

## MARKERS OF OXIDATIVE STRESS IN ANGIOGRAPHICALLY PROVED CORONARY ARTERY DISEASE PATIENTS

K. Sowmya<sup>a</sup>, Jothi Malar<sup>a</sup>, Nalini.G<sup>a</sup>

### ABSTRACT:

**Background & Objective:** Growing evidence indicates that overproduction of reactive oxygen species (ROS) under pathophysiologic conditions is integral in the development of cardiovascular diseases. ROS mediates various signaling pathways that underlie vascular inflammation in atherogenesis - from the initiation of fatty streak development through lesion progression to ultimate plaque rupture. Hence this pilot study was conducted to evaluate the level of oxidative stress markers like malondialdehyde (MDA), superoxide dismutase (SOD) and ascorbic acid in angiographically proved coronary artery disease (CAD) patient compared with healthy individuals along with routine plasma glucose & lipid parameters

**Materials & Methods:** The study group consisted of 60 subjects, of which 30 were healthy individuals & age matched 30 angiographically proven coronary artery disease patients were taken as cases. Peripheral venous blood was used for analysis. Oxidative stress markers - malondialdehyde, superoxide dismutase & ascorbic acid were analyzed in

Hitachi U-2001 spectrophotometer. Lipid profile & glucose values were analyzed in Bayer's Express plus automated system using kits supplied by Accurex India Ltd. The statistical analysis was done using students t- test and p-value of < 0.05 was considered significant

**Result:** Dyslipidemia [Increased Total cholesterol, Triglyceride (TGL), Low density lipoprotein-cholesterol (LDL-C) and decreased High density lipoprotein-cholesterol (HDL-C) & increased plasma glucose was seen in CAD patients. Plasma malondialdehyde - a marker of lipid peroxidation was significantly elevated and the levels of ascorbic acid and superoxide dismutase were significantly reduced in CAD patients compared to the healthy group.

**Interpretation & Conclusion:** There are an increased oxidants and decreased antioxidant levels among the CAD patients when compared to healthy individuals, indicating oxidative stress in CAD patients.

**Key words:** oxidative stress, coronary artery diseases, antioxidants

SRJM 2011;4:20-23

### INTRODUCTION :

Coronary artery disease is the major cause of morbidity & mortality in the entire world.<sup>[1]</sup> The majority of cardiovascular disease results from complications of atherosclerosis. Progress made in the medical field has attempted to address this insidious disease on all fronts - etiology, pathogenesis, treatment and prevention. Despite all-round efforts, it remains a major challenge to the health managers and scientists. In developing countries, the incidence of CAD is increasing alarmingly.<sup>[2]</sup> India is on the verge of cardiovascular epidemic. In India, epidemiological studies have revealed that the prevalence of CAD has increased from 4% in 1960 to 11% in 2001. It has been predicted that by the year 2020 CAD will persist as the major and the most common threat to human life.<sup>[3,4]</sup>

The underlying cause of coronary artery disease is atherosclerosis. An important initiating event for atherosclerosis is impaired endothelial function. The endothelial function is impaired by Reactive Oxygen Species (ROS) and various risk factors.<sup>[5]</sup> Reactive Oxygen Species reduces the endothelial nitric oxide production. Lack of nitric

oxide contributes to impaired vascular relaxation & increased platelet aggregation thereby leading to atherosclerosis. Role of dyslipidemia in causing CAD is well established by various studies.<sup>[6,7,8]</sup> Moreover oxidized LDL-C plays an important role in the pathogenesis of atherosclerosis<sup>[9]</sup> and this oxidative modification of LDL-C is brought about by free radicals. Goldstein<sup>[10]</sup> in his work has proved oxidized LDL-C as an important atherogenic factor. The oxidized LDL-C bypasses the normal feedback control of LDL-C receptor and is avidly endocytosed by the scavenger receptor pathway of macrophages. The scavenger receptor pathway is not regulated by the intracellular cholesterol concentration thus leading to loading of macrophages with cholesterol thus converting them to foam cells and thereby leading to atherosclerosis.

Malondialdehyde is a breakdown product of peroxidation of long chain fatty acids.<sup>[11]</sup> Increased lipid peroxides lead to endothelial cell damage, uncontrolled lipid uptake, and increased thrombogenicity thus leading to atherosclerosis.<sup>[12]</sup> Endothelial cells have a comprehensive array of antioxidant defense mechanisms to reduce free radical formation or limit their damaging effects. These include enzymes such as superoxide dismutase and catalase to degrade superoxide and peroxides respectively, and essential free radical scavengers like ascorbic acid. superoxide dismutase is a secretory glycoprotein found in blood vessel walls which represents an important vascular enzymatic antioxidant defense system. It dismutates superoxide ions to H<sub>2</sub>O<sub>2</sub> and thereby improves endothelial function.<sup>[13]</sup> Ascorbic acid (vitamin C) is an important antioxidant in plasma, it consumes free radicals and helps to preserve alpha

### CORRESPONDING AUTHOR :

**Dr. K. Sowmya**

Associate Professor,  
Department of Biochemistry, SRMC & RI  
Sri Ramachandra University,  
Chennai - 600 116

Email : sowmyasathyan@yahoo.co.in

<sup>a</sup>Dept. of Biochemistry, SRMC & RI

tocopherol in lipoproteins.<sup>[14]</sup> Thus the antioxidants tend to reduce the risk and severity of atherosclerosis by inhibiting lipid peroxidation.

