Biochemical markers for early detection of cardiovascular disease in smokers

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Abstract

Introduction: The prevalence of coronary heart disease (CHD) is increasing in urban as well as rural areas in India especially during the last decade due to adoption of smoking habits. Tobacco smoking is currently ranked fourth world-wide in contributing to mortality. The increased risk of atherothrombotic vascular events seen in smokers could be due to increased serum homocysteine and abnormal lipid profiles.

Aim: To study serum homocysteine and lipid profile in smokers and non-smokers with coronary heart disease below 40 years age.

Materials and Method: A total of 90 men all having CHD and below 40 years were included in the study. They were divided into two groups of 45 each. All patients with history of cigarette smoking were included in test group and the non-smokers were included in control group. Serum of test and control groups was estimated for homocysteine and lipid profile in addition to other routine investigations.

Results: Age 36-40 years was the most common age with history of CHD. The body mass index was almost same for both the groups. The mean values of serum homocysteine in smokers with CHD was significantly raised (P<0.001) than the control group of non-smokers. Total cholesterol and LDL in smokers were also significantly elevated (<0.05) when compared with non-smokers.

Conclusion: Hyperhomocysteinemia is an independent risk factor for CHD and serum homocysteine levels can act as **"**Potential Biochemical Markers**"** for early detection of CHD.

Keywords: Hyperhomocysteinemia, Coronary heart disease, Lipid profile, Tobacco smoking

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Introduction

Coronary artery disease (CAD) has a world-wide distribution and is rapidly increasing in economically developed societies. This disease is more prevalent above 20 years of age. CAD affects the coronary arteries and atherosclerosis is the main cause for this disease, accounting for more than 90% cases.(1) Although any artery can be affected the coronary vessels are the main targets.

Although atherosclerotic coronary heart disease or ischemic heart disease (IHD) is the most common cause of death in developed countries, it is widely prevalent in industrialized countries too. Extensive epidemiological investigations have revealed various risk factors that predispose to the development of clinical atherosclerosis.(2) These risk factors usually act synergistically rather than singly. The basic lesion, atheroma or fibro-fatty plaque consists of a raised focal plaque within the intima having a lipid core and a covering fibrous cap.

Epidemiologic studies indicate that certain genetic or acquired factors increase the risk of atherosclerosis. The most important risk factors are hyperlipidemia, hypertension, cigarette smoking, hyper-homocysteinemia and diabetes mellitus. Hyperhomocysteinemia and hyperlipidemia have been reported to be associated with increased incidence of ischemic heart disease.(3) Hyperhomocysteinemia and hyperlipidemia may be secondary to some well-known causes like cigarette smoking, nutritional deficiency of B_6, folic acid, and B_{12} deficiency.

Severe homocysteinemia is rare. Mild homocyteinemiais relatively common and occurs in approximately 5 to 7% of general population. Patients with mild hyperhomocysteinemia have none of the clinical signs and are typically asymptomatic until 3rd or 4th decade of life when premature coronary artery disease develops. There is a strong association between cigarette smoking, hyperhomocysteinemia, hyperlipedemia and susceptibility to CHD.(4) In people who smoke one or more packs of cigarettes per day, the death rate from CHD is 70-200% higher than that of non-smokers, and the risk is particularly significant in younger men. At autopsy the degree of aortic and coronary atherosclerosis is found to be greater in smokers than in non-smokers.

A positive relationship has been suggested between elevated serum homocysteine, elevated total cholesterol, LDL, VLDL and triglycerides; and risk of coronary heart disease in younger people below 40 years. Abundant epidemiological evidence has demonstrated that the presence of mild hyperhomocysteinemia is an independent risk factor for atherosclerosis in coronary vascular disease.(5)

In view of the known risk of CHD in smokers, the present study was undertaken to determine the serum homocysteine and lipid profile in smokers and non smokers below 40 years with history of CHD in our population. In many individuals with inborn errors of homocysteine metabolism, kidney or liver disease, nutrient deficiencies or ingestion of certain pharmaceuticals and cigarette smoking, homocysteine levels can increase beyond normal levels and lead to coronary artery disease in individuals below 40 years.

