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Research Article

EVALUATION OF LIPID PEROXIDE AND ANTIOXIDANTS IN SMOKERS

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ABSTRACT
Oxidative stress has been implicated in smokers. The oxidative stress is in terms of serum lipid peroxidase level and antioxidant activity in terms of superoxide dismutase and glutathione peroxidase levels were compared in smokers and age matched healthy controls. The mean values of serum lipid peroxidase were increased and superoxide dismutase and glutathione peroxidase levels were decreased in smokers as compared to controls. The oxidative stress was increased in smokers.

Keywords: *Smokers, Glutathione Peroxidase, Lipid Peroxidase, Oxidative Stress, Superoxide Dismutase*

INTRODUCTION
Coronary artery disease (CAD) is the third leading cause of mortality all over the world. Atherosclerosis is the principle cause of myocardial and cerebral infarction (Mukul and Kanta, 2005). Smoking is one of the major risk factor for CAD and other risk factors for CAD are diabetes mellitus, obesity, hypertension and dyslipoproteinemias (Judith *et al.,* 1999). A cigarette smoker has two to three times the risk of having a heart attack than a non smoker. At least 80% of heart attacks in men under 45years of age thought to be due to cigarette smoking.

At this age heavy smokers have 10 to 15 times the rate of fatal heart attacks than non smokers. Smoking is a major risk factor for atherosclerosis and coronary heart disease (CHD), there being a dose response relationship between cardiovascular morbidity and mortality and the number of cigarettes smoked. Smoking is known to produce free oxygen radicals in our body. An excess of free oxygen radical production due to lack of antioxidants, may increase the risk of coronary artery disease. Superoxide dismutase (SOD) is metalloenzyme which catalyses superoxide radical and glutathione peroxidase (GPx) catalyses reduction of various hydroperoxides, phospholipid hydroperoxides and other free hydroperoxides (Sharma *et al.,* 2005).

Oxidative stress is defined as an imbalance between the production of reactive oxygen species (ROS) or free radicals and antioxidant defense, which may induce tissue injury. Oxidative stress can be assessed by measurement of reaction products of oxidative damage, like lipid peroxidation, DNA oxidation and protein oxidation (Halliwell, 1997). Lipid peroxidation is a reaction of oxidative deterioration of polyunsaturated fatty acids involving direct reaction of oxygen and lipid to form free radical intermediates and to produce semistable peroxides.

MDA (malondialdehyde) is considered as bio marker of lipid peroxidation. SOD is a naturally occurring enzyme that protects the body against active oxygen free radicals by scavenging excess superoxide. Lower undetectable levels of SOD allow oxygen radicals to form in anaerobic bacteria and to inactivate other bacterial enzyme systems. SOD has particular value as an antioxidant that can help to protect against cell destruction (Math *et al.,* 2004).

The GPx enzyme takes part in a system that converts intra cellular free radicals in to less reactive or neutral components (Maria *et al.,* 1999). Thus oxidative stress may play important role in pathogenesis of CAD risk factors. So the present study was planned to evaluate the levels of lipid peroxide & antioxidants Smokers.

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MATERIALS AND METHODS

Subjects

The present study was carried out in the Department of Biochemistry S.R.T.R. Medical College, Ambajogai. The cases were selected from those attended the Medicine OPD, at S.R.T.R. Medical College, and Hospital, Ambajogai. The investigations were carried out in Biochemistry Laboratory, S.R.T.R. Medical College, and Hospital, Ambajogai.

Design

The total number of subjects included in the study was 80 and divided into two groups. Group I consisted 40 normal healthy subjects as controls while group II consisted 40 patients with smokers as cases. The study included subjects of age in between 30-60 years for both groups.

Criteria for Selection

Smokers = Smoking more than 15 cigarettes or bidis per day for 5 years or more.

Methodology

Under all aseptic precautions 10 ml of morning blood sample was collected from anticubital vein of controls & cases after an overnight fasting. Five ml blood was collected in the bulb with heparin and 5ml were collected in plane bulb. Blood samples were centrifuged at 3000 rpm for 10 minutes. Heparinized whole blood was collected for estimation of enzyme erythrocyte SOD, erythrocyte GPx while serum was used for estimation of MDA.

1. Serum Lipid Peroxide (MDA) was estimated by Satoh method (Satoh, 1978).
2. Superoxide Dismutase activity was measured by using RANSOD method by Randox Laboratories Ltd.
3. Glutathione peroxidase activity was measured by using RANSEL method by Randox Laboratories Ltd.

Statistical Analysis

The biochemical parameters were expressed as mean $\pm$ SD. Statistical significance was evaluated by student's t - test. P value of less than 0.001 and 0.05 was considered to indicate statistical significance.

RESULTS AND DISCUSSION

Results

Our objective was to evaluate the levels of serum Lipid peroxide, Erythrocyte Superoxide Dismutase and Erythrocyte Glutathione peroxidase in Hypertension. Hypertensive cases (5.47 $\pm$ 0.76 nmoles/ml) showed statistically significant ($P < 0.001$) increase in mean serum lipid peroxide level (MDA) as compared to controls (3.28$\pm$0.54 nmoles/ml) (Table no. 1). We also found statistically significant ($P < 0.001$) decrease in mean erythrocyte SOD level in cases (714.25$\pm$ 72.42 U / gm Hb) as compared to controls (952.58$\pm$ 92.25 U/gm Hb) (Table no. 2) as well as statistically significant ($P < 0.001$) decrease in mean erythrocyte GPx level in cases (25.78$\pm$ 4.36 U / gm Hb) as compared to controls (30.75$\pm$ 4.12 U / gm Hb) (Table 3).

