Section A -Research paper
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Analysis of Oxidative Stress Markers in Premature Coronary Artery Disease

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ABSTRACT:

Background & Objective: Formation of atherosclerotic plaques is the major cause of coronary artery disease (CAD). Oxidative stresses which consume smooth muscle cells and macrophages for the formation of foam cells, is a significant factor in the development of atherosclerosis.

A pro-oxidant and anti-oxidant imbalance can produce highly reactive and unstable free radicals; which target proteins and DNA. Degradation of Polyunsaturated lipids by reactive oxygen species produces malondialdehyde (MDA) is a potential biochemical marker. Antioxidant enzyme activity is also decreased in CHD, which is associated with a higher risk of developing the disease. The hospital based cross sectional observational study was conducted to evaluate the

Level of oxidative stress markers like malondialdehyde (MDA) & antioxidants markers superoxide dismutase (SOD), catalase (CAT) in premature coronary artery disease (CAD) patients & compared with healthy individuals.

Materials and Methods: The total study group consists of 400 subjects, of which 200 were healthy individuals (controls) & 200 premature coronary artery disease patients were taken as cases. Venous blood was used for analysis. Oxidative stress marker (MDA) & Antioxidants (SOD & CAT) were analyzed on Spectrophotometer. The data analysis was done by using mean, standard deviation & student t-test.

Results: MDA (4.10 ± 0.47 vs. 2.08 ± 0.35) was higher, & SOD (17.47 ± 1.433 vs. 26.81 ± 3.04) and catalase (115.39 ± 5.07) vs. 176.99 ± 9.31) were lower in CAD patients than control subjects. The level of Plasma malondialdehyde was significantly increased & the level of superoxide dismutase & catalase were significantly reduced in CAD patients as compared to healthy individuals.

Conclusion: The present study concluded that increased level oxidants & decreased antioxidant levels among the CAD patients as compared to healthy individuals.

Key words: coronary artery disease; Malondialdehyde; superoxide dismutase; catalase.

Introduction:

Coronary artery disease (CAD) occurs when arteries of the heart become clogged with atherosclerotic plaques, which results in less blood reaching the heart muscle. There are several cardiovascular diseases, but CAD is the most common. [1] In the majority of nations coronary artery disease (CAD) is the leading cause of mortality and morbidity.[2] As per WHO statistics, 17.1 million deaths were caused by CVDs in 2004 and by 2030, 23.6 million deaths will be caused by CVDs, mainly coronary artery disease (CAD) and stroke.[3] Obesity, hypertension, cigarette smoking, type 2 diabetes mellitus, elevated cholesterol level is a major risk factor for CAD.[4] Incidence of CAD is increasing at an alarming rate in developing countries.[5] India is in danger of experiencing a cardiovascular pandemic. According to predictions, by the year 2030, CAD will continue to be the biggest and most frequent threat to human existence. [6, 7]

It is now generally accepted that oxidative stress plays a significant role in the development of atherosclerosis. According to various studies have shown that Increased ROS generation has been linked to endothelial dysfunction (ED) in both experimental and clinical atherosclerosis.[8,9] Malondialdehyde (MDA) is an intermediate end product of LPO; has the ability to cross linking membrane components containing Amino-groups. MDA can be detected using its reaction with thiobarbituric acid (TBA), which has been the most frequently used LPO indicator.[10]

Unsaturated aldehydes produced by lipid peroxidation, such as acrolein and malondialdehyde (MDA), have toxic effects due to their reactivity with nucleophile substances and their capacity to form protein and DNA adducts without any previous metabolic activation. These aldehydes are thought to act as agents of vascular dysfunction and inflammation.[11] SOD and catalase play a crucial role in the enzymatic detoxification of some extremely reactive LPO compounds. Superoxide radical is dismutated by SOD to produce oxygen and hydrogen peroxide. Hydrogen peroxide is broken down by catalase to produce oxygen and water. These enzymes that scavenge free radicals serve as the first line of protection for cells against oxidative damage.[12] The present study was designed to study the levels of oxidants (MDA) & antioxidants (SOD, catalase) in Premature CAD patients & healthy controls; and compared the oxidative stress biomarkers in Premature CAD patients & healthy controls.

