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IJPBS |Volume 1| Issue 2 |APRIL- JUNE |2011

IDENTIFICATION AND PREDICTION OF CORONARY HEART DISEASE IN PATIENTS WITH APOLIPOPROTEIN LEVELS

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Research Article

RECEIVED ON 15-05-2011 ACCEPTED ON 28-05-2011

ABSTRACT

Coronary Heart Disease (CHD) is the leading cause of deaths in the developed and developing countries. CHD is always due to atherosclerosis. A number of risk factors have been reported for atherosclerosis. Of these, the major risk factors are high cholesterol level, hypertension, smoking and diabetes mellitus. Fifteen patients with coronary heart disease were chosen for the study. Blood samples were collected from the overnight fast CHD patients and the levels of lipid profile were analyzed and were compared to the age and sex matched normal persons. In the result, increased levels of cholesterol, triglycerides, VLDL-cholesterol, LDL-cholesterol and moderately elevated levels of HDL-cholesterol were seen. While in apoproteins, increased level of Apo-B and decreased level of Apo-A_1 and lowered Apo-A_1/Apo-B ratio were seen in CHD patients, when compared to the normal subjects. Increased cholesterol, LDL-cholesterol and decreased HDL cholesterol are considered to be high risk factor for CHD. These conventional lipid profiles were used to identify the CHD. The measurement of apolipoprotein-A_1 and B may be more useful in the identification and prediction of CHD. Therefore, conclude that the quantization of apolipoproteins is superior to the traditional lipid profile in the identification and prediction of CHD.

KEYWORDS: *Apolipoprotein-A_1, apolipoprotein-B, cholesterol, HDL-cholesterol, triglycerides.*

Introduction

Coronary Heart Disease (CHD) is the leading cause in most industrialized countries, accounting for one out of every two deaths in the United States. Disorders of the lipid transport system resulting from complex interactions among nutritional environmental and generic factors. It has been proposed low density lipoproteins (LDL) cause cholesterol deposition in the arterial wall whereas high density lipoproteins (HDL) promote effect of cholesterol from this site. The low levels of HDL and/or high levels of LDL have been associated with increased risk of CHD.

Atherosclerosis, a disease literally means hardening of the arteries and accumulation of lipids characterizing the typical lesions. It is the principal cause of death in western world, a progressive process that begins in childhood with clinical manifestations in middle to late adulthood[1]. Arteries like the aorta, coronary and the cerebral are the prime

targets with myocardial infarction and cerebral infarction, which are major consequences. In small arteries, atheromos are occlusive, comprising ischemic injury, while in large arteries; they are destructive, favoring thrombosis. Many lesions are dense and fibrous while others containing large amount of lipids and necrotic debris[2, 3].

By reducing the lumen of coronary arteries, atherosclerosis causes an absolute decrease in myocardial perfusion in the vasal state. Fatty streaks develop as circulating monocytes migrates into intima, taking oxidized LDL from plasma, becoming lipid larden foam cells. A nature plaque contains a core of extracellular lipid surrounded by smooth muscle cells separated from lumen by collagen rich thick-cap. Such plaques may ruptures; allowing blood which disrupts the arterial walls comprising thrombosis and local vasospasm, further causing acute coronary syndromes[4]. A reduction in the oxygen carrying capacity of the blood, as in extremely severe anemia, is a rare cause of myocardial ischemia. When there is decreased in the supply of blood to the heart, thus resulting in heart attack and cell death [5].

