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A study of association of oxidative stress and antioxidant status in cardio vascular diseases

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Abstract: *Oxidative stress is defined as an imbalance between free radicals and the ability of the body to counteract or detoxify their harmful effects through neutralization by antioxidants. Involvement of oxygen radical has been suggested in many clinical conditions including Cardio vascular disease (CVD).*
The present investigation identified the SOD, catalase activity and describes the lipid profile by identifying the lipid peroxidation. The result shows that Lipid peroxidation as measured by the level of MDA was increased, where as SOD and catalase activities were decreased in patients with Cardio vascular disease.
Key Words: *Trace elements, Lipid peroxidation, Cardio vascular disease, Superoxide dismutase, Catalase.* ***MDA-*** *Malondialdehyde.*

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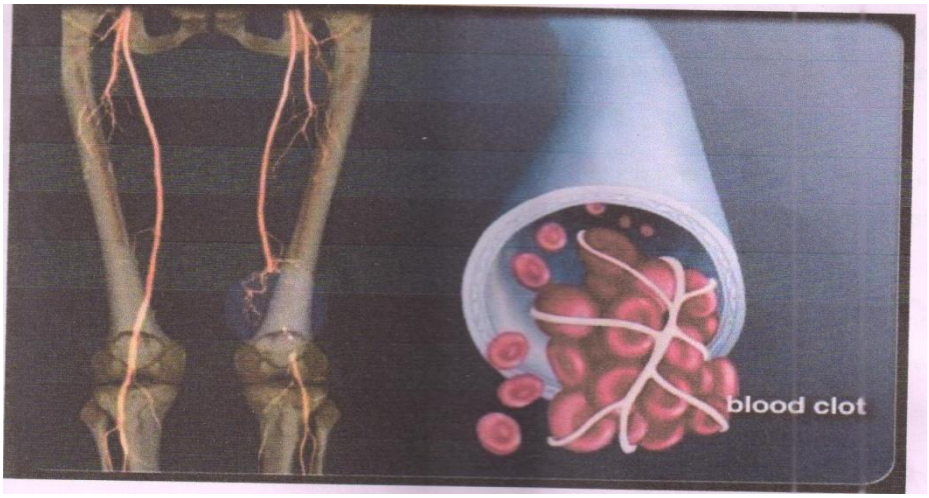
I. Introduction

Cardiovascular disease (CVDs) is a collective term for diseases of the heart & blood vessels. The term commonly includes disease such coronary heart disease, heart failure, cardio myopathy, congenital heart disease, peripheral vascular disease and stroke. Many of these conditions can be life threatening.

Epidemiology of CVDs

- CVDs are the number 1 cause of death globally: more people die annually from CVDs than from any other cause.
- An eatimated 17.5 million people died from CVDs in 2012, representing 31% of all global deaths. Of these deaths, an estimated 7.4 million were due to coronary heart disease and 6.7 million were due to stroke.
- Over three quarters of CVD deaths take place in low- and middle-income countries.
- Out of the 16 million deaths under the age of 70 due to noncommunicable diseases, 82% are in low and middle income countries and 37% are caused by CVDs.



(**Figure-1:** Schematic Representation of Cardio Vascular Disease (CVDs) showing blood clots)

Risk factors of CVDs

The risk factors of CVDs, such as High blood pressure, cholesterol, overweight/obesity, Tobacco use, lack of physical Activity and Diabetes. However, there are also some major CVD risk factors that cannot be

controlled. In terms of attributable deaths, the leading CVD risk factor is raised blood pressure to which 13% of global deaths is attributed, followed by tobacco use (9%), raised blood glucose (6%), Physical inactivity (6%) and overweight and obesity (5%).

Oxidative Stress

Oxidative stress is defined as an imbalance between free radicals and the ability of the body to counteract or detoxify their harmful effects through neutralization by antioxidants. It not only causes hazardous events such as lipid peroxidation and oxidative DNA damage, but also physiologic adaptation phenomena and regulation of intracellular signal transduction. From a clinical standpoint, if biomarkers that reflect the extent of oxidative stress were available, such markers would be useful for physicians to gain an insight into the pathological features of various diseases and assess the efficacy of drugs.