Some studies have found hyperhomocyteinemia to be a stronger risk factor for CAD than hyperlipidemia. The risk of CAD becomes four-fold when hyperhomocysteinemiais combined with hyperlipidemia. Research has shown that most patients with myocardial infarction (MI) having normal cholesterol levels and without diabetes mellitus and/or hypertension, did show high plasma homocysteine levels.

Materials and Method

This was a prospective study done in the department of Cardiology and department of Biochemistry, Gandhi Medical College and Hospital, Secunderabad, over a two and half year period. The cases for the present study were selected from the department of Cardiology. A total of 90 male patients admitted with acute myocardial infarction were selected.

Inclusion criteria:

1. Age less than 40 years
2. Patients with confirmed acute myocardial infarction

Exclusion criteria:

1. Age more than 40 years
2. Patients with symptoms of myocardial infarction but with negative findings on electrocardiogram and biochemical cardiac markers.
3. Impaired renal function
4. Patients with hypo/hyperthyroidism
5. Hemolyzed blood samples

The 90 men were divided into two groups of 45 each as smokers, and non-smokers. The smokers had a history of cigarette smoking >20 cigarettes /day for the last 15 years. Remaining 45 patients who were non-smokers were taken as controls.

Complete clinical details including family history, past history, personal history, smoking habits with duration and number of packs smoked were noted. Investigations done were Electrocardiography, complete blood picture, ESR, urine analysis, coagulation studies, C-reactive protein, fasting blood glucose, plasma biochemical markers CK-MB, troponins, fibrinogen levels, myocardial perfusion scanning, stress echocardiography and coronary arteriography.

Under strict aseptic conditions, 5 ml of venous whole blood sample was collected from each subject in a plain, dry and properly labeled container. Precautions were taken to prevent hemolysis. Samples were brought to Clinical Biochemistry Lab, Gandhi Hospital and centrifuged after clotting and retraction at room temperature. Clear serum was collected and subsequently analyzed for serum homocysteine and serum lipid profile.

The quantitative estimation of serum homocysteine was determined by the Axis homocysteine enzyme immunoassay (EIA), on a 9-12 hours fasting blood sample. Total cholesterol was estimated by Cholesterol Oxidase-PAP method, HDL cholesterol by PEG-CHOD-PAP, end point assay with lipid clearing factor (LCF), triglycerides by GPO-PAP, end point assay and for LDL-cholesterol using Friedewald's equation.

Results

Descriptive statistical analysis was used to study the data to find out the Means, Standard deviation values and the variations of the study subjects in control and experimental groups. In addition, the correlation coefficients were also found out for both groups to understand important variables pertaining to biochemical parameters like serum homocysteine and lipid profile.

Table 1: Demographic details in study

Age interval in years	Controls	Smokers
15-20	0	1(1.2%)
21-25	0	1(1.2%)
26-30	1(1.2%)	2(2.4%)
31-35	3(3.7%)	4(9.6%)
36-40	36(45%)	32(40%)
BMI(mean±SD)	23±2.4	22±3.1

Age group of 36-40 years was the most common age group for myocardial infarction and there was no significant difference in both groups. BMI was also insignificant in both groups.