Hypercholestrolemia,hypertriglyceride mia and other abnormalities in lipid metabolism contribute a major risk factor. The most striking association is with elevated levels of LDL and VLDL containing 70% of cholesterol in the blood[6]. Smoking is the dominant cause of atherosclerosis in women mainly increasing the degree of aortic and coronary atherosclerosis[7]. Two fold increases is seen in incidence of myocardial infarction in diabetes than non-diabetic. Obesity is an independent risk factor which is associated with hypertension, diabetes and physical inactivity. Regular physical activity shown to reduce risk for CHD by increasing HDL-cholesterol, lowering BP and reducing blood clotting etc[8]. Increase in age and male sex is the risk factors which cannot be corrected. Expression of anger affects CHD risk because of increased cardiovascular reactivity[9]. Blood concentrations of antioxidant affect the susceptibility of LDL and lipoprotein-A to oxidation, may increase atherosclerosis risk, when decreased. Hyperhomocystinuria has been established as an independent risk factor for coronary vascular disease etc. High levels of fibrinogen, coagulation factor VII increases the risk for CHD[10].

Lipoproteins are conjugated protein, consists of a lipid core of mainly non-polar or hydrophobic triglyceride, cholesterol ester and free fatty acid surrounded by a single layer of polar or hydrophilic phospholipid and cholesterol molecules [11]. Apolipoprotein is the protein part, which characterize the lipoprotein. The amount and the nature of the apolipoprotein vary with that of lipoprotein. Apolipoprotein carry out several important roles like enzyme co-factors, lipid transfer proteins, solubility of lipid molecules and ligand for interaction with lipoprotein receptors in tissues.

Table 1: Structure and Functions of Different Classes of Apoproteins

Apoprotein	Lipoprotein	Function
Apo-A_1	HDL and CM	**Activator of LCAT**
Apo-A_2	HDL & CM	**Inhibitor of LCAT**
Apo-B_{100}	LDL, VLDL & IDL	**Acts as ligand for LDL receptor**
Apo-B_{48}	CM & CM reminants	**Synthesized in intestine**
Apo-C	VLDL, LDL & IDL	**Apo-C_1 – activator of LCAT**
		Apo-C_2 – activator of lipoprotein lipase
Apo-D	Subfraction of HDL	**May acts as lipid transfer protein**

Apo-E	VLDL, HDL & CM	Cholesterol transportation

Increase in LDL, IDL and β-VLDL lead to atherosclerosis. The most common lipoprotein abnormalities like hyperlipidemia and hyperlipoproteinemia lead to accumulation of excessive lipids. While in apoproteins, increased levels of Apo-B and decreased level of Apo-A_1 have been associated with increased risk for atherosclerosis. It has been postulated for many years that apolipoproteins mainly Apo-A_1 and Apo-B could be better predictors than lipids for CHD. It is the main lipid found in the blood, bile and brain tissues. It is also one of the most important steroids of the body and is a precursor of many steroid hormones. Two thirds of cholesterol present in the blood is esterified. The liver metabolizes the cholesterol and it is transported in the blood stream by lipoproteins.

Increased levels are found in hypercholesterolemia, hyperlipidemia, hypothyroidism, uncontrolled diabetes, nephritic syndrome and cirrhosis. Decreased levels are found in malabsorption, malnutrition, hyperthyroidism, and anemia and liver diseases. Triglycerides (TG) are a family of lipids absorbed from the diet and produced endogenously from carbohydrate. Measurement of triglycerides is important in the diagnosis and management of hyperlipidemia. These diseases can be genetic or secondary to other disorders including nephrosis, diabetes mellitus and endocrine disturbances. Elevation of triglycerides has been identified as a risk factor for atherosclerotic disease.

Cholesterol distribution in the different fractions of lipoproteins i.e. HDL, LDL, VLDL and chylomicron (CM) are of particular interest in understanding the metabolic status and risk of various diseases like artherosclerosis, coronary heart disease, etc. Lower levels of HDL-cholesterol are associated with higher risks of atherosclerosis i.e. deposition of cholesterol in cells of blood vessels and complications like hypertension, coronary heart disease related to it.

Coronary heart disease and stroke (e.g. myocardial infarction) are the leading cause of deaths in the industrialized countries. CHD is always due to atherosclerosis. Atherosclerosis is the accumulation of fatty deposits in arteries, thus leads to coronary artery disease. Research indicates number of risk factors for CHD. These includes age, sex, hypertension, family history, lipid disorders, diabetes mellitus, smoking, high cholesterol level, haemostatic variables, sudden life style, obesity and poly unsaturated fatty acid deficiency.

