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A Study to Evaluate the Severity of Coronary Artery Disease (CAD) in Relation to Oxidative Stress and Antioxidant Status

Short Running Title: Association of lipid peroxidation with CAD severity

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Abstract: **Coronary artery disease (CAD) is a multifactorial disease with no known definite cure, but preventable and treatable. Many studies have linked excess generation of reactive oxygen species (ROS) with cellular damage and CAD. No systematic studies were conducted to evaluate the role of oxidative stress and antioxidant status on CAD severity. Hence we have undertaken this study. One hundred and twenty four angiographically proved CAD patients were included in this study. CAD severity was assessed by angiography. The patients with 0 vessel disease were taken as control to study the correlation of lipid peroxidation with CAD severity. Lipid peroxidation product, Malondialdehyde (MDA), antioxidants such as Super Oxide Dismutase (SOD), Glutathione (GSH) and Ascorbic acid of all patients were estimated in the serum. Our results revealed that there was higher lipid peroxidation with CAD severity as indicated from lower values of MDA and higher values of SOD in 0 vessel disease compared to single vessel, double vessel and triple vessel disease patients ($p<0.05$). The severity of CAD is closely related to increased lipid peroxidation and decreased antioxidants status as indicated by the MDA and SOD levels.**

Keywords: **Coronary artery disease (CAD); Reactive oxygen species (ROS); CAD Severity.**

1. INTRODUCTION

Coronary artery disease (CAD) is a complex multifactorial and polygenic disorder where multiple environmental and genetic factors are involved simultaneously (1**)**. Second half of the 20th century witnessed a global spread of the coronary artery disease epidemic especially in developing countries, including India (2). A constant supply of oxygen is indispensable for cardiac viability and function. As oxygen is a major determinant of cardiac gene expression and a critical participant in the formation of free radicals commonly known as reactive oxygen species (ROS) which plays a major role in the pathogenesis of cardiac dysfunction (3).

In the vascular system, the formation of ROS from endothelial cells, smooth muscle cells and macrophages may be of major relevance in atherogenesis. This oxidative stress is recogonised as a key mechanism for atherogenesis and in the development of atherosclerosis (4,5). Under normal conditions, numerous cellular antioxidant systems exist to defend against oxidant stress and maintain the redox balance of the cell. ROS are cleared from the cell by enzymatic systems including superoxide dismutase, catalase, and glutathione peroxidase or the non– enzymatic system including vitamin E, ascorbic acid and glutathione (6).

Lipid peroxidation is a free radical mediated process, which is potentially harmful to membranes, lipoproteins and polyunsaturated fatty acids leading to the formation of malondialdehyde (MDA). The physical habits like cigarette smoking and alcohol consumption of patients also contributed to lipid peroxidation (7,8).

A good antioxidant status may be important for human health and especially for the prevention of chronic diseases such as cancer and CAD. Low plasma levels of antioxidant as well as low intake of dietary antioxidants have been associated with an increased risk of atherosclerotic heart disease patients (9). The potential damage that can be caused by free radicals is minimized by a combination of biological antioxidant systems both enzymatic and non-enzymatic (10).

Several studies were conducted to evaluate the effect of oxidative stress and the role of antioxidants in coronary artery patients (11). But no detailed studies were carried out to evaluate the association of lipid peroxidation with the CAD severity in coronary artery disease patients. So we conducted a systematic study to correlate the association of lipid peroxidation with CAD severity in these patients.

2. MATERIALS AND METHODS

Materials:

The study was conducted in the Department of Biochemistry, Pushpagiri Institute of Medical Sciences and Research Center, Thiruvalla, Kerala, India from Jan'2010 to Aug'2012.

Subjects: One hundred and twenty four patients - 83 men and 41 women - who attended the Pushpagiri Heart Institute during this study period were included in the study.

Inclusion Criteria: Angiographically proved CAD patients below the age of 65 years.

Exclusion Criteria: Patients with liver and renal dysfunctions were excluded.

