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LIPID PEROXIDATION AND THE LEVELS OF ANTIOXIDANT ENZYMES IN CORONARY ARTERY DISEASE

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ABSTRACT

Coronary Artery Disease is the major cause of mortality and morbidity worldwide. Traditional risk factors account for only half of the morbidity and mortality from coronary artery disease. There is substantial evidence that oxidative stress plays the major role in the atherosclerotic process. The present study was undertaken to evaluate the level of lipid peroxidation (by measuring malondialdehyde) and antioxidant enzymes (ceruloplasmin, glutathione, superoxide dismutase) in coronary artery disease. Serum malondialdehyde levels and serum ceruloplasmin levels were significantly raised in all the subgroups of study group as compared to control group ($p<0.001$). Whole blood glutathione levels and hemolysate superoxide dismutase activity was significantly decreased in all the subgroups of study group as compared to control group ($p<0.001$). Above results suggests that the patients of coronary artery disease show increased oxidative stress and decreased levels of antioxidant enzymes. So it is recommended that the management protocol for coronary artery disease patients should include antioxidant supplementation along with simultaneous lowering of lipid peroxidation.

KEY WORDS

Coronary artery disease, Lipid peroxidation, Antioxidants, Ceruloplasmin.

INTRODUCTION

Oxidative stress induced by reactive oxygen species (ROS) is implicated in the pathogenesis of a variety of vascular diseases, including atherosclerosis, hypertension and coronary artery disease. The redox state is finely tuned to preserve cellular homeostasis through the expression of antioxidant enzymes and hence regulation of oxidants. Mammalian cells have a complex network of antioxidants like catalase, Superoxide dismutase (SOD), reduced glutathione etc to scavenge reactive oxygen species (1). Oxidative stress ensues when ROS evade or overwhelm antioxidants (2). Due to their highly reactive and non-specific nature, ROS can attack almost all biomolecules including lipid membranes (3). Lipid peroxides are derived from the oxidation of polyunsaturated fatty acids of membranes and are capable of further lipid peroxidation by a free radical chain reaction (4). Malondialdehyde (MDA) is a breakdown product of peroxidation of long chain fatty acids which accumulates when lipid peroxidation increases (5). The effects of lipid peroxides i.e. endothelial cell damage, uncontrolled lipid uptake, decreased prostaglandin synthesis and associated thrombogenecity are strongly implicated in the pathogenesis of atherosclerosis (6).

Ceruloplasmin, the major serum copper containing glycoprotein, can perform its antioxidant function as a ferroxidase and superoxide scavenger (7). It has been proposed that ceruloplasmin can serve as independent marker for the progression of coronary atherosclerosis (8). Ceruloplasmin is an acute phase protein and increased levels may be possibly due to stress induced by angina or acute myocardial infarction (AMI) (9). Biochemical studies have also shown that it is a potent catalyst of invitro oxidation of low density lipoproteins (LDL) (10). Glutathione (GSH) is an intracellular tripeptide that directly quenches the ROS and protects against deleterious effects of free radicals (11). Superoxide dismutase exists in three isoenzyme forms, all of which suppress oxidative stress under normal conditions and cause catalytic removal of superoxide anions (4).

Studies in animal models suggest that these antioxidants may be anti-atherogenic, but specific therapeutic applications

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targeting these enzymes yet remain unimplemented. Therefore, the present study was undertaken to evaluate the levels of lipid peroxidation, ceruloplasmin, and antioxidant enzymes (glutathione, superoxide dismutase) in CAD in human subjects.

MATERIALS AND METHODS

The present study comprised of 50 random cases of CAD from department of cardiology, Rajindra Hospital, Patiala and 30 age and sex matched healthy controls. Study group was divided into three subgroups i) Cases of acute MI (n=28), ii) Cases of unstable angina (n=15), iii) Cases of stable angina (n=7). The diagnosis of acute M I was based on WHO criteria which required the presence of atleast 2 of the following 3 elements: i) Ischaemic type of chest pain, ii) Changes on serial ECG tracings, iii) Increase in serum cardiac markers (CKMB) (12). Stable angina was characterized by deep, poorly localized chest or arm discomfort that was reproducibly associated with physical exertion or emotional stress and was relieved in 5 – 15 minutes by rest and/or sublingual nitroglycerin (13). Unstable angina was defined as angina pectoris or an equivalent type of ischaemic discomfort with atleast one of the following features: i) It occurred at rest (or with minimal exertion) usually lasting for > 20minutes (if not interrupted by nitroglycerin), ii) It was severe and described as flank pain and was of new onset (i.e. within one month) and iii) It occurred with a crescendo pattern (i.e. more severe, prolonged or more frequently). The patients with associated renal disease, liver disease, lung disease, thyroid disease, gastrointestinal disease etc. that could alter the required parameters were excluded from the study. A detailed clinical history was taken and all the routine investigations including Hb, TLC, DLC, blood urea and serum creatinine were carried out. In addition, lipid profile, MDA and antioxidant enzymes were estimated in all the cases and controls. Standardized procedures were followed for various estimations -Total Lipids (14), Serum Cholesterol (15), Serum Triglycerides (16), Serum HDL (17), Serum LDL (18), Serum MDA (19), Serum Ceruloplasmin (20), Hemolysate SOD Activity (21), Hemolysate protein content (22) and Whole blood GSH Levels (23). Statistical analysis was carried out by Student's paired 't'-test. The p value < 0.05 was taken as significant.