(**Figure-2:** Schematic Representation of Free Radicals, Active Oxygen Species, and Oxidative Stress)

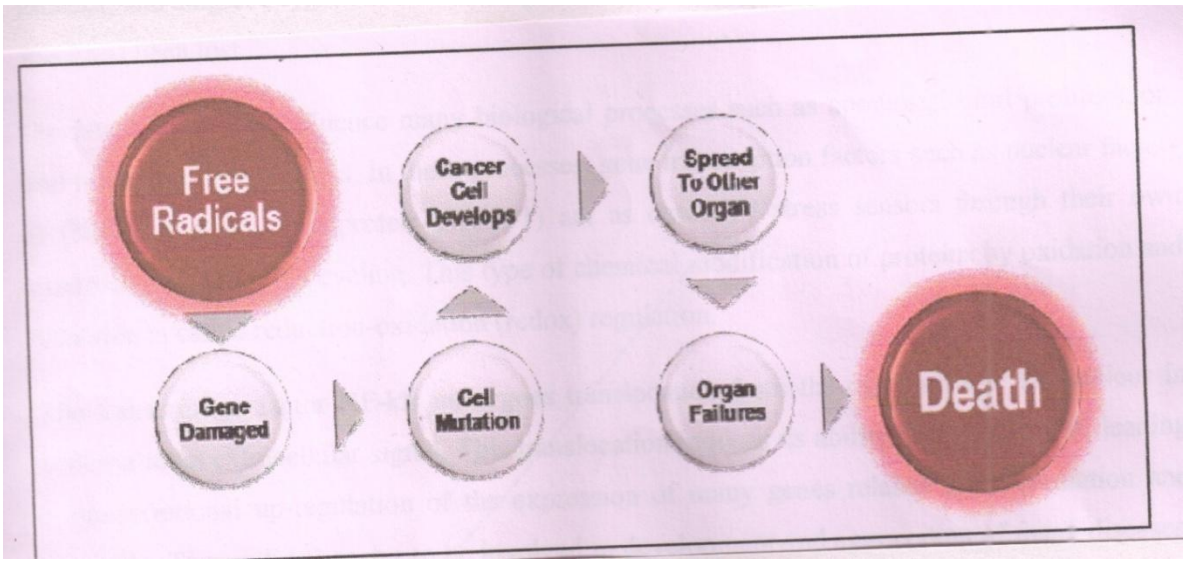


Figure-3: Schematic Representation of organ failure by the effect of free radicals

Biomarkers of Oxidative Stress

The biomarkers that can be used to assess oxidative stress have been attracting interest because the accurate assessment of such stress is necessary for investigation of various pathological conditions, as well as to evaluate the efficacy of drugs. Assessment of the extent of oxidative stress using biomarkers is interesting from clinical standpoint. The markers found in blood, urine and other biological fluids may provide information of diagnostic value. Because the body is not necessarily fully protected against oxidative damage, some of its constituents may be injured by free radicals (ROS), and the resultant oxidative products have usually been used as markers.

Many markers have been proposed, including Superoxide dismutase (SOD), catalase (CAT), Glutathione S-transferase (GST), lipid peroxides (LPO), malondialdehyde (MDA), and 4-hydroxynonenal as markers as indicators of oxidative damage to DNA; and various products of the oxidation of protein and amino acids including carbonyl protein, hydroxyleucine, hydrovaline and nitrotyrosine.

Aim

In the present study our aim is to identify the antioxidant enzyme activities such as superoxide dismutase (SOD) and Catalase (CAT) activities and also the lipid profile by determining the LPO levels in serum of Cardiovascular Disease (CVD) patients.

II. Materials and Methods

Estimation of Protein by Lowry's Method

AIM: Estimation of protein in the given sample by Lowry's method

Estimation of antioxidant activity

Superoxide dismutase (SOD):

It is an enzyme that alternately catalyzes the dismutation of the super oxide (O-2) radical into either ordinary molecular oxygen (o2) or hydrogen peroxide. For synthesizing SOD we require the following chemicals in an appropriate amount. For preparing 50ml of tris buffer we require 0.605 gm of tris base and 1mM of EDTA dissolving these above chemicals in distilled water and the pH is maintained at 8.5.

The next solution to be prepared is pyrogallol that is 0.025 gm of pyrogallol dissolved in 10 ml of distilled water. The above solutions are prepared to optimize an enzyme activity.

Catalase (CAT):

Catalase is a common enzyme found in nearly all living organism exposed to oxygen. It catalyses the decomposition of hydrogen peroxide to water and oxygen. It is a very important enzyme in protecting the cell from oxidative damage by reactive oxygen species (ROS) likewise catalase has one of the highest turnover number of all enzymes. One catalase molecule can convert approximately 5 million molecule of H2O2 to water & oxygen each minute.