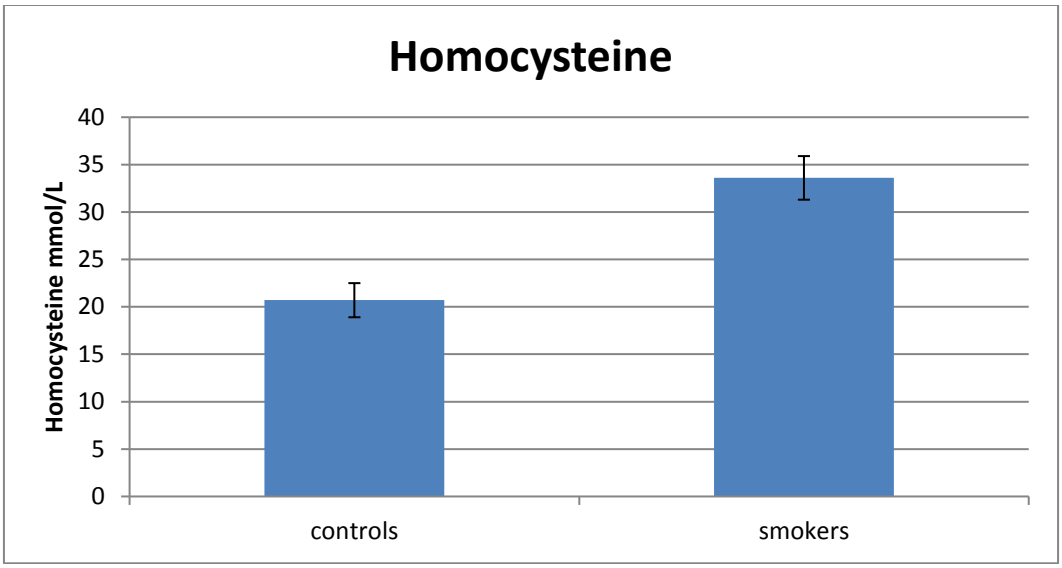

Fig. 1: Homocysteine in Non Smokers and Smokers with CHD

It was observed that the mean values of serum homocysteine in smokers and non-smokers with CHD below 40 years age group was 33.60 ± 2.3, and 20.7 ± 1.8mmol/L respectively.

Further, it was observed that in smokers with CHD below 40 years age, the Serum Homocysteine was significantly raised than the control group (P<0.001).

Table 2: Serum Lipid profile in Non Smokers and Smokers with CHD

	Non Smokers (Mean ± SD)	Smokers (Mean ± SD)	P-value
Total Cholesterol (mg/dL)	175.67 ± 5.2	179.87 ± 5.8	<0.05
HDL (mg/dL)	39.47 ± .90	37.96 ± 1.0	0.211
Triglyceriods (mg/dL)	142.93 ± 7.5	143.00 ± 8.1	0.37
VLDL (mg/dL)	28.84 ± 1.5	28.91 ± 1.5	0.76
LDL (mg/dL)	106.67 ± 5.3	110.84 ± 5.5	<0.05

Total cholesterol and LDL in smokers were significantly elevated (<0.05) in comparison to controls

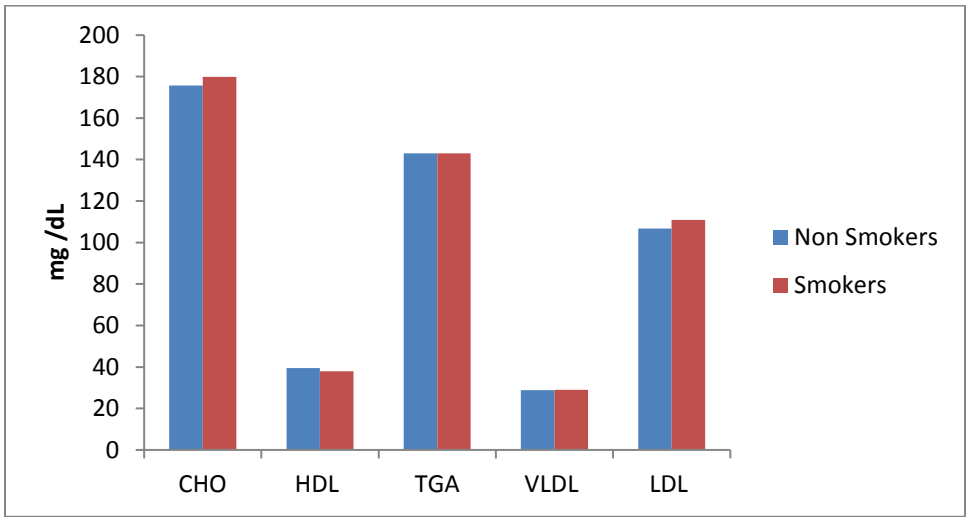

Fig. 2: Lipid profile comparison in controls and smokers

Discussion

Results of the study indicate that there is significant difference in serum homocysteine levels in smokers and non-smokers below 40 years having coronary artery disease. There is no significant increase in the serum lipid parameters except for total cholesterol and LDL in smokers as compared to non-smokers.

