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Status of Lipid profile, MDA and protein carbonyl in patients with cardiovascular diseases

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ABSTRACT

Total 185 patients of both sexes were included in study and further classified into 3 groups as hypertensive, Ischemic heart disease and cerebrovascular disease/stroke. The 60 healthy subjects in the control group were not on any kind of prescribed medication or dietary restrictions were included in the control group. In this study, we investigated first; the possible involvement of MDA and protein carbonyl as the end product of oxidative stress. and second we investigated serum lipid profiles (total, HDL and LDL cholesterol, trygliceride) to determine the relationship between these parameters in atherosclerosis. Serum triglycerides, total cholesterol, LDL cholesterol ,VLDL-cholesterol were significantly high in all groups than controls ($p < 0.001$) while HDL-cholesterol was found to be decreased significantly in all groups as compared to controls ($p < 0.001$). Total cholesterol, TC/HDL-C ratio, Triglycerides, LDL-cholesterol, LDL-C/HDL-C ratio were higher in all three groups ($p<0.001$). HDL-C concentration was significantly lower in all three groups than controls ($p<0.001$). Higher ratio of TC/HDL-C, TG/HDL-C and LDL-C/HDL-C was observed in all three groups compared to controls. MDA is estimated as a marker of lipid peroxidation, levels were significantly increased in all groups than controls ($P <0.001$). Carbonyl content is estimated as a marker of protein oxidation in red cell extract is found to be significantly increase in all groups when compared with normal ($P <0.001$). The present study concludes the importance of assessing the lipid ratios even in a normal individual as it is one of the atherogenic factors for development of myocardial infarction and other coronary complications. The existing evidence supports the view that oxidative stress may play a crucial role in cardiac and vascular abnormalities in different types of cardiovascular diseases and that the antioxidant therapy may prove beneficial in combating these problems.

Key words: Lipid profile, MDA (Malonyldialdehyde), Protein carbonyl, HDL-C,LDL-C, Triglycerides.

INTRODUCTION

Coronary heart disease (CHD) is widely prevalent both in the developed and developing countries and continues to be a leading cause of mortality despite recent advances in diagnostic facilities and treatment modalities. It is a multifactor disease where atherosclerosis and dyslipidaemia are the prominent causes involved[1].

On the basis of various long term prospective studies, a number of risk factors of CHD have been established. One of the best documented is the association between elevated blood lipids and coronary heart disease. Since the major lipids of the blood circulate as lipoproteins, there has been a considerable interest on the relationship between serum lipoproteins and CHD.

To date, a number of cardiovascular risk factors have been identified that may affect endothelial function and in turn mediate vascular disease and its complications.
Hyperçholesterolemia and oxidized LDL are factors which are well known in this pathogenesis. It was suggested that oxygen derived free radicals and NO can initiate lipid peroxidation in LDL and so contribute to the pathogenesis of atherosclerosis. During lipid peroxidation, unstable hydroperoxides resulting from peroxyl radical-dependent chain reactions involving unsaturated fatty acyl moieties later break down to smaller and more stable products like malonyldialdehyde (MDA) or thiobarbituric acid-reactive substances (TBARS), which are considered to be oxidative stress markers. [2]

The usage of protein CO groups as biomarkers of oxidative stress has some advantages in comparison with the measurement of other oxidation products because of the relative early formation and the relative stability of carbonylated proteins. oxidatively modified protein is a complex function of a multitude of factors that govern (a) the rates of formation of various kinds of reactive oxygen species (ROS); (b) the levels of antioxidant defenses that guard against ROS-mediated protein damage; (c) the sensitivity of proteins to oxidative attack; and (d) the repair or elimination of damaged proteins. However, once they are formed, the ability of these ROS to modify proteins may be prevented by the action of various enzymic and nonenzymic antioxidants that can neutralize their prooxidant capacities. The antioxidant defenses are not normally sufficient to prevent significant oxidative damage to occur. Moreover, depending upon the nature of the modifications, oxidation can lead to forms that are preferentially degraded by several intracellular pro-teases (the 20 S proteosome, calpain, cathepsin B),2–4 whereas some protein modifications may lead to derivatives that are not only resistant to proteolysis but may inhibit the ability of proteases to degrade other forms of oxidatively modified proteins. [3,4]. The accumulation of oxidized proteins is therefore dependent not only on the levels of proteases that selectively degrade oxidized proteins, but also on the levels of metabolites and metal ions that activate or inhibit their activities. Finally, it must be realized that most of the many factors that govern the rates of protein oxidation and degradation of oxidized proteins are of genetic origin. Their concentrations and activities are therefore subject to genetic fidelity, which is also subject to compromise by ROS-mediated DNA damage.[5-7].