For preparing phosphate buffer (50 ml of potassium phosphate buffer, Ph 7, at 25 c prepare 11.4 mg/ml solution in ultrapure water using potassium phosphate dibasic trihydrate. Adjusted the Ph to 7 at 25 c using 1 molar HCL. Hydrogen peroxide (H O) solution (0.036% w/w) prepare in phosphate buffer using hydrogen peroxide (30% w/w), determine the A240 of this solution using phosphate buffer as a blank. The A240 must be between 550 & 520 absorbance units. If necessary add hydrogen peroxide to increase the absorbance and add phosphate buffer to decrease the absorbance.

Lipid Peroxides (LOP):

We estimated LPO using 250 ul serum samples and next incubated for 2 hrs at 37 c. The sample was mixed with 0.25 ml of 10% w/v trichloro acetic acid to ppt protein. The mixture was centrifuged at 2000 rpm for 10 minutes and aliquote of 250 ul supernatant was reacted with 0.250ul of 0.1 M EDTSA and 0.5ml of 0.67% Thio Barbituric acid in boiling water bath for 10 minutes. After cooling it was diluted with 0.5ml of distilled water. The absorbance was read at 532 nm and 600 nm.

Study parameters	Category	Controls	CVD(n)
Age	**30 - 50**		**9**
	50 – 70		**9**
	70 – 90		**3**
Gender	**Male**		**10**
	Female		**11**
BMI	**<18.5(U/W)**		**1**
	18.5 – 24.9 (N)		**13**
	25 – 29.9 (O/W)		**1**
	30 – 39.9 (Obese)		**5**
	40 or above (S obese)		**1**

Smokers	**Smokers**		**5**
	Non-Smokers		**16**
Alcoholic	**Alcoholic**		**6**
	Non-Alcoholic		**15**
Food habit	**Veg**		**2**
	Non-Veg		**19**
Place of Residence	**Urban**		**12**
	Rural		**9**
Clinical parameters			
Potassium			**3.65**
Sodium			**134.14**
Diabetic	**Diabetic**		**6**
	Non-diabetic		**15**
LDL			
HDL			

Results and Discussion

Subjects

To evaluate the oxidative stress and antioxidant status such as SOD, CAT and Lipid Peroxide in patients with CVD. In this study, we take 20 patients with CVD was compated with 20 normal healthy volunteers. The demographic data showed that higher number of males in this study was 11 and no of females were 10 in CAD patients, while as no. of males in control was 12 and females 8 as shown in fig.1

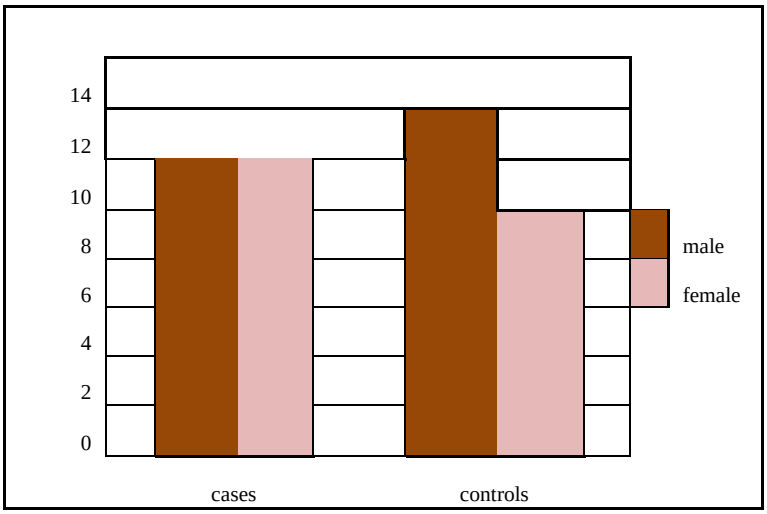


Figure-1: Gender ratio in CAD patients and controls

Age Distribution

Age group shows the number of patients in a definite age group. In this study we found that the number of patients was 20. In age group of 30 – 40, cases 3 and control 2, in between age group 41-60. Cases 4 and control 2, and in age 60+, cases 3 and control 4.

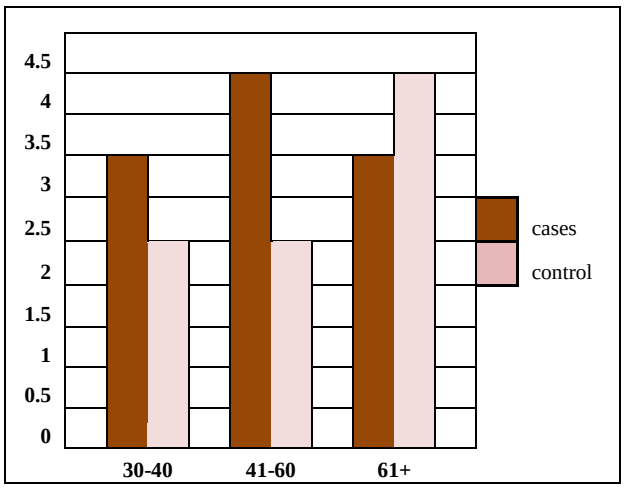


Figure-2: Age distribution of Female

Alcoholic and non alcoholic

In this study we found that among the 20 CAD patients and 20 controls, Alcoholic were 12 and non alcoholic were 8 while as in control 5 was alcoholic and 15 were non alcoholic.