Materials and Methods

Study Design:

The present study was hospital based cross sectional observational study, which had carried out in the Department of Biochemistry, SGT Medical College, Gurugram. The subjects for the study included from Medicine OPD SGT Medical College, Gurugram & Cardiology department of SGPGI, Lucknow. The

written consents were taken from the patients prior to the study & the objectives of the study were fully explained. The written informed consent was taken from the subjects to be included in the study.
The clearance was taken from institutional ethics committee of FMHS, SGT University.

Sample Size:

Sample Size Calculation for prevalence study:

$$n = \frac{Z^2 P(1-P)}{d^2}$$

n = Sample Size
Z = Z Statistic for a level of confidence
P = Expected prevalence or proportion
d = Precision

Level of confidence = 95%
Disease prevalence in India = ~ 9%
Sample size calculated from the above given formulae = 197

Study groups

The study included a total 400 subjects; which was divided in to two groups. The first group has 200 cases & second group has 200 controls. Selection of cases was done on the basis of ECG graph & cardiac markers.
Group I (Controls): This group had 200 Age & gender matched healthy individuals
Group II (Cases): This group had 200 Patients of either gender suffering from premature CAD (Males <55 years & female<65years in females)
Premature coronary artery disease according to American Heart Association (AHA) defined as artherosclerotic narrowing of coronary arteries. (Males <55 years & in females <65years)

Exclusion criteria for cases

- Any other Acute /Chronic inflammatory disorder
- Smoking & Alcoholism
- Recent use of lipid lowering drugs & corticosteroids
- Pregnant or lactating women.

Exclusion criteria for Controls

- Any other Acute /Chronic inflammatory disorder
- Smoking & Alcoholism
- Pregnant or lactating women.

Objectives:

- Estimate oxidative stress biomarkers; catalase, malondialdehyde, superoxide dismutase in premature coronary artery disease patients & controls.
- Compare the result of oxidative stress biomarkers in premature coronary artery disease patients & controls.

Sample collection: - Five ml blood was collected from the patients as well as controls after taking appropriate aseptic precaution. The sample was collected in EDTA & plain vacutainer for the estimation of various parameters.

Methods:

- Estimation of serum Malondialdehyde (MDA) by Satoh, 1978 [13]
- Estimation of SOD by Marklund and Marklund 1974[14]
- Estimation of plasma catalase by Aebi 1983 [15]

Statistical Analysis: - Data and various parameters will be analyzed on SPSS software (USA inc.) version 23. Mean and standard deviation of all parameters was calculated. Chi square test will be applied to non-parametric variables. Student t-test will used to compare averages in two groups

Results:

Table 1: Comparison of Oxidative stress markers in Control Group & Cases Group

Parameters	**Group-I (Healthy Control) Mean± SD**	**Group -II (Premature CAD) Mean± SD**	*P value*
Malondialdehyde (MDA) (nmole/ml)	2.08±0.35	4.10 ±0.47	(p<0.001) Highly significant
Superoxide dismutase (SOD) Units/mg	26.81±3.0	17.47 ±1.43	
Catalase (CAT) (IU/L)	176.99±9.31	115.39 ±5.07	