Conventional lipid profile cholesterol, HDL, LDL, TG, VLDL levels are used to identifying risk factor for CHD. Increased cholesterol, LDL-cholesterol arid decreased HDL-cholesterol is considered to be a high risk factor for CHD. However in certain cases the conventional lipid fractions particularly LDL and HDL levels do not recognize CHD. Recently quantation of apolipoproteins specifically Apo-A_1 and Apo-B has been suggested to be a more specific and a sensitive marker for profiling individual CHD risk than corresponding levels of lipoprotein fractions. Apo-B is a carrier protein for LDL-cholesterol; Apo-A_1 is a carrier protein for HDL-cholesterol. Therefore the present study analysis apolipoprotein levels in people with CHD.

The main objectives of the present study are to review various etiological factors in the development of atherosclerosis and CHD, analysis the conventional lipid profile - cholesterol, TG, HDL, LDL and VLDL in CHD patients and controls, analysis the levels of Apo-A_1 and Apo-B and to correlate the levels of Apo-A_1 and Apo-B with CHD.

Materials and Methods

2.1 Subjects and Samples

The subjects taken for the study of altered cholesterol, TGL, HDL, LDL were coronary heart disease. Blood samples were collected from fifteen newly diagnosed CHD patients from Vel R.S. Medical College and Hospital, Avadi, Chennai. These samples of patients were compared with normal individual blood of the same sex and age. Blood was collected from 15 normal individual persons and used as controls. Both controls and patients were ranging in age from 35-60 years. Blood were collected by fasting sample in a tube serum was separated by centrifugation at 3000 rpm for 15 min.

2.2 Estimation of Cholesterol

The serum cholesterol levels were estimated by Ziakiti's-Zak and Boyle method. Plasma was treated with ferric chloride acetic acid reagent to precipitate the protein. The protein free supernatant was treated with concentrated H_2SO_4. A reddish purple color was developed which was measured at 560nm using a suitable standard and a reagent blank. Take 0.2m1 of serum in a 15ml glass stopper centrifuge tube. Added 9.8m1 of working ferric chloride acetic acid reagent mixed well and allowed to stand for 15 mins and centrifuged. Take 0.5m1 of supernatant and added 3ml of concentrated sulphuric acid, added 1ml of ferric chloride acetic acid reagent. Make up the working standard solution to 6ml with ferric acetic acid reagent. To all the tubes, added 3ml of concentrated sulphuric acid and mixed well. Measured the color which developed was read at 560nm after 15 mins. The results were expressed as mg/dl.

2.3 Estimation of HDL-cholesterol

The HDL-cholesterol was estimated by Wybenga and Pileggi method. In hot acidic medium, cholesterol oxidize ferric ion to a colored complex which can be measured at 830nm was directly proportional to the cholesterol concentration. Serum 0.2ml was taken in the test tube and added 0.2ml of the HDL reagent, mixed well for 15 mins and centrifuged separated the clear supernatant and estimated the cholesterol level of the supernatant. Series of the test tubes were taken and marked them as blank, standard, test as 1, 2, 3 respectively. 5ml of the cholesterol reagent was pipette out into the series of three tubes. Then add the HDL reagent into the first tube and second tube in the level of 0.2m1 respectively. Then supernatant 0.2ml was added into the third tube considered as test cholesterol standard reagent (20 mg/dl). 0.02ml was added to the second test tube which was considered as a standard. First tube was considered as a blank. Mixed well and immediately kept in boiling water bath for 90 seconds then cooled. After cooling the given color developed was read at 530nm against blank HDL. The results were expressed as mg/dl.