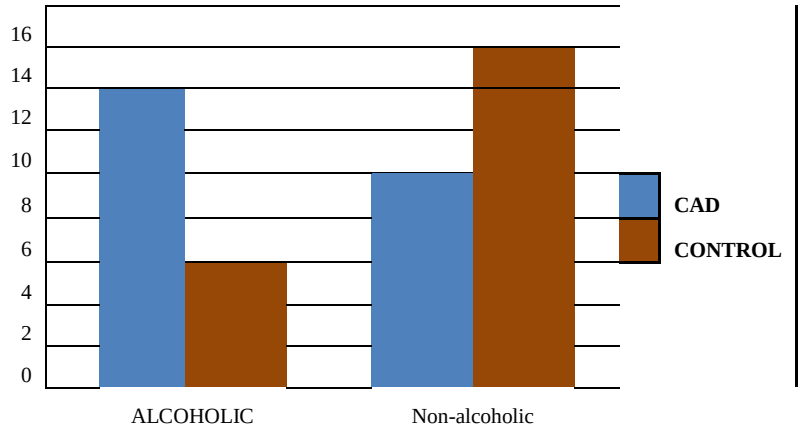


Figure-3: Distribution of Alcoholics and Non-Alcoholics in CAD patients and controls

Smokers and Non- Smokers

In this study we found that among the 20 CAD patients and 20 controls, Smokers were 10 and non smokers were 10 while as in control 8 was smokers and 12 were non smokers.

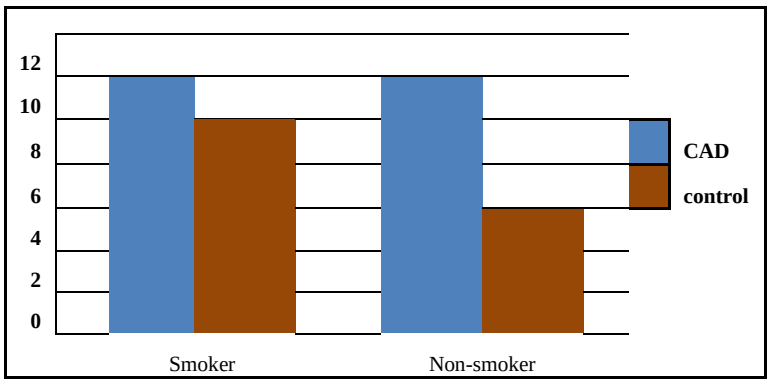


Figure-4: Smokers and non smokers

Antioxidant activity:

We evaluated the oxidative stress and antioxidant status such as SOD, CAT and Lipid peroxide in patients with CVD. The study was conducted on patients suffering from CVD. When the mean values of the serum enzymes were compared, a marked decrease in the levels of SOD, CAT was found (Figure-5 & Figure-6), whereas the level of LPO, was markedly increased (Figure-7) in Cardiovascular patients compared to healthy controls indicating increase oxidative stress.