There are very few studies on markers of oxidative stress in angiographically proved CAD patients. Hence this pilot study was undertaken to estimate the level of malondialdehyde, superoxide dismutase and ascorbic acid, in angiographically proved CAD patients and compare it with healthy individuals. Thus the estimation of routine parameters, together with oxidant-antioxidant profile in an individual will help in risk assessment and management of CAD.

## MATERIALS & METHODS

All the tests were performed in the laboratory of Sri Ramachandra Medical College & Research Institute and the procedures followed were in accordance with the ethical standards of the committee on human experimentation of the institution. The written consents were taken from the patients prior to study and the objectives of the study were fully explained. The complete clinical and personal history of the subjects was recorded in the form of Questionnaire. Patients with smoking habits and those suffering from renal disease, chronic obstructive pulmonary disease, hypertension and hepatitis were excluded from the study. Selection of the patients was done after confirmation of CAD by electrocardiogram and angiogram. The control group comprised of 30 apparently healthy individuals in the age group of 40-60 years of both sexes (14 males and 16 females) and age matched 30 angiographically proven cardiac cases of both sexes (18 males and 12 females) from the Department of Cardiology, Sri Ramachandra Medical College & Research Institute, Porur, Chennai were taken as cases. Blood samples were drawn after overnight fasting. Fasting fluoride plasma and serum samples were collected using BD vacutainer tubes for the estimation of glucose and lipid profile respectively. Heparinized plasma samples were used for the analysis of malondialdehyde and ascorbic acid & haemolysate was used for estimation of superoxide dismutase.

Lipid profile & glucose values were analyzed in Bayer's Express plus automated analyser using kits supplied by Accurex India Ltd. Fasting plasma glucose values were measured by glucose oxidase-peroxidase method, serum Total cholesterol was measured by cholesterol oxidase-peroxidase method, TGL was measured by glycerol kinase, glycerol phosphate oxidase, peroxidase method, HDL-C was determined by cholesterol esterase, cholesterol oxidase & catalase method - a direct enzymatic method & LDL-C was calculated using Friedewald's equation.

Plasma ascorbic acid was estimated by the method of Omaye et al<sup>[15]</sup> and expressed as mg/dl. SOD was estimated by the method of Marklund & Marklund<sup>[15,16]</sup> in which the degree of inhibition of auto-oxidation of pyrogallol by superoxide dismutase was used as a measure of enzyme activity and expressed as U/ml. Malondialdehyde was estimated as a marker of lipid peroxidation by the method

of Yagi et al<sup>[17]</sup> and expressed as nmol/ml. SOD, malondialdehyde & ascorbic acid were analyzed in Hitachi U-2001 spectrophotometer. Statistical analysis was done using students t- test and p -value of < 0.05 was considered significant

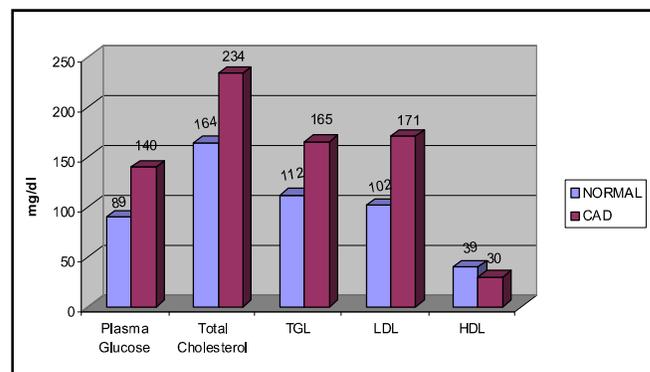
## RESULT

The present study was conducted on 30 CAD patients and the results were compared with healthy controls. Mean age in the study group was  $46 \pm 4.4$  years and that in control group was  $48 \pm 4.8$  years. The controls of our study group were not a diabetic, hypertensive nor had the habit of smoking. Among the cases, 36 % (11 out of 30 cases) were diabetic and were on oral hypoglycemic drugs (metformin). Table I and Fig.1 shows the comparison of the Biochemical parameters of Conventional risk factors. The mean values of Plasma glucose, Total cholesterol, LDL-C, and TGL were found to be higher in CAD cases & the mean HDL cholesterol was significantly low in CAD cases as compared to normal group. The difference in mean values among the cases and controls were calculated and was found to be statistically significant for all the parameters. Table II and Fig. 2 shows a statistically significant ( $p = 0.001$ ) increase in malondialdehyde levels and significant decrease in SOD ( $p = 0.001$ ) & vitamin C ( $p = 0.001$ ) levels in CAD cases compared to the normal group