2.4 Estimation of Triglycerides

Triglycerides were estimated by enzymatic method. Increased levels of triglycerides well commonly found in hyperlipoproteinemia, nephrotic syndrome, coronary heart disease, hyperthyroidism, liver disorders and diabetes mellitus. Dissolve one enzyme vial with 10ml buffer. Series of three test tubes were taken and marked as 1,2,3 for the preparation of blank. Into the first tube, 0.05m1 of distilled water was added 1ml of reconstituted reagent was added to the same tube. To the second test tube, 0.05m1 of standard solution was added and 1ml of reconstituted reagent was added to that tube. Serum 0.05ml was added to the third tube and added 1ml of the reconstituted reagent. This was considered as test. All these tubes considered reagents were mixed and incubated for 10 mins at 37°C. After

exactly 10 mins incubation, 2m1 of distilled water was added to all these tubes. The color developed in the tube was read at 560nm after 30 mins. The results were expressed as mg/dl.

2.5 Estimation of Apolipoprotein A_1

The apolipoprotein-A_1 was estimated by immuno turbidimetric method. The apolipoproteins contained in the human serum form immune complexes with their corresponding antibodies. The concentrations are determined by turbidimetry assay. The results are evaluated using reference curve prepared with the aid of dilution of calibrator. The control and sample are diluted manually or automatically with saline solution (0.9% NaCl). Diluted antiserum Apo-A 1:41 with buffer solution. The working reagent is stable for 2 weeks at 2-8°C. Diluted samples and controls 1:21 with saline solution. To 20µl of diluted calibrator, control and sample is pipette out with 1ml of working reagent. Mixed and incubated for 10 mins at 37°C. Read at 340nm against blank. The results were expressed in mg/dl.

2.6 Estimation of Apolipoprotein-B

The apolipoprotein-B was estimated by immuno turbidimetric method. The apolipoprotein contained in the human serum form immune complexes with their corresponding antibodies. The concentrations are determined by turbidimetry assay. The results are evaluated using a reference curve prepared with the aid of solution of calibrator. Diluted antiserum Apo-B 1:41 with buffer solution. The working reagent is stable for 2 weeks at 2-8°C. Diluted sample and control 1:15 with saline. Dilute the calibrator with saline. To 20 µml of diluted calibrator, control and sample is pipette out with 1ml of working reagent. Mixed and incubated for 10 mins at 37°C. Read at 340nm against blank. The results were expressed in mg/dl.

2.7 Statistical Analysis

All the quantitative measurements were made on each patient and normal. The values are expressed as Mean ± SD. Statistical analysis was done by student 't' test and the 'p' values to assess the statistical significance of the changes, observed in CHD patients as compared to controls.

Results and Discussions

The present study analyzed the lipid profile in 15 CHD patients. The results were compared with an equal number of age and sex matched normal subjects. **Table 2** shows the case history of the normal and CHD subjects. **Table 3** shows the plasma levels of total cholesterol, HDL-cholesterol and LDL-cholesterol in normal and CHD subjects. **Table 4 shows** the levels of triglyceride and VLDL–cholesterol in normal and CHD patients. Table 5 shows the levels of apolipoproteins and their ratio in the normal and CHD subjects. The present study focused the relation between the conventional lipid profile along with lipoproteins and CHD. Lipid profile - cholesterol, TG including lipoproteins – Apo-A_1 and Apo-B were analyzed in 15 persons who were diagnosed as CHD patients. The results were compared with an equal number of 15 male, age matched normal subjects.

The levels of total cholesterol, triglycerides, LDL-cholesterol and VLDL-cholesterol were significantly increased in plasma of CHD patients as compared to normal. The levels of HDL-cholesterol were moderately increased. The concentration of apolipoprotein-A_1 was significantly decreased while apolipoprotein-B is increased significantly in the plasma of CHD patients as compared to the normal. The Apo-A_1/Apo-B ratio was found to be significantly reduced in CHD patients as compared to normal subjects. Several epidemiological studies have confirmed that

Coronary heart disease (CHD) has associated with lipid abnormalities. The reduction of total and LDL-cholesterol and an increase of HDL-cholesterol reduce the risk of CHD.