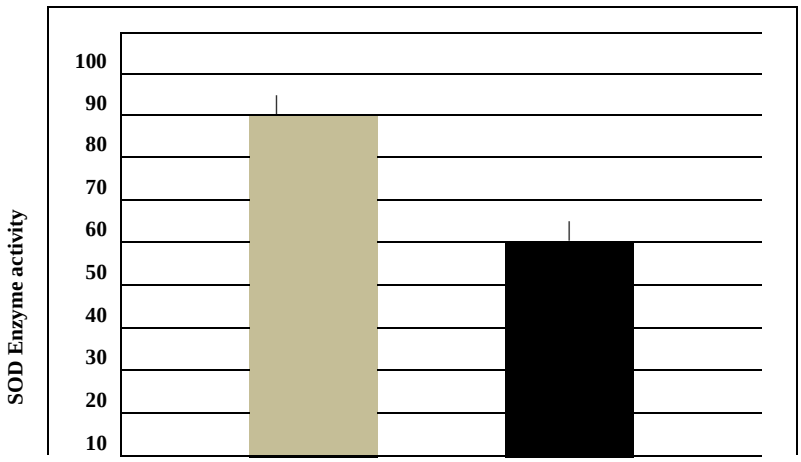


Figure-5: SOD Enzyme activities between Serum CVD patients and normal controls

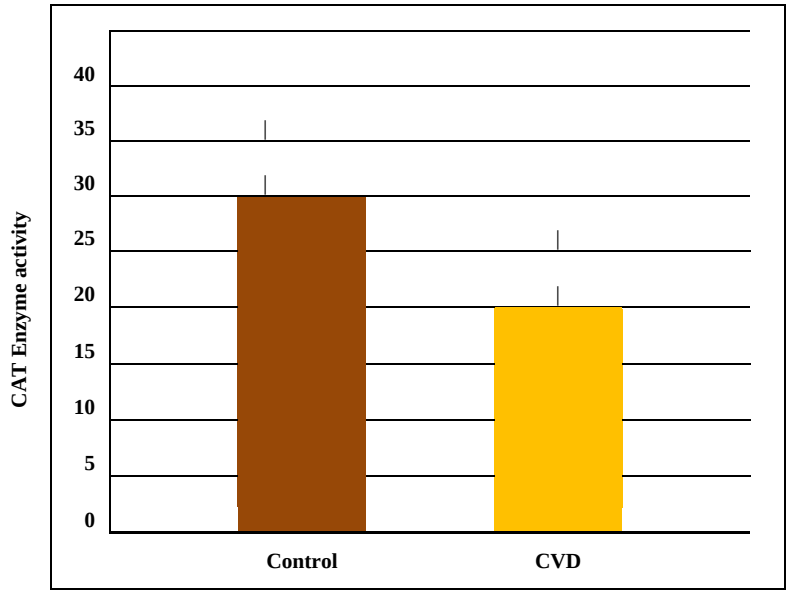


Figure-6: CAT Enzyme activities between Serum CVD patients and normal controls

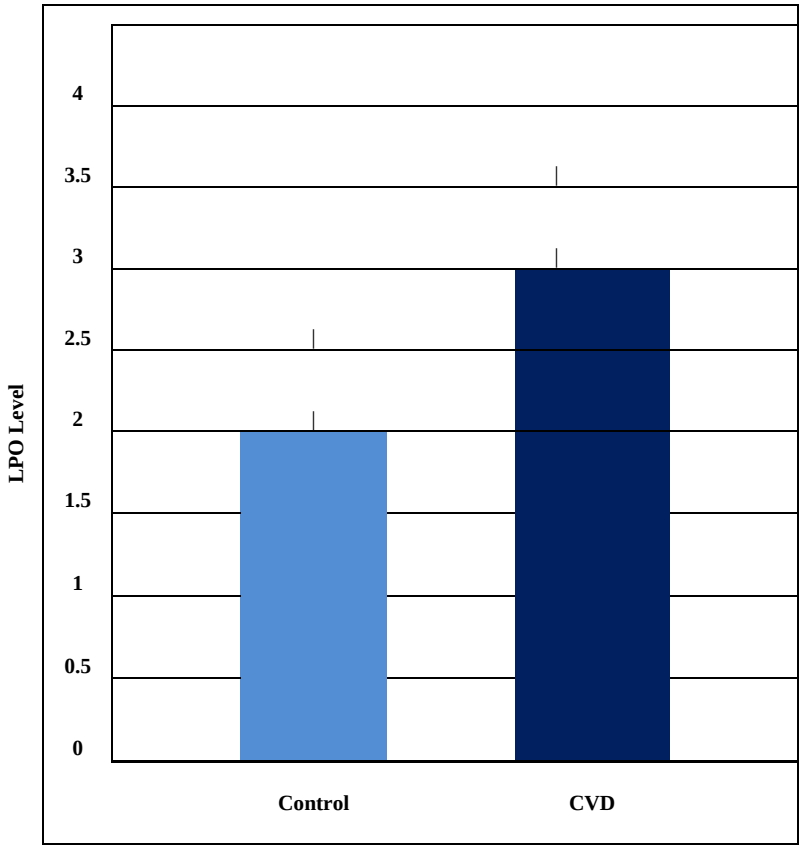


Figure-7: LPO level between Serum CVD patients and normal controls

III. Conclusion

We found significant decrease of SOD, CAT and LPO activities in Cardio vascular disease sample as compared to normal samples. Our results confirm that overproduction of reactive oxygen species especially in CVD cannot be properly balanced by the antioxidant enzymes. Therefore, decrease in the serum antioxidant

enzymes activities such as SOD, CAT and LPO in CVD may be related to the oxidative damage of membrane protein and lipid by increased oxygen free radicals in the body.

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