MATERIALS AND METHODS

Patient's attending cardiovascular OPD as well as Patients admitted in cardiac wards were taken for evalution of lipid profile, protein carbonyl and MDA content in their blood. Total 184 subjects were included for study out of which 128 were males and 56 were females. Sixty healthy age sex matched controls group, were not on any kind of prescribed medication or dietary restrictions were included in the control group.
The subjects were classified into 3 groups:

Group I- subjects with hypertension. Total patients 60 out of which 34 are males and 26 are female.
Group II- subjects with Ischemic heart disease. Total patients 68 out of which 48 are males and 20 are female.
Group III- subjects with Cerebrovascular diseases. Total patients 56 out of which 46 are males and 10 are female .
And healthy age sex matched control group . Total healthy individuals 60 out of which 36 are males and 24 are female patients.

The diagnosis of cardiovascular diseases was based on echocardiography, stress test and angiography as required for the disease.

Informed consent was taken from all subjects involved in the study and the study was approved by the Institutional Ethical committee. Blood samples (5mL) were drawn into plain vacutainers from the antecubital veins of healthy controls and patients ,after overnight fasting for lipid profile. Patients with diabetes mellitus, renal insufficiency, current and past smokers, hepatic disease or taking lipid-lowering drugs or antioxidant vitamin supplements were excluded from the study.

Concentration of serum Malonyldialdehyde (MDA) in serum [8], carbonyl content in RBC [9-10]estimated. Lipid profile TC (Total Cholesterol), TG(Triglyceride), and HDL-cholesterol were analyzed enzymatically using kits obtained from Randox Laboratories Limited, Crumlin, UK. Plasma LDL-cholesterol was determined from the values of total cholesterol and HDL-cholesterol using the following formula.[11]

Table 1 :Status of Lipid profile in various groups in mg/dl

Parameters	Group- I (Hypertension)	Group- II (Ischemic Heart)	Group- III (Cerebrovascular diseases/stroke)	Control	"P" value
Total Cholesterol	261.16 ± 36.90	230.50± 31.27	200.52 ± 42.42	187.67 ± 11.50	<0.001
Triglycerides	200.40 ± 33.90	148.87 ± 31.42	171.78 ± 42.16	108.23 ± 12.20	<0.001
HDL Cholesterol	40.10 ± 10.10	35.90± 7.60	37.98 ± 6.05	50.18 ± 6.70	<0.001
LDL Cholesterol	160.91 ± 11.31	142.44 ± 30.31	120.98.13 ± 10.77	82.90 ± 8.92	<0.001
VLDL Cholesterol	36.10 ± 9.70	28.49 ± 6.70	31.97 ± 13.98	23.96 ± 5.14	<0.001

