OXIDISED LDL, HDL CHOLESTEROL, LDL CHOLESTEROL LEVELS IN PATIENTS OF CORONARY ARTERY DISEASE

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ABSTRACT

Coronary artery disease is a major cause of morbidity and has various risk factors. Lipid profile i.e. low HDL-cholesterol, high LDL cholesterol, high total cholesterol, high triglycerides playing important role in its causation. Recently interest has been shown in the oxidized fraction of LDL as one of the risk factors. In the present study 60 age and sex matched normal healthy individuals were taken as controls and 60 patients of CAD were taken. Cholesterol was measured by enzymatic method, HDL cholesterol by phosphotungstate precipitation method. Serum levels of LDL fraction of cholesterol was measured by a new and simpler method of precipitation. Result was expressed as mol/L of diene conjugates. It was observed that LDL cholesterol, VLDL cholesterol, total cholesterol, total cholesterol:HDL cholesterol, LDL cholesterol:HDL cholesterol were significantly raised and HDL cholesterol was significantly low in patients. ($p < 0.001$). Though HDL cholesterol was significantly raised in females as compared to males in both the groups ($p < 0.001$). Serum level of total cholesterol, oxidized LDL:HDL cholesterol were also raised significantly ($p < 0.05$). The level of oxidized LDL showed an increasing trend in patients.

KEY WORDS

Oxidised - LDL, lipoprotein, coronary artery disease

INTRODUCTION

Atherosclerosis is a major health problem of middle and late adulthood and various risk factors have been sited for its occurrence e.g. sex, family history, hypertension, smoking, dyslipidemia, diabetes mellitus etc.

The various lipoprotein fractions are implicated as positive and negative risk factors. Of these the protective effect of high density lipoprotein - cholesterol (HDL-C) and harmful effect of low density lipoprotein cholesterol (LDL -C) have been well established.

Goldstein (1) has originally reported a process of LDL modification involved in the phenotypic change of macrophage to foam cells in the evolving stages of atherosclerosis, many studies have been conducted to show the atherogenic nature of this process (2) and

has established oxidative modification of low density lipoprotein (LDL) as an important atherogenic factor. Much of the recent interest in oxidized LDL comes from the discovery that it exhibits properties in vitro that could explain the migration of monocyte macrophages in to the intimal space and their conversion in to foam cells (3). A significant point is that oxidized LDL bypasses the normal feedback control of LDL receptor but is avidly endocytosed via the scavenger receptor pathway of macrophages. The internalized cholesterol does not down regulate this route of uptake and this leads to loading of these cells with cholesterol and cholesterol esters. Converting them to foam cells. Given the clinical atherogenic role of oxidized-low density lipoprotein (ox-LDL) numerous efforts have been made to detect its level in circulation. Hasegawa and workers (4), Toshima *et al.* (5) have developed immunological technique like sandwich-ELISA. Though the major obstacle of these techniques is the difficulty in detecting the minute amount of ox-LDL, and lack of specificity of the antibody, due to the large size of apoprotein B.

Due to the above shortcomings we tried to study the levels of circulating ox-LDL by a more simpler method of its precipitation in serum (6). The method apart from being simple is also inexpensive.

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MATERIALS AND METHODS

We had taken 60 clinically diagnosed cases of coronary artery disease of both sexes in the age group of 40-85 years. Brief clinical history covering the sign symptoms, past personal and family history of concerned risk factors was taken. The diagnosis was confirmed by ECG, ECHO, specific enzyme levels like-CPK-MB, CPK, SGOT, LDH. 60 age and sex matched controls were taken.

About 1 ml of fasting blood sample was collected in plain vials. It was allowed to stand for 30 min. for the clot to form and serum was separated. The serum was kept at 4°C for analysis within 72 hours. We determined the serum cholesterol level by the enzymatic method, HDL - cholesterol by precipitation with phosphotungstate and MgCl₂ method, LDL-cholesterol VLDL cholesterol by precipitation followed by enzymatic method (7). Apart from these parameters, ox-LDL was determined by a newly described method of Ahotupa *et al.* (6).

To the serum sample heparin citrate buffer (0.064 mol/L, pH 5.05) was added, mixed by vortexing and centrifuged at (2000-2500 rpm) x 30 min supernatant was decanted and used to determine HDL and very low density lipoprotein (VLDL cholesterol).

The pellet having LDL was re-suspended in phosphate buffer (pH 7.4). A mixture of chloroform : methanol (2:1) was added to it to extract the lipid fraction. This mixture was centrifuged, its upper layer was pipetted. It was dried under nitrogen at 37°C. The lipid was re-dissolved with cyclohexane. This was then read spectrophotometrically at 234 nm. Cyclohexane was taken as blank. Absorbance units were converted to molar units using the molar extinction coefficient 2.95 x 10⁴/M/cm. Correlation analysis was done and students 't' test was used to see if the correlation was significant. Students 't' test was also used for the other statistical analysis to find the significance.