CAD severity determination:

CAD severity was assessed by the coronary angiographic results.

On the basis of coronary angiographic results, the patients were classified into

Group-0: Twenty patients with no diseased vessel

Group-1: Forty patients with single – vessel disease

Group-2: Thirty three patients with double – vessel disease

Group-3: Thirty one patients with multi- vessel disease.

Control Subjects:

The group-0 (patients with no diseased vessel) was taken as control

Collection of blood:

Blood samples were collected after getting informed consent. Three ml peripheral blood was collected aseptically in an EDTA tube.

Determination of lipid peroxidation:

Lipid peroxidation was assessed by estimating the lipid peroxidation product, malondialdehyde (MDA) by thiobarbituric acid assay method (12).

Determination of antioxidant status:

The antioxidant status was assessed by estimating enzymatic antioxidant like superoxide dismutase (SOD) (13) {Reagents used: Sodium pyro phosphate buffer, Phenazine metho sulphate , Nitro blue tetrazolium, NADH ,n-butanol} and non enzymatic antioxidant like glutathione(GSH) (14) {Reagents used: precipitating reagent, DTNB reagent, phosphate solution} and ascorbic acid (15) {Reagents used: DTC reagent, H_2SO_4, TCA}.

All these parameters were estimated manually by using chemicals from Sigma Chemicals Limited and the instrument used was UV-VIS Spectrophotometer.

Statistical analysis:

Statistical analysis was done by using one –way analysis of variance (ANOVA) to test the significant difference of various parameters between groups of patients based on CAD severity. Bonferronic was used as a Post-Hoc test.

3. RESULTS

The frequency of patients:

The frequency of patients with respect to CAD severity is given in Table 1. Out of 124 CAD patients examined, the majority of patients are of single vessel disease.

The level of MDA with CAD severity:

The level of MDA with CAD severity is given in Figure.1. The level of MDA increased significantly ($p<0.05$) as CAD severity progressed from 0 vessel to 3 vessel disease (Table 2).

The level of SOD with CAD severity:

The level of SOD with CAD severity is given in Figure 2 . The level of SOD in group 0 was significantly higher ($p<0.05$) when compared to 1, 2 &3 groups but we found no significant variation in the level of SOD among 1, 2 &3 groups (Table 2).

The level of GSH with CAD severity:

The level of GSH with CAD severity was given in Figure 3. The level of GSH in group 0 was significantly higher ($p= 0.024$) when compared to group3 but no significant variation when compared to 1&2 groups. There was no significant difference in GSH levels among 1, 2&3 groups (Table 2).

The level of ascorbic acid with CAD severity:

The level of ascorbic acid with CAD severity is given in Figure 4. The level of ascorbic acid in group 0 was higher ($p=0.124$) when compared to group3 but there was no significant variation in ascorbic acid levels among 0, 1 & 2groups and also among 1, 2 & 3 groups (Table 2).

4. DISCUSSION

Coronary Artery Disease (CAD) is the major cause of mortality and morbidity worldwide (16). It was observed that increased concentrations of MDA in the circulation of CAD patients indicated increased lipid peroxidation (17). Our results showed that the level of MDA progressively increased where as the level of SOD progressively decreased from group 0 to group 3. The level of GSH also had a trend of progressive decrease from group 0 to group 3. But no such differences were observed in the level of ascorbic acid. This may be probably due to dietary factors. However there was significant decrease in the level of ascorbic acid in group 3 compared to group 0.

Rise in MDA could be due to increased generation of reactive oxygen species (ROS) due to the excessive oxidative damage in these patients. MDA is a decomposition product of auto oxidation of polyunsaturated fatty acids, which is used as an index of oxidative damage (18). The extent of lipid peroxidation was found to be higher in patients with diseased vessels than in patients without diseased vessels as shown by the significantly higher levels of erythrocyte MDA.