Table 2: Case Histories of the Subjects Investigated

General Characteristics	Normal	Abnormal
Total number of subjects	15	15
Sex	Male	Male
Age	35 – 60	35 – 60
Type of habits	-	Cigarette smoking and alcohol consumption

Table 3: Plasma Levels of Total Cholesterol, HDL-cholesterol and LDL-cholesterol in Normal and CHD Patients

Parameters	Normal	CHD Patients
Total cholesterol (mg/dl)	173 ± 10.07	238.73 ± 12.94 *
HDL-cholesterol (mg/dl)	48.06 ± 6.32	56.4 ± 7.88 •
LDL-cholesterol (mg/dl)	102.46 ± 11.74	154.47 ± 10.13 *

Data represents Mean ± SD from 15 subjects in each group

* Significantly different from normal ($p<0.001$)

• Moderately different from normal ($p<0.001$)

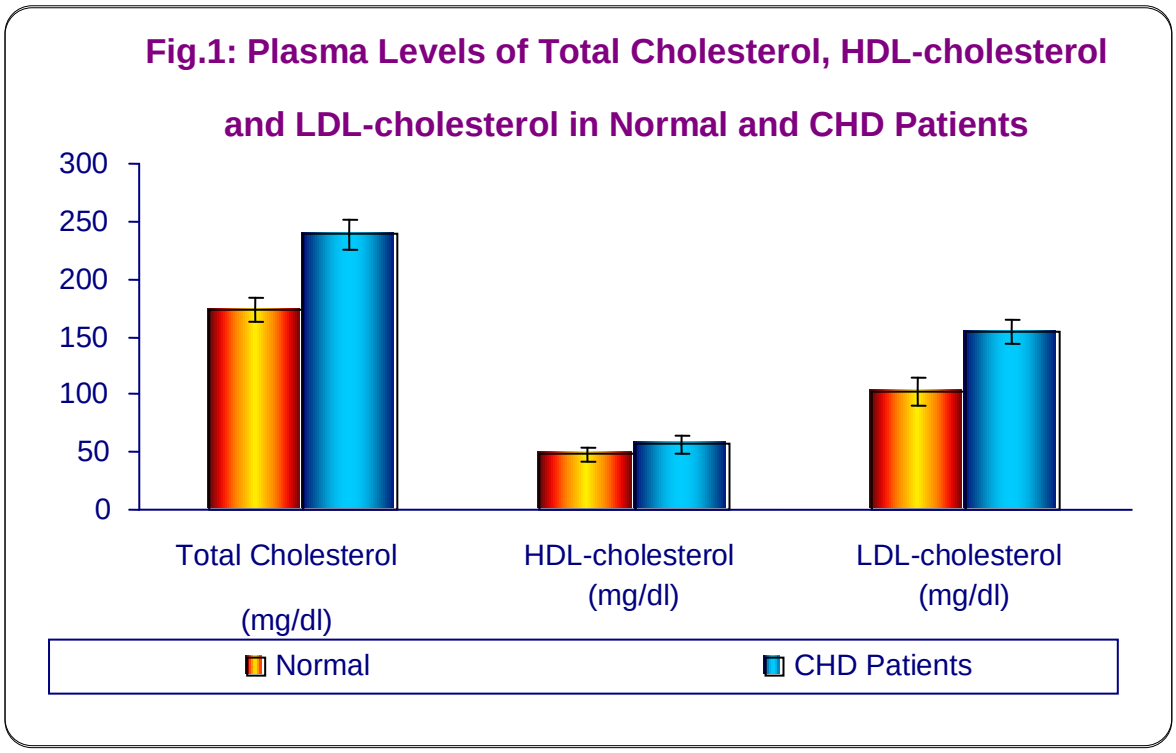


Table 4: Levels of Triglyceride and VLDL-cholesterol in Normal and CHD Patients

Parameters	Normal	CHD Patients
Triglycerides (mg/dl)	79.06 ± 19.41	**144.6 ± 13.26 ***
VLDL–cholesterol (mg/dl)	**20.86 ± 5.95**	**27.8 ± 3.81 ***