RESULTS AND DISCUSSION

Coronary artery disease is the major cause of mortality and morbidity worldwide. It is associated with various risk factors such as age group 41-60,male gender, smoking habit and hypertension, as evaluated in the present study (Table1a). Further, an adverse lipid profile (elevated serum cholesterol/ triglyceride/LDL along with low HDL) is an additional risk factor, which was also assessed in the present study (Table 1b).

Table 1a : Distribution of Risk Factors

Sr. No.	Risk Factor		Study Group
1.	Age Group	31-40	8%
		41-50	36%
		51-60	32%
		61-70	16%
		≥71	8%
2.	Sex	Male	68%
		Female	32%
3.	Smokers		24%
	Non Smokers		76%
4.	Hypertensive		56%
	Non-Hypertensive		44%

Table 1b : Comparison of Serum Lipid Profile of Study Group and Control Group

Sr.No.	Parameter (mg%)	Control Group	Study Group
1)	Total Lipid	518.10±103.14	580±81.94*
2)	Cholesterol	163.0±30.32	225.24±83.06**
3)	Triglyceride	107.43±31.38	166±72.06***
4)	HDL	49.46±7.91	45.50±4.69*
5)	LDL	97.66±23.11	126.02±29.33***

Statistical Comparison was done between control and Study group : * p<0.05; **p<0.01; ***p<0.001

Table 2 shows that there was a statistically significant (p<0.001) increase in the levels of ceruloplasmin in patients of CAD (Mean ±SD, 48.93 ± 4.44 mg %) as compared to controls (32.25 ± 4.67 mg %). Statistical analysis revealed significantly increased levels of ceruloplasmin in all the subgroups i.e. acute myocardial infarction, unstable angina, stable angina. Our results were in agreement with those reported by other authors (7).

Ceruloplasmin is an acute phase reactant and increased levels may possibly be due to stress induced by angina or AMI (9). Over the last several years, the idea that inflammation plays a key role in atherosclerosis has received considerable attention. Few reports have aimed to clarify a possible role of ceruloplasmin - a minor, long lasting acute phase protein as a cardiovascular risk factor. The liver is the major source of serum ceruloplasmin in adults. Proinflammatory agonists of the acute phase reaction such as certain cytokines and tissue

Table 2 : Comparison of Different Parameters Related to Oxidative Stress and Antioxidant Defence Systems in Control and Study Groups and among the Sub Groups of Study Group

Parameter	Control Group (n=30)	Study Group (n=50)	Myocardial Infarction (n=28)	Unstable angina (n=15)	Stable angina (n=7)
Serum Cerulo-plasmin(mg%)	32.25 ± 4.67	48.93 ± 4.44***	50.03 ± 3.57***++	49.38 ± 3.43***+	43.60 ± 5.93**
Serum MDA (μmol/L)	4.25±0.74	6.77 ± 0.66***	6.94 ± 0.67***++	6.65 ± 0.47***++	5.98 ± 0.48**
Blood GSH (mg%)	31.71 ± 3.66	20.63 ± 4.86***	19.23 ± 5.38***+	21.04 ± 3.17***++	25.32 ± 2.03**
Hemolysate SOD (U/mg protein)	6.10 ± 0.84	4.05 ± 0.56***	3.88 ± 0.46***++	3.98 ± 0.44***++	4.88 ± 0.44**

*** p<0.001 , ** p<0.01 when compared with control; + p<0.05, ++ p< 0.01,+++ p<0.001 when compared with stable angina

necrotic factor alpha (TNF- α) are known to enhance the gene expression of ceruloplasmin in hepatocytes (24). Also hypercholesterolemia is associated with high plasma levels of inflammation sensitive proteins such as ceruloplasmin and others, the presence of which may predict ischaemic stroke (25).