We observed a significant decrease in the levels of non enzymatic antioxidants like SOD and GSH in patients with diseased vessels when compared to patients without diseased vessels. The decrease in the level of these antioxidants may be due to the increased consumption to prevent oxidative damage in these patients (19, 20, and 21). Therefore there is significant increased oxidative stress and decreased antioxidant status in CAD patients with diseased vessels when compared to cardiac patients without diseased vessels. This may be due to an imbalance between the generations of reactive oxygen species (ROS) and the antioxidant system which mop up the free radicals.

From the above results it is clear that there was high lipid peroxidation and lower antioxidant levels in CAD patients with 3 vessel disease compared to 0 vessels, 1 vessel or 2 vessel diseases. From these findings we conclude that increased oxidative stress predispose for the progression of coronary artery disease.

5. FUNDING

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ABBREVIATIONS

CAD - Coronary Artery Disease

ROS - Reactive Oxygen Species

MDA – Malondialdehyde

SOD – Super Oxide Dismutase

GSH – Glutatrhione

APPENDIX – A

TABLES AND GRAPHS:

Table 1: Frequency of patients with respect to CAD severity

Groups		Frequency	Valid Percent
Valid	0	20	16.1
	1	40	32.3
	2	33	26.6
	3	31	25.0

Table 2: Statistical analysis- post-hoc test

Parameters	Type of CAD severity	N	Mean	Std. Deviation	F	Sig.
MDA	0	20	0.87	0.360		
	1	40	1.33	0.272		
	2	33	1.65	0.372		
	3	31	2.48	0.274	123.77	0.000
SOD	0	20	4.38	1.304		
	1	40	2.79	1.828		
	2	33	2.36	2.084		
	3	31	1.40	0.878	13.73	0.000
GSH	0	20	10.61	4.56		
	1	40	8.59	4.11		
	2	33	8.50	4.31		
	3	31	7.17	1.98	3.25	0.024
Ascorbic acid	0	20	0.94	0.425		
	1	40	1.04	0.547		
	2	33	0.96	0.551		
	3	31	0.75	0.398	2.07	0.108