Data represents Mean ± SD from 15 subjects in each group

*** Significantly different from normal (p<0.001)**

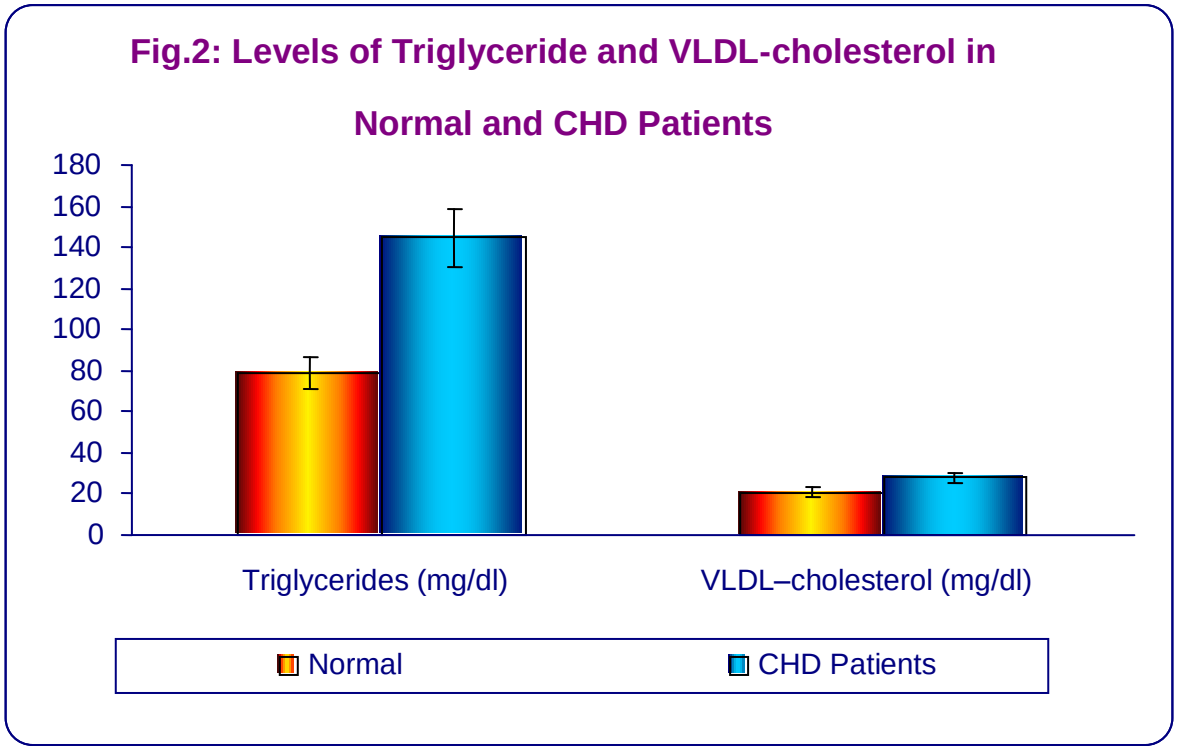
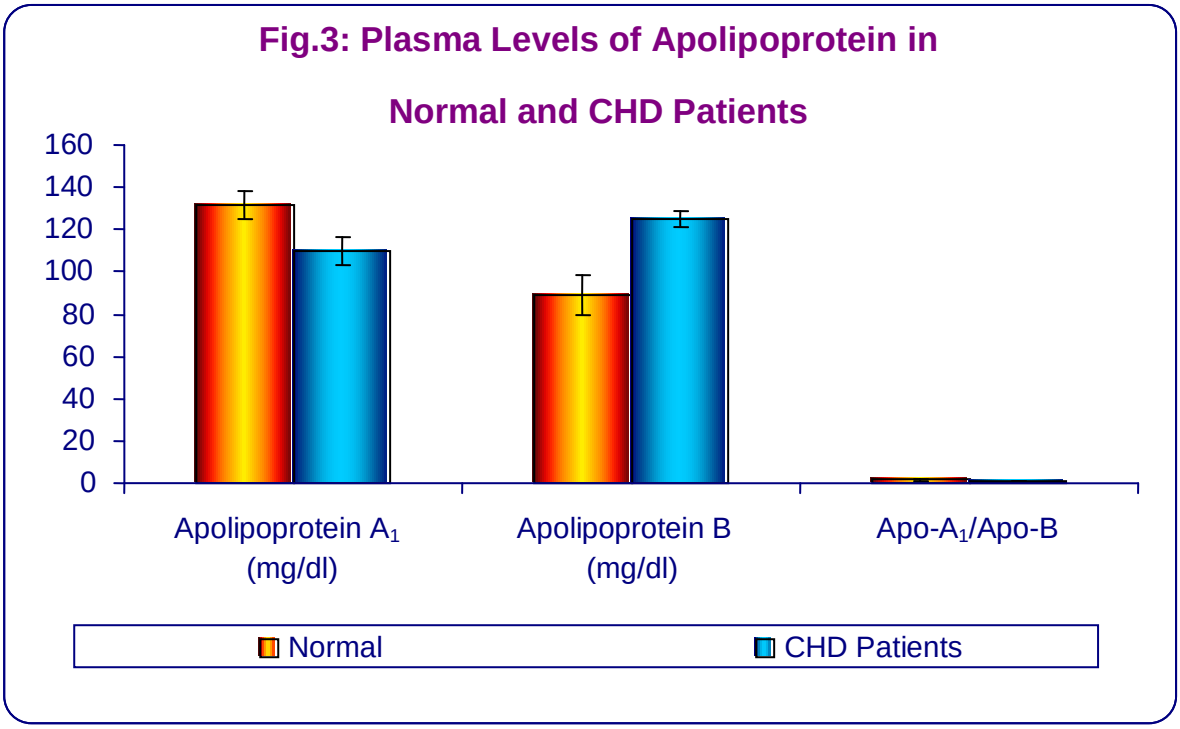