There is slight increase in total cholesterol and LDL in smokers compared with non-smokers and also there is slight decrease in HDL levels in smokers compared with non-smokers having CAD.

Many studies have reported that serum homocysteine levels were higher in smokers. This has been explained to be due to tobacco smoking which interferes with synthesis of pyridoxal phosphate that causes decreased PLP concentration in serum. This causes disturbance in methionine metabolism which causes increased serum homocysteine levels which predisposes to athero-thrombotic vascular disease.[5] In addition, smoking has adverse hemostatic and inflammatory effects including increased level of C reactive protein (CRP), and fibrinogen.

Experimental evidence suggests that the atherogenic predisposition associated with hyperhomosysteinema results from endothelial dysfunction and injury followed by platelet activation and thrombus formation. The homocystiene induced atherosclerosis is characterized by substantial platelet accumulation and platelet-rich thrombus formation in areas of endothelial injury.

The postulated effects involve oxidative damage to vascular endothelial cells and increased proliferation of vascular smooth muscle cells after oxidative metabolism of homocysteine to homocystine and homocysteine thiolactone. Oxidative modification of low-density lipoprotein (LDL) promotes the formation of foam cells, which acts as a source of reactive oxygen species.

Lentz and colleagues[6] have demonstrated that diet-induced hyperhomocysteinemia in primates leads to impaired vasomotor regulation in vivo and endothelial antithrombotic function ex vivo. These findings are supported by the work of Celermajer and colleagues,[7] who demonstrated impaired endothelium-dependent vasodilation, and also by van den Berg and colleagues,[8] who demonstrated impaired endothelial anticoagulant function in young patients with hyperhomocysteinemia and peripheral vascular disease.

Homocysteine is rapidly auto-oxidized when added to plasma, forming homocystine, mixed disulfides, and homocysteine thiolactone. Potent reactive oxygen species, including superoxide and hydrogen peroxide, are produced during the auto-oxidation of homocysteine and hydrogen peroxide; it has been implicated in the vascular toxicity of hyperhomocystinemia.

Homocysteine induced endothelial-cell injury also leads to the generation of hydrogen peroxide. Auto-oxidation of homocysteine produces other cytotoxic reactive oxygen species, including the superoxide anion radical and hydroxyl radical, these initiate lipid peroxidation, an affect that occurs at the level of endothelial plasma membrane and within lipoprotein particles. Homocysteine auto-oxidation has been shown to support the oxidation of low-density lipoprotein through the generation of the superoxide anion radical. In addition to promoting atherosclerosis through endothelial injury or dysfunction, homocysteine is also potent mitogen for vascular smooth muscle cells.

Homocysteine also directly damages the vascular matrix by affecting the biochemical and biosynthetic vascular functions of endothelial cells. Homocycteinethiolactone, a highly reactive anhydrous by-product of homocysteine oxidation, combines with low density lipoprotein to form aggregates that are taken up by the intimal macrophages and incorporated into foam cells within nascent atheromatous plaques.

Jakubowski[9] showed that cells deficient in cystathionine b-synthase produce more homocysteine thiolactone in cultured cells than normal cells and that the thiolactone is incorporated into cellular and secreted proteins through lysine acylation by the activated carboxyl group of the thiolactone.

Environmental toxins, such as tobacco smoke and carbon disulfide, can raise homocysteine levels and nutritional deficiencies of folic acid or vitamin B12 also raisehomocysteine levels. Deficiency of vitamin B6 may also be associated with high homocysteine in the blood.

Boushey and Coworkers[10] performed meta-analysis on existing studies of homocysteine and smoking. They estimated that 10 percent of the risk of coronary artery disease in the general population is attributable to homocysteine. They reported that an increase of 5 mmol per liter in the plasma homocysteine concentration raises the risk of coronary artery disease which is equivalent to an increase of 20 mg per deciliter in the cholesterol concentration. They suggested that increasing folate consumption by approximately 200 mg per day would reduce total homocysteine concentrations by approximately 4 mmol per liter, a reduction that could potentially have a major effect on cardiovascular mortality.