Significance at the level $p<0.05$

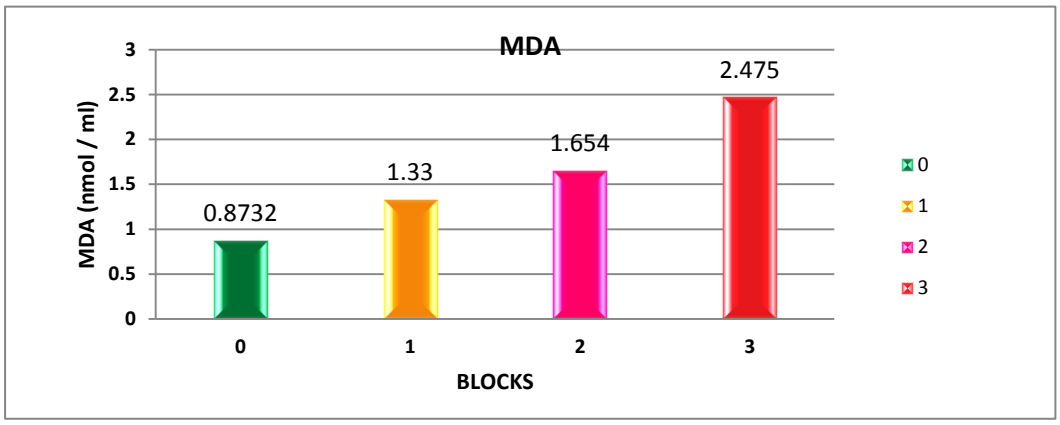

Fig.1 Level of MDA with CAD severity

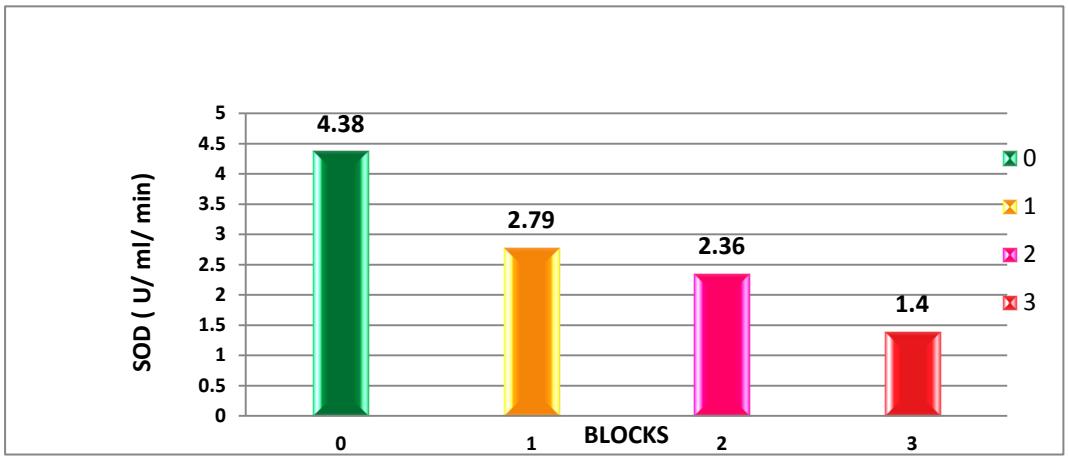

Fig.2 Level of SOD with CAD severity

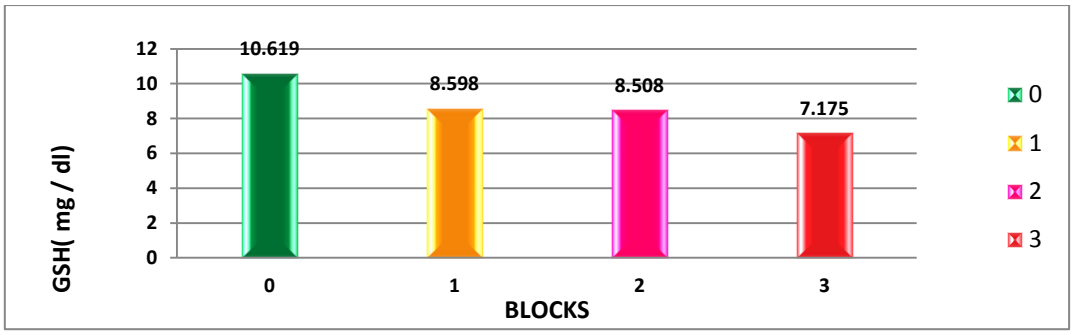

Fig.3 Level of GSH with CAD severity

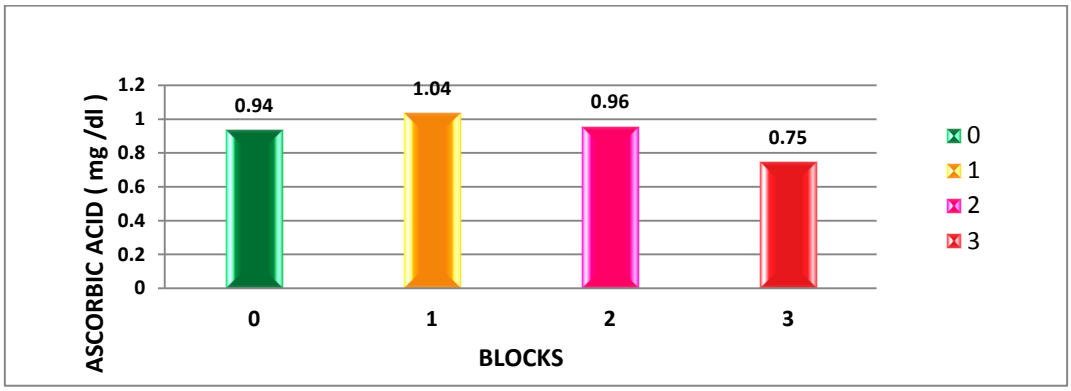

Fig.4 Level of Ascorbic acid with CAD severity