Table 5: Plasma Levels of Apolipoproteins in Normal and CHD Patients

Parameters	Normal	CHD Patients
Apolipoprotein A_1 (mg/dl)	131.50 ± 6.78	**109.76 ± 7.03 ***
Apolipoprotein B (mg/dl)	89.0 ± 9.77	**125.0 ± 3.78 ***
Apo-A_1/Apo-B	**1.48 ± 0.14**	**0.80 ± 0.04 ***

Data represents Mean ± SD from 15 subjects in each group

- **Significantly different from normal (p<0.001)**



Besides dietary factors, the plasma cholesterol level is affected by a number of factors and depends upon the presence of specific receptors on cell surface. The plasma levels of cholesterol reflect the level of lipoproteins LDL and HDL. LDL is cholesterol rich and accounts for the most of the cholesterol in the plasma. The plasma concentration of LDL is determined by the rate of uptake by LDL receptors. Cholesterol accumulation in liver may leads to reduction of LDL receptor in number and hence by increase plasma LDL level[12]. Cigarette smoking is known to increase total cholesterol and decrease HDL-cholesterol[7]. Secondary increase in plasma total cholesterol due to chronic alcoholism has been reported. Most of the patients investigated were known to be smokers and alcohol drinkers. Therefore our reports have agreement with these risk factors to CHD. Increased levels of plasma LDL-cholesterol and Apo-B are risk factors for atherosclerosis. The critical role of LDL in atherosclerosis has been confirmed in genetically altered mice. The mechanism is that normal LDL does not cause foam cell formation when incubated, but when LDL undergoes lipid peroxidation result in the formation of cholesterol laden foam cells.

