosis by directly producing injury via mechanical stress on endothelial cells in the arterial tree, by markedly increasing the lysosomal enzyme activity that lead to increased cell degradation and release of highly destructive enzymes into the arterial wall.

The relationship between the levels of dietary and serum cholesterol has attracted the attention of the scientific community. The most important association of atherosclerosis and ischaemic heart disease is the elevated level of LDL (Weiss et al, 1972) but hyperlipidemia with increased concentration of VLDL also appear to increase the risk. In contrast, serum HDL level are inversely related to the ischaemic heart disease risk (Heirs et al, 1980).

**HYPOTHESIS OF ATHEROGENESIS**

Current theories of the pathogenesis of the lesion of atherosclerosis relate back to early proposals made by Virchow, Rokitansky and Duguid. Virchow believed that a form of low grade injury to the artery wall resulted in a type of inflammatory insudation which in turn caused increased passage and accumulation of plasma constituents in the intima of the artery. Rokitansky's belief, subsequently elaborated upon by Duguid was then an encrustation of small mural thrombi went onto organize by the growth of smooth muscle cells into them and they would become incorporated into the lesions and thus serve on sites where the lesions would progress.
In 1973, these two notions about atherogenesis were combined with new knowledge of the cellular and molecular biology of the artery wall in a hypothesis termed "the response to injury hypothesis of atherosclerosis". A second hypothesis that was also formulated in 1973, the monoclonal hypothesis, suggests that the lesions of atherosclerosis may represent some form of neoplasm.

It has been demonstrated that advanced semi-occlusive lesions of human coronary atherosclerosis can also regress. In a quantitative image analysis study of coronary angiogram from a series of patients being aggressively treated with lipid lowering regimens of either ninficin and colestipol or lovastatin and colestipol. Brown and colleagues have demonstrated statistically significant regression in association with decreases in plasma cholesterol and LDL. This provides a clear evidence that the lesions of atherosclerosis are able to regress at apparently all stages of lesion development. A number of investigators have proved the capacity of fish oils, which contain large amount of Omega-3 fatty acid, to decrease plasma cholesterol level and potentially to induce lesion regression when added to the diet of hypercholesterolemic individuals.

**Chemistry of Plasma Lipoproteins**

The major classes of plasma lipoproteins are cholesterol, cholesteryl ester, triglycerides and
phospholipids. Although lipids are vital components of many of the body's tissues, they are insoluble in water. To reach those tissues, lipids must be transported in the blood stream by complex water soluble molecules called lipoproteins. Structurally, lipoprotein consists of a core of non polar cholesteryl ester and triglyceride covered by a polar surface monolayer made of phospholipids, free cholesterol and the protein or polypeptide moieties called apolipoproteins. Each of the plasma lipids has one or more apoproteins that performs specific function essential to lipid transport and cellular uptake.

The five principal lipoprotein classes are defined according to their density on ultracentrifugation and by their mobility on agarose gel electrophoresis. In addition, they can be classified on the basis of size and relative concentration of cholesterol or triglyceride and by their apoprotein content. The major lipoprotein classes are chylomicron, very low density lipoproteins (VLDL), intermediate density lipoproteins (IDLs), low density lipoprotein and high density lipoproteins.

**CHANGES IN LIPOPROTEIN LEVEL**

**AFTER HIGH CHOLESTEROL DIET**

Most studies have established that dietary cholesterol and fats have definite relation to serum lipids. Plasma cholesterol and LDL level are sensitive to the amount of saturated fat and cholesterol in the diet.
Ingested fats usually contain about three times as much saturated fatty acids (mainly Palmitic and Stearic) as polyunsaturated fatty acids (namely linoleic and linolenic).

If a healthy young adult switches from this diet to one contains the same amount of total fat in which the ratio of polyunsaturated to saturated is closer to unity and the cholesterol content is less than 300 mg/day the STC level will drop 10-15 percent within 2 weeks and remain depressed on continuation of the diet.

But effect of long term and short term feeding of diet rich in cholesterol shows variable responses and in subject to individual variation.