RESULTS

The mean total and LDL, VLDL cholesterol level in CAD cases was found to be significantly higher .The

mean HDL cholesterol was significantly low in CAD cases as compared to control group. The serum oxidized - LDL though found to be higher in cases was not statistically significant see Table 1. The mean HDL cholesterol was significantly higher in females as compared to males in both the groups of cases and controls see Table 2.

Ratio of total cholesterol to HDL cholesterol and LDL to HDL cholesterol was very significantly raised in CAD cases, as compared to controls. The ratio of ox-LDL to HDL cholesterol was significantly raised in cases see table 3. There was no significant correlation of age and parameters under study between patients and age matched controls.

DISCUSSIONS

The study was conducted on sixty confirmed cases of CAD and sixty age and sex matched control. Indirect studies like protective effect of cholesterol lowering agents against CAD have also been studied (8). In our study there was a significant difference in HDL-cholesterol between males and females (p<0.001). This difference in the HDL-C level between the males and females is due to significantly higher levels of HDL2 and HDL3 in females as shown by Martini *et al.* (9). Watkins *et al.* has shown a decrease in the risk of CAD by 2.3% with every 1 mg/dl rise in serum HDL-cholesterol (10). In our study level of serum VLDL was very significantly raised in cases (p<0.001). Studies have shown high risk of CAD in patients with increased serum TGL levels (11). Since VLDL carries the highest amount of TGL this may be the reason for very high level of VLDL in our study. The main mechanism by which LDL particles act as a risk factor have been shown by Brown and Goldstein (12). They showed a receptor mediated uptake of LDL -C by cells subsequent to incorporation of cholesterol in cells. The LRCCPP trial and Blankenhorn *et al.* have shown a decreased lesion progression by lowering LDL-C levels (13, 14).

Holvoet *et al.* (15) have shown significantly increased level of MDA in patients of atherosclerosis. Subsequently many immunological techniques. e.g.

Table 1. Serum Total Cholesterol, HDL-Cholesterol, LDL- Cholesterol, VLDL and Oxidized LDL in patients and controls

	TC (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	VLDL-C (mg/dl)	Ox-LDL (mol/L) of DC
Control	180±26.77	53.03±10.34	107.7±37.33	17.80±1.51	41.95±8.27
Cases	206.2±47.83*	42.77±7.61**	136.2±43.10**	29.2±9.27**	49.87±24.32

Results are given as Mean ± SEM

* p value < 0.05; ** p value < 0.001; n(sample size) = 60.

Table 2. Comparison of HDL-Cholesterol between males and females in patients and controls

	HDL-C in patients (mg/dl)	HDL-C in controls (mg/dl)
Males	38±1.94 (n=30)	45.96±9.12 (n=30)
Females	69±21.70** (n=30)	57±11.02** (n=30)

Results are given as Mean±SEM

** p <0.001

Table 3. Ratio of Total Cholesterol, HDL-Cholesterol, LDL-Cholesterol, VLDL and Oxidized LDL in patients and controls

	TC: HDL-C	LDL-C: HDL-C	OX-LDL: HDL-C
Controls	3.5±0.98	2.1±0.96	0.0317±0.009
Cases	4.9±1.2**	3.2±1.04**	0.047±0.030*

Results are given as Mean±SEM

* p value < 0.05 ; ** p value < 0.001

n(sample size) = 60.

Table 4. Baseline Characteristics of the Study Group

	n = 60	Percentage
Diabetes	50	83.33
Stroke	2	3.33
Hypertension	60	100
Smoking	48	80
Family history	40	66.66

sandwich ELISA have been developed to detect levels of circulating ox-LDL (4, 5), we have estimated ox-LDL by a method which is much simpler and inexpensive. In our study we found a definite increasing trend in ox-LDL level in cases though it was not statistically significant. The trend would be established on including a large number of subjects. Recent studies by Holvoet *et al.* have shown that CAD patients had a higher level of circulating ox-LDL as compared to controls and the sensitivity of this circulating ox-LDL was 76% for cases of CAD (16). It has also been seen that patients with CAD have elevated titre of autoantibodies against ox-LDL as compared to healthy

controls (17).

A study conducted by American Medical Association (18) had shown increased risk of CAD in patients with total : HDL cholesterol > 4. Similar evidence was given by Ladeia *et al.* (19).