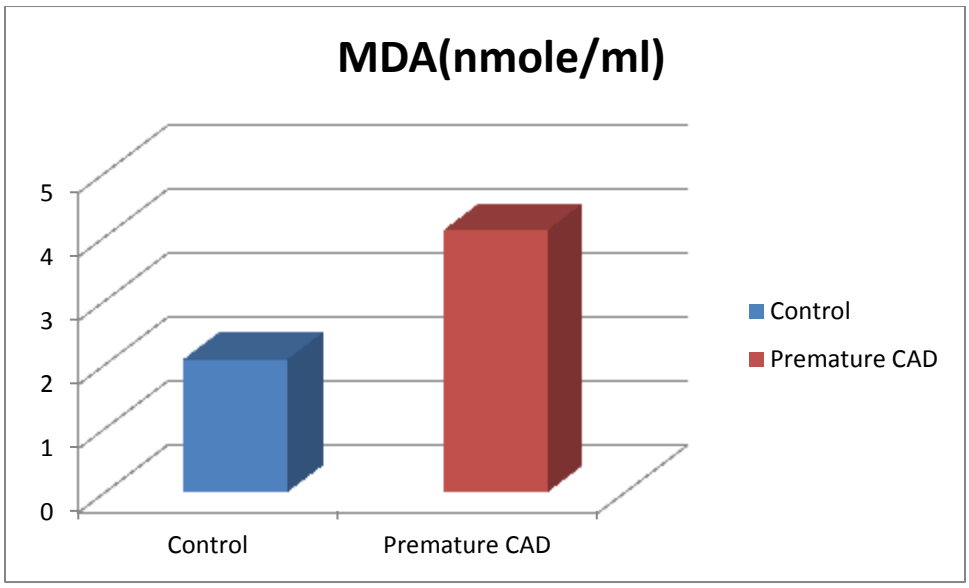

Fig 1: Shows Plasma MDA level in control & Case group

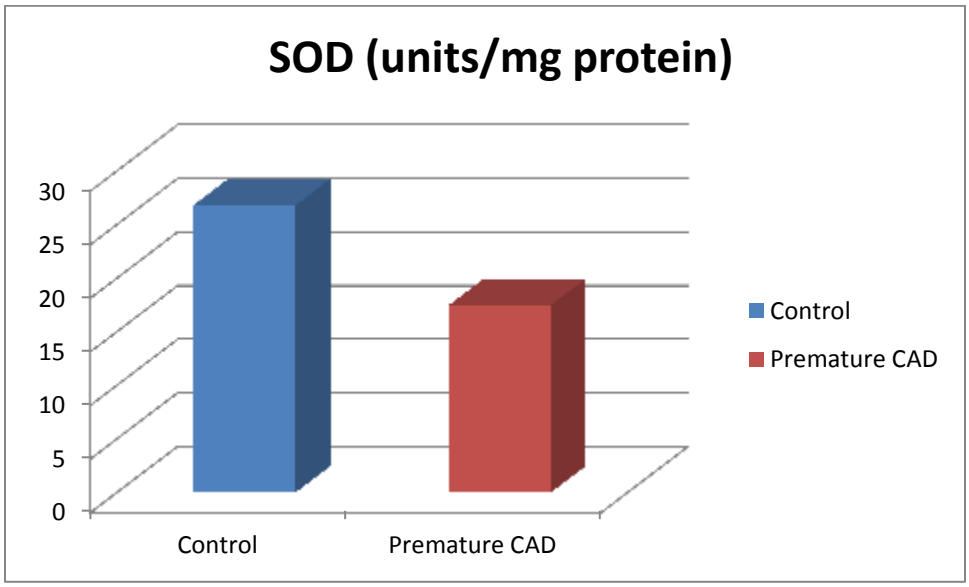

Fig 2: Shows SOD level in control & Case group

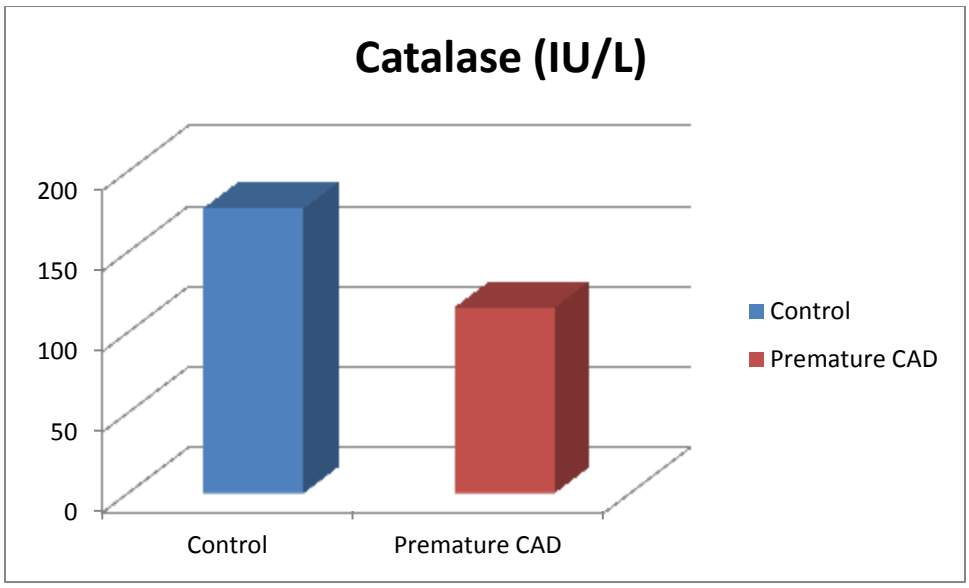

Fig 3: Shows catalase level in control & Case group

Discussion:

Coronary artery disease is leading cause of death among Indian population. It is associated with various risk factors. The role of oxidative stress plays an important role in the development of coronary artery disease is well known.[16] Reactive oxidant species can harmfully affect on all types of biomolecules including; lipids, proteins and DNA. Antioxidative defence mechanism consists of of enzymatic and non enzymatic defence to inactivate reactive species.[17, 18] MDA which is an end product of lipid peroxidation, is commonly used as as bio marker of oxidative stress. Free radical mediated peroxidation of unsaturated fatty acids and auto-oxidation is reflected by MDA .[19]

The level of plasma MDA in the cases was significantly higher than in the controls. The results shown in Table 1 of MDA in controls & CAD patients. The result was compared with CAD patients. The increased plasma MDA is an end product of lipid peroxidation.

Routhu Kathyaini et al 2011; [20] conducted a prospective study of 30 patients diagnosed as AMI & 30 healthy controls. Significantly increased mean values of plasma MDA levels in ($p < 0.001$) in the study group as compared to controls.

Bastani AB, Rajabi S, Daliran A, Saadat H, Busheri FK 2011; [21]conducted a study sample consists of 90 subjects who were divided into three equal groups: patients with acute coronary syndrome (ACS) patients with chronic CAD and healthy subjects as control. The results indicated a significant increase in MDA level and the percentage of MDA release ($P<0.05$) in the patient groups compared controls.