Studies have shown that ceruloplasmin can be considered an important risk factor predicting AMI and cardiovascular disease. Evidence suggests that LDL can be oxidized to an atherogenic form (oxidized-LDL) within arterial wall by macrophages and other cells. This oxidation may be mediated by copper ions released from ceruloplasmin in atherosclerotic lesions (10). The pro-oxidant activity requires an intact surface and a single copper atom at the surface of the protein near the His 266. Under conditions where inhibitory proteins are present (such as albumin), LDL oxidation by ceruloplasmin is optimal in presence of superoxides, which reduce surface copper atom of ceruloplasmin (10). Different mechanisms have been proposed for oxidation of LDL. Copper ion induced oxidation of LDL results in the release of hydroperoxides that are converted to reactive aldehydes. Interaction of these aldehydes with lysine residues in the apolipoprotein B-100 moiety renders the LDL more negatively charged which results in decreased affinity for LDL receptor and increased affinity for scavenger receptor (26). So serum ceruloplasmin levels can be suggested to be an independent risk factor for CAD operating through the oxidative modification of LDL.

Serum glycoproteins including ceruloplasmin have been determined in coronary atherosclerosis. According to Gensini scoring system (severity score for a stenosed vessel depending on degree of luminal narrowing and its location), ceruloplasmin can serve as independent marker for the progression of coronary atherosclerosis (8).

Table 2 also shows that there was a statistically significant (p<0.001) increase in the levels of MDA in patients of CAD (Mean ± SD, 6.77± 0.66 mmol/L) as compared to controls (4.25 ± 0.74 mmol/L). Statistical analysis revealed significantly increased levels of MDA in all the subgroups i.e. acute myocardial infarction, unstable angina, stable angina. Results of our study are akin to other authors who found increased MDA levels in all the patients (27, 28). MDA is a decomposition product of autooxidation of polyunsaturated fatty acids which is used as an index of oxidative damage (28). The high concentration of MDA in all the patients indicates increased membrane lipid peroxidation. Enhanced lipid peroxidation may occur as a result of the fact that naturally occurring scavenging mechanisms are suppressed and the free radical generation processes are enhanced (7). It has also been suggested that hyperlipidemia, specially hypercholesterolemia, can cause an increase in lipid peroxidation (29).

Table 2 reveals that there was statistically significant (p<0.001) decrease in the levels of GSH in patients of CAD (Mean±SD, 20.63 ± 4.86 mg %) as compared to controls (31.71±3.66 mg %). Statistical analysis revealed significantly decreased levels of GSH in all the subgroups i.e. myocardial infarction, unstable angina, stable angina. Similar results have been shown by other authors (30, 31). Ischaemia induces metabolic alterations capable of reducing the defense mechanisms of heart against oxygen toxicity, depending on the severity of ischaemic damage. Ischaemia shifts the redox state of cell towards oxidation, GSH and protein- SH content being significantly reduced (32) .The significantly decreased levels in our study could be secondary to increased oxidative stress.

Table 2 also shows that there was a statistically significant (p<0.001) decrease in the activity of SOD in patients of CAD (Mean±SD, 4.05±0.56 U/mg protein) as compared to controls. (6.10±0.84 U/mg protein). Statistical analysis revealed significantly decreased levels of SOD in all the subgroups. Other eminent authors also reported a significantly decreased activity of SOD in CAD patients (7,29,33). Decrease in SOD activity may be attributed to hypoxia (due to ischemia) and

reperfusion. There is an enhanced production of superoxide anions by ischaemic cells. Also increased concentration of LDL causes uncoupling of endothelial nitric oxide synthase and consequently increased production of superoxide anions in vessel wall (34). In patients with CAD, secretion of TNF-α is increased and it contributes to depression of extra cellular SOD activity**.** This leads to increased free radical load and increased inactivation of NO by superoxide anions forming peroxynitrite thus contributing to endothelial dysfunction in patients with CAD (33).

Thus present study indicates that CAD patients have increased oxidative stress and a compromised antioxidant defense system. The raised ceruloplasmin could be a risk factor of CAD by modifying of LDL to an atherogenic form). This means that increased ceruloplasmin could behave as a pro-oxidant (a negative correlation was observed between ceruloplasmin and GSH/SOD by Kaur et al, unpublished results). Raised ceruloplasmin could serve as a marker of chronic inflammation (as ceruloplasmin is a long lasting acute phase glycoprotein) or it might also be elevated as a consequence of oxidative stress in CAD.These aspects needs to be confirmed by more rigorous studies. So, it is recommended that the management strategy for the patients of CAD should include specific antioxidant supplementation alongwith lowering of lipid peroxidation. Once the role of ceruloplasmin is unequivocally established therapeutic applications targeting modification of ceruloplasmin levels can be devised for such patients.

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