The studies to evaluate the changes in lipoprotein profile after ingestion of high cholesterol fat diet in selected number of subjects, showed that the basal levels of various lipoproteins except serum triglycerides were within normal range at their age (Arora et al, 1987). The studies also indicated that after prolonged high cholesterol breakfast (HCFB for 7 days), young subjects showed predominant rise in STC is in the form of HDL cholesterol while in old subjects rise in STC was due to LDL cholesterol (Arora et al, 1989, 1985). Some persons behaved differently showing fall in their STC level. The individual responses to HCFB varies enormously but remains constant for an individual over along period of time (Kinsburry et al, 1960).
CHYLOMICRONS

Chylomicrons are the largest of the lipoprotein. Their primary functions is to transport dietary or exogenous triglyceride and cholesterol from the intestinal lumen to sites of metabolism or storage. The chylomicrons are formed in the gastrointestinal (G.I.) tract. In the lumen of the GIT, dietary fat is degraded into free fatty acids and monoglycerides. These substances enter the intestinal villi, where they are reconstructed into a triglyceride particle. Dietary cholesterol absorbed into the intestinal wall is then esterified to cholesteryl esters, mainly cholesteryl oleate, by the enzymatic reaction catalysed by acyl-cholesterol acyl transferase. The triglyceride and cholesteryl esters are then combined with apo B48, AI and AN within the intestinal wall to form chylomicron particles. The nascent chylomicron particles enter the systemic circulation by the way of lymphatics. Apo-E and C are then added to the particles. Normally chylomicrons are cleared rapidly from the blood and are virtually absent in the fasting state. The clearing of chylomicrons is modulated by the enzyme lipoprotein lipase (LPL). LPL catalyzes hydrolysis of the triglyceride core of the chylomicron, leaving a remnant particles rich in cholesterol, apo-C, apo-E and apo-B48. These remnants are thought to be atherogenic. Delayed clearance of the remnant particles may damage the vascular endothelium and thus predispose to atherosclerosis. The
detection of chylomicron particles in fasting plasma is always abnormal and indicate the presence of hyperlipidemia. The overall result of chylomicron transport process is to deliver dietary triglyceride to adipose tissue and cholesterol to the liver.

**VERY LOW DENSITY LIPOPROTEIN (VLDL)**

VLDLs are intermediate in size between chylomicrons and IDL. VLDLs are produced in the liver. Their primary lipid is triglycerides but cholesterol, cholesteryl ester and phospholipids are also present. The synthesis of VLDL is increased by excess carbohydrates, alcohol or caloric consumption. The function of VLDL is to transport endogenously synthesized triglyceride and cholesterol into the peripheral tissues, where the lipids fatty acids can be utilized for energy or stored as triglyceride. VLDL particle upon hydrolysis by the enzyme LPL changes into IDL.

IDL which carry both cholesterol and triglyceride, are the product of the enzymatic break down of VLDL. Elevations of IDLs are thought to predispose to premature CAD and peripheral artery disease.

**LOW DENSITY LIPOPROTEINS (LDL)**

LDL which is 45 percent cholesterol by weight is the major carrier of cholesterol to the nerve tissue, cell membrane and other cells that require the cholesterol for metabolic function. LDL usually is formed from VLDL break down. Increased LDL synthesis may occur by means
of enhanced conversion of VLDL remnants or direct hepatic production of apo-B containing lipoproteins. Apo-B_{100} is the only protein found in LDL and makes up about 20 percent of the LDL mass.

About 75 percent of the LDLs in the blood stream are removed by the specific receptor mediated binding. The remaining LDL particles are cleared by scavenger or macrophages, receptors or by nonreceptors mediated mechanisms.

**HIGH DENSITY LIPOPROTEINS (HDL)**

HDLs are produced by the liver and the GI tract and by the peripheral catabolism of chylomicrons and VLDLs. HDL particles carry cholesteryl ester as their major lipid and apo AI and AII as their major proteins.

Much of the apoprotein component of HDL is transferred in the systemic circulation of HDL or chylomicrons. By weight, HDL particles are about 30 percent cholesterol, 45 percent proteins and 25% percent phospholipid. HDL particles exist in several subtypes. For clinical purposes, HDL$_2$ and HDL$_3$ are the major circulating subfractions. HDL$_2$ is the subfraction most clearly associated with statistical protection against premature atherosclerosis. Alcohol consumption increased both HDL subfractions with a greater impact on HDL$_3$. Lower levels of both subfractions are associated with male gender, hypertriglyceridemia, diabetes mellitus, obesity, uremia, use of androgens, progestins and tobacco product and diets rich in polyunsaturated fat but low in total fat.
content. A complete lipoprotein profile including determination of HDL levels should be obtained in any patient with established CAD. In practice, HDL cholesterol level should be measured in any one with a total cholesterol value exceeding 200 mg/dl.