In addition to these, oxidized LDL acts in the vessel wall to stimulate the secretion of cytokines, growth factors by endothelial cells, smooth cells and monocyte derived macrophages. The consequence is attachment of more monocytes to the lesions and proliferation of smooth muscle cells which synthesise and secrete increased amount of matrix such as collagen. Although mice are resistance to atherosclerosis, increased plasma levels of reminants of lipoproteins or LDL leads to atherosclerosis in these species. In contrast to atherogenic Apo-B lipoprotein, the Apo-A containing lipoprotein HDL, appear to be antiatherogenic. HDL cholesterol levels are as strong indicator of protection of CHD, as LDL-cholesterol levels are indicators of risk. HDL mediated reverse cholesterol transport from peripheral tissue to liver is thought to be the

primary mechanism by which HDL protects from atherosclerosis.

In rare cases, low plasma HDL is due to a genetic deficiency of one of the structural components of HDL, such as Apo-A but low HDL levels usually, the secondary constituents of increased plasma levels of VLDL and IDL(CM and its reminants)[13]. Elevated levels of TG and VLDL were observed in the present study. TG make up the bulk of the VLDL 55% to 85% by weight and the size of the VLDL is determined by the TG available,hence very large TG rich VLDL is secreted in state of caloric excess in diabetes mellitus and with alcohol consumption. Small VLDL is secreted when fewer TG are available. The role of VLDL in atherogenesis is uncertain. The major reason for this uncertainity is due to the inverse relationship between elevated levels of TG rich lipoproteins and reduced levels of antiatherogenic HDL cholesterol. Hypertriglyceridemia, may not be directly atherogenic but the surrogate of other lipoprotein abnormalities[14]. The concentration of Apo-A_1 was decreased significantly and Apo-B was increased in all CHD patients as compared to normal. Recent reports suggest that apolipoprotein-A_1 may be better diagnosis of CHD than cholesterol levels and low ratio of apolipoprotein-A_1 and B may be best predictor[15]. Further, Apo-A_1 levels have been found to be more useful in identifying patients with CHD than HDL[16, 17].

In our study, the result showed increased HDL level with decreased Apo-A_1 in CHD patients as compared to normal subjects. Apo-B is a carrier protein for LDL-cholesterol. Apo-B has seen increased in the following states like nephrotic syndrome, pregnancy, biliary obstruction, cigarette smoking and haemodialysis, while decreased in abetalipoproteinemia, estrogen therapy, liver disease, exercise, drug therapy and infections. Apo-A_1 is a major carrier protein of HDL-cholesterol, increases during pregnancy, exercise, alcohol consumption and estrogen therapy and decreased by 'fish-eye' disease, familial LCAT deficiency, infection, cholestasis, cigarette smoking, and diabetes and drug therapy. Recent studies have revealed that decreased levels of Apo-B and subsequently increased level of HDL-cholesterol are directly associated with angiographic reduction and favorable clinical outcome[15].

Research further showed that patients with CHD exists a group with normal LDL-cholesterol but with high concentrations of Apo-B. Measurement of LDL-cholesterol does not recognize the coronary in these individuals. Therefore, Apo-B has been shown to be a more reliable indicator of risk. Therefore Apo-B measurement can be useful in identifying patients at increased risk of atherosclerotic cardiovascular disease could otherwise go unidentified by the traditional lipoprotein profile[18]. The Apo-A_1 and Apo-B ratio of all CHD patients lowered significantly as compared to normal. Studies have shown that Apo-A_1/Apo-B ratio distinguishes unequivocal between patients with and those without CHD. Therefore, apolipoprotein-A and B studies are superior to conventional total cholesterol or LDL, and HDL cholesterol studies for identifying the risk of CHD.

Acknowledgement

Authors sincerely thank Dr.R.Rangarajan, Chairman, Dr.Sakunthala Rangarajan, Vice-Chairman, VELTECH Group of Educational Institutions and Dr.K.Siddappa Naidu, Principal, VELTECH MULTITECH Dr.Rangarajan Dr.Sakunthala Engineering College, Chennai–600062 for their unremitting encouragement and valuable advices for publishing this paper successfully.

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