Data are given as Mean ±SD

Graph

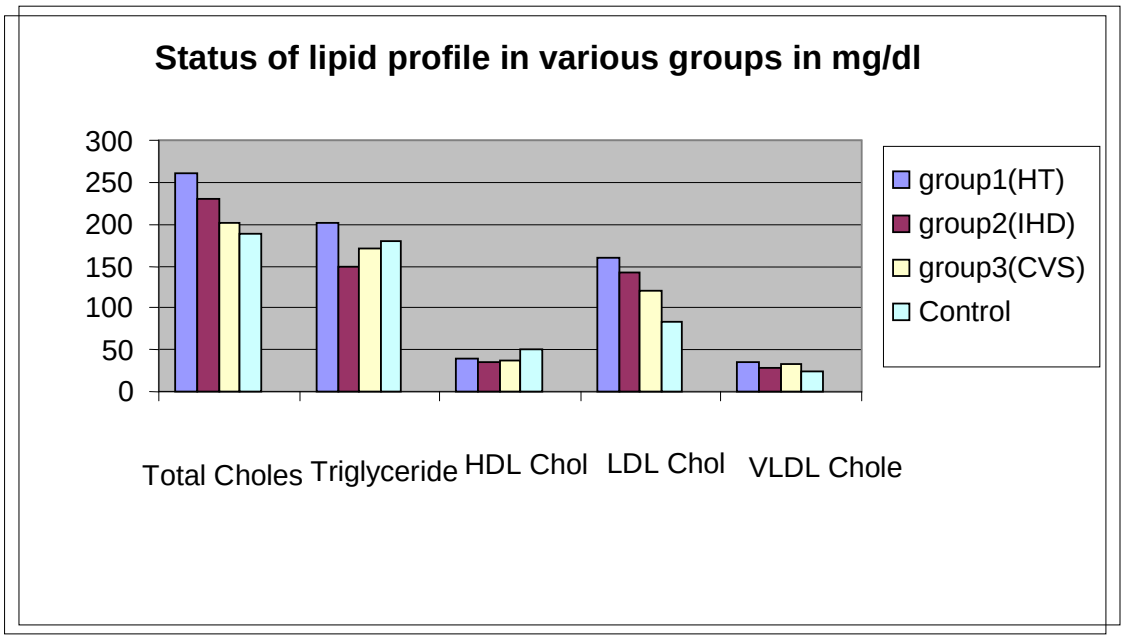

Table 2: TC/HDL-C, LDL-C/HDL-C and TG/HDL-C ratio in various groups

Parameters	Group- I (Hypertension)	Group- II (Ischemic Heart)	Group- III (Cerebrovascular diseases/stroke)	Control	"P" value
TC / HDL- C	6.52 ± 0.65	6.42 ± 0.11	5.27 ± 0.11	3.73 ± 0.71	<0.001
LDL-C/ HDL-C	4.01 ± 0.12	3.97 ± 0.98	3.18 ± 0.78	1.65 ± 0.33	<0.001
TG/HDL-C	4.99 ± 0.35	4.14 ± 0.13	4.52 ± 0.69	2.15 ± 0.82	<0.001

Data are given as Mean ± SD

Table 3 :Status of MDA and protein carbonyl in various groups

Parameters	Group- I (Hypertension)	Group- II (Ischemic Heart)	Group- III (Cerebrovascular diseases/stroke)	Control	"P" value
MDA (nmol/mg Protein)	4.39 ± 0.98	4.64 ± 0.88	4.79 ± 0.61	3.89 ± 0.94	<0.001
Carbonyl (nmol/mg protein)	4.21 ± 0.88	4.01 ± 0.38	3.97 ± 0.61	1.81 ± 0.58	<0.001

Data are given as Mean ± SD

RESULTS AND DISCUSSION

The status of lipid profile is given in Table.No.1. There is significant difference found in lipoproteins level between the different groups of cardiovascular diseases when compared with control group. Total cholesterol , Triglycerides , LDL-C and VLDL- C levels were significantly increased in all groups when compared with controls($p<0.001$).Group I shows the highest values for lipoproteins then the Group- II & III. HDL- C level was significantly lower in all groups when compared with controls($p<0.001$).Group- II shows the lowest values for HDL-C then Group- I and III.

Table 2 shows TC/HDL-C, LDL-C/HDL-C and TG/HDL-C ratio in various groups. Total cholesterol, its ratio to HDL-cholesterol (TC/HDL-C) , LDL-cholesterol and its ratio to HDL-cholesterol (LDL-C/HDL-C) and TG/HDL-C ratio a were higher in all groups when compared with controls ($p<0.001$).

Table 3 shows The MDA levels were found to be significantly increased in all three groups ($p<0.001$).Carbonyl content in red cell extract were found to be significantly increased in all three groups ($p<0.001$) when compared with normal. Statistical analysis was performed by students t test in the SPSS statistical programme. Dyslipidemia is one of the major risk factors of cardiovascular disease, which can be modified either by proper life style changes or medical management or by the combination of both**.** Lipoprotein abnormalities play an important role in the causation of atherosclerosis . Dyslipidaemia causes morbidity and mortality in patients with elevated triglyceride and LDL, and decreased HDL cholesterol concentrations. The modifications of LDL lipoprotein increase atherogenicity and available data suggest that LDL is more atherogenic .[12]. Dyslipidemia characterized by elevated TC, LDL-C and lowered HDL-C, is a conventional risk factor observed in myocardial infarction. Patients[13-15] and is the major cause of atherosclerosis are suggested to act synergistically with non-lipid risk factors to increase atherogenesis. Low-density lipoprotein cholesterol (LDL-C) is the main therapeutic target in the prevention of CVD(Cardiovascular disease). Increased triglycerides (TG) and decreased high-density lipoprotein (HDL-C) are considered to be a major risk factor for the development of Insulin resistance and metabolic syndrome. Although the TG/HDL-C ratio has been used as a clinical indicator for Insulin resistance, results were inconsistent. The TG/HDL-C ratio is also widely used to assess the lipid atherogenesis. How ever the utility of this ratio for predicting coronary heart disease (CHD) risk is not clear.