Our results for plasma MDA are supported by Routhu kathyaini *et al* & bastani AB suggest greater risk of atherosclerosis and cardiovascular disease. The antioxidant enzyme system is a protective mechanism against LPO. The result shown in table 1 of SOD & catalase in control & CAD Patients. The levels of plasma SOD & catalase were significantly decreased in CAD patients as compared with controls as shown in Table 1. **Sowmya K, Malar J, Nalini G in 2011**; [22] Conducted a study consisting of 60 subjects of which 30 were healthy individuals & 30 angiographically proven coronary artery disease (CAD) patients.

Plasma Malondialdehyde (MDA) was significantly elevated & the level of ascorbic Acid, catalase & SOD were significantly reduced in CAD patients as compared to healthy group.

Cheraghi M et al in 2018; [23] Conducted a study included 50 CHD patients and 50 healthy volunteers. Serum GSH level and CAT and GPX activities were significantly greater in healthy controls than in CHD patients. Serum MDA, NO, and FBS levels were significantly greater in CHD patients than in healthy controls. Our results for plasma SOD & catalase are supported by **Cheraghi et al & Sowmya K**. The decreased level of antioxidants is associated with a higher risk of atherosclerosis & developing the CAD.

Conclusion:

The present study concluded that the levels of MDA (oxidants) are increased in CAD patients due to lipid peroxidation while the levels of antioxidants (SOD & Catalase) are decreased when compared with those of normal healthy individuals. It has also been observed that both SOD and catalase are intracellular Enzymes to protect against reactive oxygen species in coronary artery disease.

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