HDL particles are thought to participate in the reverse transport of free cholesterol from the peripheral tissues by way of HDL receptor. Gram and co-workers reported that apo AI and AII interact with this receptor. This receptor mediated reverse transport could explain why patients with elevated HDL concentration are less prone to CAD.

**Lipoprotein (a)** \( [LP(a)] \)

LP(a) has been established as an independent CAD risk factor. The structure of LP(a) is similar to that of an IDL molecule. If the serum level of both LDL and LP(a) are elevated, the risk of CAD is markedly increased. Recent angiographic studies have documented a positive correlation between LP(a) levels and the severity of coronary atherosclerosis.

**Diet and serum total cholesterol (STC)**

Bruhn (1940) observed a 20% rise in mean cholesterol level after a fat load.

Keys et al (1956) showed that serum cholesterol level is independent of the cholesterol intake over the whole range of natural diet.
But later on it was proved that feeding cholesterol rich diet for 2-8 weeks raised STC in blood (Arora et al., 1987, Messinger et al., 1950, Conner et al., 1961, and Deborah Applebaum et al., 1974). Effect of high cholesterol feeding diet on postprandial cholesterol levels studied in the past showed insignificant difference between post prandial value and basal value (Albrink et al., 1936, Pomeranze et al., 1954, Schilling et al., 1964). Patients were studied for STC value upto 24 hour after a test meal.

Nikkila and Konttinen (1962) showed a significant decrease in cholesterol level six hour after a test mean in healthy soldiers.

**DIET AND HIGH DENSITY LIPOPROTEINS (HDL)**

Borden et al. (1964) reported enhanced level of HDL in rats after feeding cholesterol while Haft et al. (1962) and Kritchevsky (1965) showed little change of HDL level in cholesterol fed rats.

High intake of cholesterol in the form of 3-6 eggs yolk/day has been reported to increase apo-lipoprotein-E containing HDL subspecies in human (Mahley et al., 1978). This effect was seen whether or not there was an increase in plasma total cholesterol.

Diet is an important modulator in the synthesis, secretion and concentration of serum lipoproteins.

There are reports that substitution of large amounts of polyunsaturated containing fats for saturated
fat in diet can decrease the level of HDL lipids and protein (Nichamen et al, 1967). An increase in the P/S fat ratio from 0.25 : 1 to 4:1 in diet fed to four normal subjects for five weeks resulted in the reduction of HDL cholesterol and apolipoprotein A-I concentration of 33 and 21 percent respectively with an associated reduction in HDL₂ : HDL₃ ratio (Shephered et al, 1978). Other numerous studies reported either no change (Lewis, 1978 and Shore et al, 1981) or increase (Jackson and Glueck, 1980) in the levels of HDL cholesterol with feeding of diet enriched in polyunsaturated fat.

It has been shown that apolipoprotein A-I level was increased when fats were consumed in divided doses over a 10 hour period, but not when the same amount of fat ingested as a single load (Keys et al, 1980).

Serum Triglycerides (STG) and Very Low Density Lipoproteins (VLDL)

The level of serum triglycerides varies considerably after ingestion of fat rich diet. Rise in STG level has been reported after giving different amount of fat load and measuring the STC level at different time interval. (Mikkila and Konttinen, 1962, Denborough, 1963). Clefsky et al (1975) noted biphasic STG curve with an initial peak occurring 1-3 hours after feeding and a second peak after 4-7 hour. The initial peak was thought to be due to an increase in chylomicron level in more than 98 percent of cases, whereas second peak represented rise in VLDL in 82 percent cases.
Havel (1957) concluded that increment in the level of STG following ingestion of fat is entirely the result of an increase in their concentration in VLDL.

Excess production of VLDL and triglyceride are more often due to secondary abnormalities rather than primary factors, and perhaps the most common cause is high carbohydrate intake, excess consumption of alcohol. Increased levels are also found in diabetes mellitus, nephrotic syndrome and hypothyroidism.

Delayed clearance of triglyceride from the plasma is noted in case of IHD after high fat diet (Arora et al., 1987, David and Brown et al., 1961).

VLDL remnants, also known as beta VLDL having smaller particles size than normal VLDL particles and contain more cholesterol. Both of these features impart atherogenic potential to VLDL remnants.