In the present study, triglycerides, VLDL-C, LDL-C, and total cholesterol in all three groups are significantly higher as compared to those in controls ($p < 0.001$). HDL-C in all three groups is lower as compared to that in controls ($p < 0.001$). Elevated TG has been found to be significant and independent risk factor for major coronary events even after adjustment for LDL-C and HDL-C levels and other risk factors. Similar results have been reported by some other authors [16,17]. Decreased HDL-C in patients indicate decreased rate of reverse cholesterol transport and therefore accumulation of TG rich lipoproteins leading to increased risk of atherosclerosis and CVD in all groups.

Thus, higher TG and VLDL-C and lower HDL-C levels are better indicators of CVD than the classical risk factors like total cholesterol and LDL-C supporting the hypothesis that lipoprotein metabolism and their catabolic rate play a crucial role in the development and progression of atherosclerosis.

Total cholesterol, TC/HDL-C ratio, Triglycerides, LDL-cholesterol, LDL-C/HDL-C ratio were higher in all groups ($p<0.001$). HDL-C concentration was significantly lower in all groups controls ($p<0.001$). Higher ratio of TC/HDL-C, TG/HDL-C and LDL-C/HDL-C was observed in all groups compared to controls. Increased LDL-C and reduced HDL-C are considered to be highly atherogenic. Thus the increased level of LDL-C/HDL-C would indicate an increased risk of developing atherosclerosis. A cut of level of 1.6 has been suggested . [18]. Increased TG and

decreased HDL-C are also thought to be atherogenic and thus increased ratio of TG/HDL-C would indicate an increased atherogenic risk.

CVD have been generally described as having high levels of oxidative stress. Oxidative stress generally causes damage to the membrane polyunsaturated fatty acids leading to the generation of MDA, a thiobarbituric acid reacting substance (TBARS). Increased lipid peroxidation products in with vascular complications, have been reported.[2]. All the biomolecules like lipids,proteins and nucleic acids may be attacked by free radicals, but lipids are probably the most susceptible. The oxidative destruction of lipids (lipid peroxidation) is a destructive, self perpetuating chain reaction, releasing Malonyldialdehyde (MDA) as the end product .Significant increase of MDA concentration in serum is found in all groups when compared with normal.

Cellular proteins are the main targets of oxidation resulting in the formation of aldehyde and ketone residues,of this post translational process is a measure of carbonyl content in proteins, which is an indicator of oxidative stress. Carbonyl groups formation are considered as an early and stable marker for protein oxidation. Significant increase of Carbonyl content in red cell extract is found in all groups when compared with normal.

Protein oxidation, in contrast to lipid peroxidation, does not have the features of chain reactions. The plasma proteins destroyed by peroxidation have a quite long period. Therefore, the evaluation of POX (Protein oxidation) in plasma is a respected marker of free radical intensity . Reactive oxygen species modify amino acid side chains of proteins such as arginine, lysine, threonine and proline residues to form protein carbonyls .[18]. Carbonyl group formation is considered an early and stable marker for POX, and elevated protein carbonyl levels are detected in CVD. This indicates carbonyl group formation and thus evidence of free radical modification of proteins.

The present study concludes the importance of assessing the lipid ratios even in a normal individual as it is one of the atherogenic factors for development of myocardial infarction and other coronary complications. The practice of computing the ratio should be practiced even in a normal health check up packages. The existing evidence support the view that oxidative stress may play a crucial role in cardiac and vascular abnormalities in different types of cardiovascular diseases and that the antioxidant therapy may prove beneficial in combating these problems.

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