Wald et al[11] observed that males below 40 years who had ischemic heart disease and were smokers had higher serum homocysteine than non-smokers with IHD. Graham et al[13] measured serum homocyteine in smokers and non-smokers. Mean fasting serum homocysteine was significantly higher in smokers than controls. In a study, 35-65 year old patients with smoking and without smoking who had myocardial infarction were followed up over a 3 year period. The risk of myocardial infraction increased 6% to 7% for every 1µmol/L increase in total homocysteine in smokers than non smokers.

A systematic review of studies with the same types of patients and controls and the same methods and outcomes can provide a more accurate estimate of the true association between homocysteine and smoking as a vascular risk. Assuming that strong, dose-dependent, and positive association does exist, there is reasonable evidence, from regression analysis, that the association is independent of other factors known to be associated with raised serum homocysteine (e.g.: age, sex, low physical activity, blood pressure, DM and cholesterol).

Furthermore, the more positive results of retrospective studies may reflect a consistent bias resulting from homocysteine levels measured after the acute vascular event, which may be higher than levels measured beforehand. Finally, it is also possible that serum homocysteine is just a coincidental marker of another causal risk factor such as folate status, B_{12} and B_6, which can explain the higher rates of Coronary heart disease (CHD) among people with lower intakes of folate and B_6.[12]

The results of the lipid profile in the present study indicate that there is no significant change in smokers with coronary heart disease as compared with non-smokers. It is suggested that it is the carbonmonoxide and not nicotine in tobacco smoke which is responsible for much greater risk of smokers developing atherosclerosis in comparison to non-smokers.

Myocardium is affected by small carboxy hemoglobin concentration since utilization of available oxygen is very high. There is also strong relation of infarction risk to the number of cigarettes smoked daily. It is suggested that smoking may cause acute but reversible influence on platelet survival and adhesiveness, coagulation factors, carboxy hemoglobin levels and myocardial irritability.[13]

Cells maintain their cholesterol content and both influx and efflux are important in hypercholesterolemia. Most of the plasma cholesterol is as cholesterol ester. Cholesterol ester can be readily transported between lipoproteins through APOD, the increased free cholesterol levels in smokers are probably related to decreased activity of LCAT in smokers.[14]

Smoking is associated with atherosclerosis. The data from animal studies suggests that nicotine causes endothelial damage. Data from human studies indicates that smoking increases the number of damaged endothelial cells, tendency to thrombus formation, fibrinogen levels and all this leads to clotting abnormalities. When we look at the various types of lipoproteins, it is the level of LDL cholesterol that is most directly associated with CHD. Recent evidence indicates that levels of plasma lipoprotein (A-I the major HDL protein) apolipoprotein-B (the major LDL protein) are better predictors of CHD than HDL cholesterol or LDL cholesterol respectively.

In the present study while HDL cholesterol levels were similar in both smokers and non-smokers with CHD and also the serum triglycerides and VLDL were not significantly raised among smokers with coronary heart disease as compared to non-smokers.

Lower HDL levels in cigarette smokers have been reported by Gosset LK l[15] while others have not reported any significant relationship of HDL to cigarette smoking. Increased LDL cholesterol and decreased HDL cholesterol levels in cigarette smoking have been studied by Waheeb DM.[16] There are many studies to emphasize the positive relationship of total cholesterol, LDL, VLDL and triglycerides to the risk of CHD. HDL has inverse relation to the risk of CHD. LDL has been suggested as a stronger risk factor for CHD especially in younger males.

In this direction, it is also felt that further studies are needed on larger population to understand the detailed mechanisms / interactions between these biochemical molecules and development of coronary heart disease.

Conclusion

Tobacco smoking remains the most important cause of preventable morbidity in smokers. Coronary heart disease remains an important cause of morbidity and mortality in young age group with smoking. Results of the study indicate a significant rise in serum homocysteine, total cholesterol and LDL in smokers as compared to non-smokers below 40 years with CHD. Serum homocysteine and serum lipid profile levels can act as "Potential Biochemical Markers" for early detection of CHD, thereby, helping in initiating early treatment to minimize and or avoid complications of CAD.

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