**Table 1: Conventional risk factors in normal individuals & Angiographically proved CAD Patients**

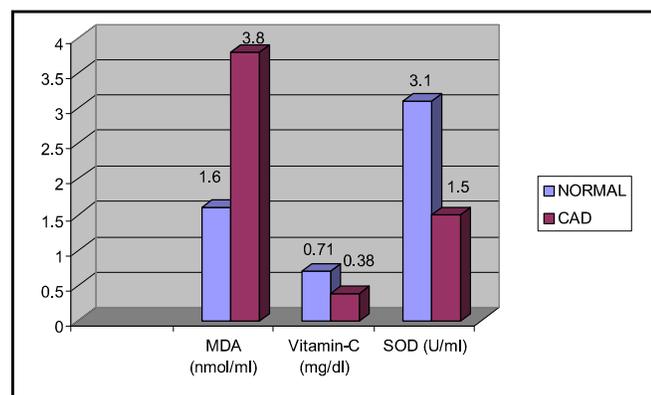
Parameters (mg/dl)	NORMAL (n = 30)	CAD (n = 30)	p-Value
Plasma Glucose	$89 \pm 12$	$140 \pm 39$	<0.001
Total Cholesterol	$164.7 \pm 31.06$	$234.4 \pm 38.41$	<0.001
TGL	$112.6 \pm 30.75$	$165.9 \pm 38.20$	<0.001
LDL	$102.9 \pm 30.01$	$171.02 \pm 38$	<0.001
HDL	$39.26 \pm 7.22$	$30.2 \pm 4.2$	<0.001
p-value < 0.001 is considered significant			

**Fig.1: Graphical representation of conventional risk factors in normal individuals & Angiographically proved CAD Patients**



**Table 2: Oxidant & antioxidant levels in healthy individuals and Angiographically proved CAD Patients**

Parameters	NORMAL (n = 30)	CAD (n = 30)
MDA (nmol/ml)	1.6 ± 0.32	3.8 ± 0.76***
Vitamin-C (mg/dl)	0.71 ± .21	0.38 ± 0.09***
SOD (U/ml)	3.1 ± 0.59	1.5 ± 0.39***
***p-value < 0.001		

**Fig.2: Graphical representation of oxidant & antioxidant levels in healthy individuals and Angiographically proved CAD Patients**

## DISCUSSION

Coronary artery disease continues to be a leading cause of death among the Indian population. It is associated with various risk factors. Dyslipidemia is a major risk factor for CAD & the leading cause of death world wide.<sup>[7]</sup> In this study the levels of risk associated lipids, i.e. Total cholesterol, Triglycerides and LDL-C were high and levels of HDL-C were low in CAD patients compared with the normal individual, thus proving the association of dyslipidemia with CAD. A positive relationship between dyslipidemia and risk of CAD has been well established by many studies<sup>[16, 18, 19, 20]</sup> thus favouring the study finding. Plasma glucose levels were found to be significantly high among the cases than among the controls and the levels of lipid peroxidation were found to be high among the cases indicating that diabetes is associated with increased generation of reactive Oxygen Species. malondialdehyde is a product of auto oxidation of polyunsaturated fatty acids which is used as an index of oxidative damage.<sup>[21]</sup> Enhanced lipid peroxidation is the result of increased free radical generation and suppressed scavenging mechanisms.<sup>[22]</sup> In this study malondialdehyde levels were significantly increased in CAD patients compared to the normal individuals thus confirming increased lipid peroxidation in CAD patients. Mendis<sup>[23]</sup> & Kostner et al<sup>[24]</sup> in their study have reported high malondialdehyde levels in CAD patients compared to controls. This study results also akin to the above study finding.

The levels of antioxidants such as ascorbic acid & superoxide dismutase were found to be low in CAD patients compared to healthy controls in this study. Ascorbic acid is

a water-soluble vitamin and a powerful antioxidant that acts as the body's primary defense against peroxyl radicals formed during the metabolic process. Gokce<sup>[25]</sup> and Santillo<sup>[14]</sup> in their study have proved the beneficial effect of ascorbic acid supplementation in CAD patients and its protective role against the peroxidative damage of lipids, favouring this study finding. Superoxide dismutase is a major enzyme in plasma which removes the superoxide radical. In this study the level of superoxide dismutase was low in CAD patients compared to healthy controls. The decreased levels of superoxide dismutase in CAD patients may be because of reduced production or increased utilization of the enzymes, thereby rendering an individual susceptible to oxidative damage due to decreased clearance of free radicals. Landmesser<sup>[26]</sup> in his study have proved, reduced superoxide dismutase activity as a major contributor of endothelial dysfunction in patients with CAD. Wang<sup>[27]</sup> in their study have established the association of decreased superoxide dismutase levels in CAD patients, supporting this study finding. Thus the levels of superoxide dismutase may be envisaged as a potential marker of risk assessment for CAD in addition to glucose & lipid profile.

## CONCLUSION

The study results show an increased lipid peroxidation, decreased antioxidant status and dyslipidemia among the cases, thus indicating increased oxidative stress among coronary artery disease patients. Hence oxidative damage due to lipid peroxidation can be managed by increasing the antioxidant status by supplementation of antioxidants and by taking measures to correct dyslipidemia, thereby we can reduce the events of coronary artery diseases.

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