It has been demonstrated that apo A-1 the major HDL protein inhibits LDL oxidation (21). Thus low HDL-C levels with low apo A-1 will increase the LDL oxidation and thus decrease the ratio of ox-LDL : HDL-C. so the ratio is a better indicator. In our study though ox-LDL was not significantly raised its ratio with HDL was raised significantly in cases (p<0.05) .

REFERENCES

1. Glomset, J.A. (1989). the plasma lecithin : cholesteryl acyl transferase reaction. J. lipid Res. 9, 155-167.
2. Steinberg, D. and Lewis, A. (1997). Conner Memorial Lecture : oxidative modification of LDL and atherogenesis. Circulation 95, 1062-1071.
3. Esterbauer, H., Wag, G. and Pahl, H. (1993). Lipid peroxidation and its role in atherosclerosis. Brit. Med. Bull. 49 (3), 566-576.
4. Hasegawa, A., Toshima, S., Nakano A. and Nagai, R. (1999). Oxidized LDL in patients with coronary heart disease and normal subjects. Nippon Rinsho. 57 (12), 2754-2758.
5. Toshima, S., Hasegawa, A., Kurabayashi, M, Itabe, H., Takano, T., Sugano, J., Shimamura, K, Shimnamura, K, Kimura, J, Michishita, I., Suzuki, T. and Nagai, R. (1999). Circulating Oxidized Low Density Lipoprotein levels A Biochemical Risk Marker for Coronary Heart Disease. Arteriosclerosis, Thrombosis, and Vascular Biology. 20 (10), 2243.
6. Ahotupa, M., Ruutu, M. and Mantyla, E. (1996). Simple methods of quantifying oxidation products and antioxidant potential of lipoproteins Clinical Biochemistry. 29, 140.
7. Varley, H. (1991). Practical clinical biochemistry, 5th edn. CBS Publishers, Delhi, India. Vol 1, p. 650-669.
8. Hoppichlor, F, Leclitner, M. and Patanch, J.R. (1992). Provastain a new cholesterol synthesis inhibitor for lowering increased serum cholesterol. Zgesmte. Inn. Med. 47 (11), 523-527.
9. Martini, S., Baggio. G. *et al.* (1984). Evaluation of HDL2 and HDL3 cholesterol by a precipitation procedure in a normal population and hyperlipedemic phenotypes. Clinical Chimica Acta. 137, 291-298.

10. Watkins, I.O., Neaton, J.D. and Phillips, A.N. (1986). High density Lipoprotein cholesterol and coronary heart disease incidence in black and white MRFIT usual Care men. *Am. J. Cardiol.* 57, 538-545.
11. Austin, M.A. (1991). Plasma Triglycerides and coronary artery disease Arteriosclerosis Thromb. 11, 2-14.
12. Brown, M.S. and Goldstein, J.L. (1984). How LDL receptors influence cholesterol and atherosclerosis. *Sci. Amer.* 251 (5), 58-66.
13. Lipid Research Clinic Programme: the lipid Research clics. Coronary Primary Prevention Trial Results. I. Reduction in incidence of coronary heart disease. (1984) *JAMA.* 251, 351-364.
14. Blankenhorn, D.H., Nessim, S.A. and Johnson, R.L. (1987). Beneficial effect of combined colestipol Niacin therapy on coronary atherosclerosis and coronary venous by-pass grafts. *JAMA* 257, 3233-3240.
15. Holvoet, P., Perez, G. and Zhao, Z. (1995). Malondialdehyde - modified low density lipoproteins in patients with atherosclerotic disease. *J. clin. Invest.* 95, 2611-2619.
16. Holvoet, P., Mertens, A. *et al.* (2001). Circulating ox-LDL is a useful marker for identifying patients with CAD Arteriosclerosis, Thrombosis and Vascular Biology (2), 844-850.
17. Evaggelia S, Lourida, *et al.* (2002). Elevated titre of autoantibodies against oxidized LDL forms in patients with CAD. *J. Cardiol.* (43), 38-46.
18. Americal Medical Association, Council of Scientific Affairs: Dietary and Pharmacologic therapy for lipid risk factors. (1983) *JAMA*, 250, 1873-7.
19. Ladeia, A.M., Guimaraes, A.C. and Lima, J.C. (1994). The lipid profile in coronary artery disease. *Am. Braz. Cardiol.* 63 (2), 101-106.
20. Manninen, V., Elo, M.O. and Frick, M.H. (1988). Lipid alterations and decline in incidence of CAD in Helsinki Heart Study. *JAMA* 260, 641-651.
21. Ohta, T., Takata, K. and Horiuchis, W. (1989). Protective effect of lipoproteins containing apoprotein A-1 on Cu⁺⁺ catalyzed oxidation of human low density lipoprotein. *Febs. Lett.* 257, 435-438.