Beta VLDL lipoprotein level can be increased by cholesterol feeding in man. The beta VLDL, either chylomicron remnants or hepatic lipoprotein, represent the atherogenic particle postulated several year ago by Zilversmit.

**DIET AND LOW DENSITY LIPOPROTEINS (LDL)**

The core of LDL is composed almost entirely of cholesterol esters, and the surface coat contains only one apoprotein - apoprotein B₁₀₀. In human a relatively high fraction of LDL escapes hepatic uptake and subsequently humans have a relatively high circulating level of LDL.
Indeed, about three fourths of the total cholesterol in normal plasma is contained within the LDL particles. One function of LDL is to supply cholesterol to a variety of the extrahepatic parenchymal cells such as adrenal cortical cells, lymphocytes and renal cells. These cells have LDL receptors, localized on the cell surface. In human 70 to 90 percent of LDL is removed from the plasma each day by the LDL receptor pathway. Most of the remainder is degraded by a scavenger cell system in phagocytic cells in the reticuloendothelial system.

Diet, high in fat and cholesterol cause an elevation in LDL in most animals (Mehley, 1978). The response in man varies, but in these subjects who have an elevation in plasma cholesterol, there is an elevation in plasma LDL concentration also.

Deborah, Applebaum et al (1979) demonstrated a significant rise of LDL level in human after feeding 500 mg of egg yolk cholesterol per day for 30 days. Age related difference in rise of LDL was demonstrated by Arora and Gupta et al, 1987). They showed that rise of total serum cholesterol after feeding high fat, high cholesterol breakfast for one week was much more pronounced in young subjects (20-30 yrs) with major portion of rise being contributed by increased HDL. To the contrary, in older subjects, rise of serum total cholesterol was less marked and LDL cholesterol contributing mainly in the increased level.
Bandet et al (1981) demonstrated that there was significant fall in LDL level in five subjects on 3 hours and five hours after taking butter diet. They proved that this fall was due to defect in VLDL hydrolysis by serum lipase and due to metabolic blocking in liver or adipose tissue.

There is an alteration in the LDL apoprotein particles induced by high cholesterol diets. In normal LDL, beta apoprotein (B₁₀₀) is the major detectable moiety. However, in several species, the LDL contain a variable amount of the 'E' apoprotein following high cholesterol feeding (Mehley et al, 1977 and Rudel et al, 1979). The identification of LDL receptor by Joseph Goldstein (1977) and its role in the control of serum cholesterol metabolism has greatly enhanced our understanding of cholesterol turnover. Cholesterol no more remains an inert substance as thought before.

In fact, disappearance of intravenous radioactive cholesterol within 20 minutes of injection from the vascular compartment reflects its high dynamic states with the tissue cholesterol. Perhaps, this dynamic equilibrium is achieved by the presence of LDL receptors and a lot of undefined hormonal or neurogenic factors affecting these receptors.

A single high cholesterol diet induced changes in various lipoprotein fractions and its relevance in
predicting an individual's risk for the development of atherosclerosis in future still remains an unexplored field.

Many studies have been published establishing the relationship of diet to serum lipid profile. Short term and long term feeding of diet rich in cholesterol showed significant responses but whether these responses are maintained for a longer period or could be reproduced after a gap of 4-6 years has emerged as a subject of great controversy.

Lipoprotein Profile in Familial Hypertension

Simon, Friedlander and Kark (1988) showed that in presence of a family history of heart attack, the mean level of HDL was 5 mg/dl lower in cases of coronary heart disease than in control. No such difference existed in the absence of a family history of heart disease. In multiple logistic regression, HDL-c was a significant negative predictor of CAD but only in subjects having a positive family history. A one standard deviation (10 mg/dl) increment in HDL-c was associated with a two third reduction in heart disease risk. In an overall logistic model combining family history with other risk factors, the significant predictor of heart disease were age, STC, hypertension, family history and HDL-c.

Carr, Thomas, Larker and Wilkinson (1990) found that in hyperlipidemic hypertensive patients levels of
plasma cholesterol, triglycerides, low density lipoprotein cholesterol, very low density lipoprotein cholesterol were increased and high density lipoprotein cholesterol was reduced and their normotensive first degree relatives also showed abnormal plasma lipids (raised cholesterol, triglycerides and LDL and reduced HDL cholesterol).

Boyle et al (1992) showed that children with a parental history of hypertension was associated with greater STC, LDL. Krezesinki et al (1993) showed that when the family history of hypertension was present the HDL cholesterol was lower.