Discussion

In the present study the levels of lipid peroxide significantly increased in smokers as compared to controls. There has been considerable interest in the concept that the uncontrolled lipid peroxidation is a key contributing factor in the pathophysiology of CAD especially in smoking. Lipid peroxidation eventually is a sequence of the injury caused by reactive oxygen species. Free radical damages can accumulate over time and may thereby contribute to cell injury and development of human diseases. Free radical has been implicated in the development of several diseases including atherosclerosis, diabetes, hypertension, obesity (Delanty and Dichetr, 1998; Sastre *et al.*, 2000). The increased production and / or activity of oxygen-derived free radicals contribute to endothelial dysfunction in chronic smokers. Cigarette smoke contains abundant amount of oxidants and superoxide (O^{-2}) anion from cigarette smoke may rich the vascular endothelium and can then react with nitric oxide (NO^{-}) to form peroxynitrite anion, a highly reactive intermediate with strong cytotoxic potency. Thus the damaging free radicals in cigarette smoke may cause either direct arterial wall injury or protein peroxidation and activation of phagocyte platelet endothelial cell interaction (Kharb *et al.*, 2001). Our study confirms this view i.e. lipid peroxidation is increased in smokers with a parallel reduction in antioxidant enzyme SOD and GPx.

Hulea *et al.,* (1995) demonstrated that prooxidant / antioxidant imbalance exists in the blood of smokers (Hulea *et al.,* 1995).

Ozbay and Dulger (2002) reported that significant increase in lipid peroxidation activity and significant decrease in antioxidant enzyme activity in smokers (Ozbay and Dulger, 2002). Our study result matches with the results reported by Hulea *et al.,* (1995), Ozbay and Dulger (2002).

We observed that the level of erythrocyte SOD was significantly lower in smokers as compared to controls.

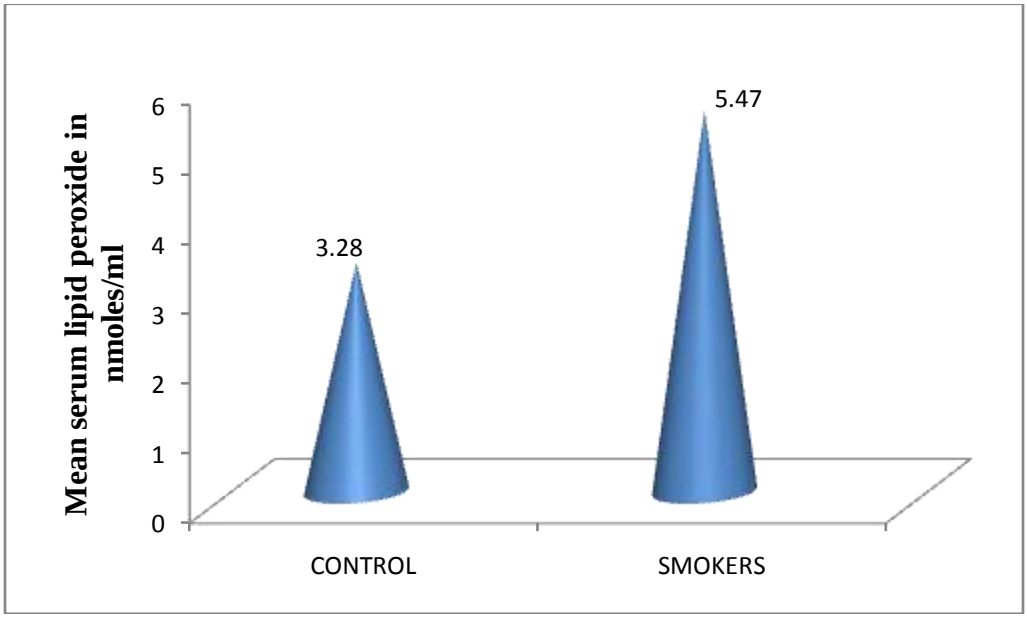
Mechanism for the smoking induced low plasma SOD level is unknown. Inhaled NO or O_2^- produced by cigarette smoking decrease circulating SOD or alternatively other components of smoking may down regulate SOD production.

Increased lipid peroxidation and antioxidant depletion in smokers may be significant contributors to biomolecular endothelial vascular damage. Although antioxidant supplementation might be helpful in preventing or attenuating some cigarette smoke related adverse effects (Reilly *et al.,* 1996). Granger *et al.,* (1981) demonstrated complete protection against ischemic tissue injury following intravenous administration of SOD.

It may therefore be concluded that decrease in level of SOD in smokers indicate that either the scavenging system has been consumed during smoking or is suppressed (Granger *et al.,* 1981). Our study result matches with studies done by Zhou *et al.,* (2000) and Alam *et al.,* (1996).

Table 1: Showing comparison of serum Lipid peroxide levels in control and smokers

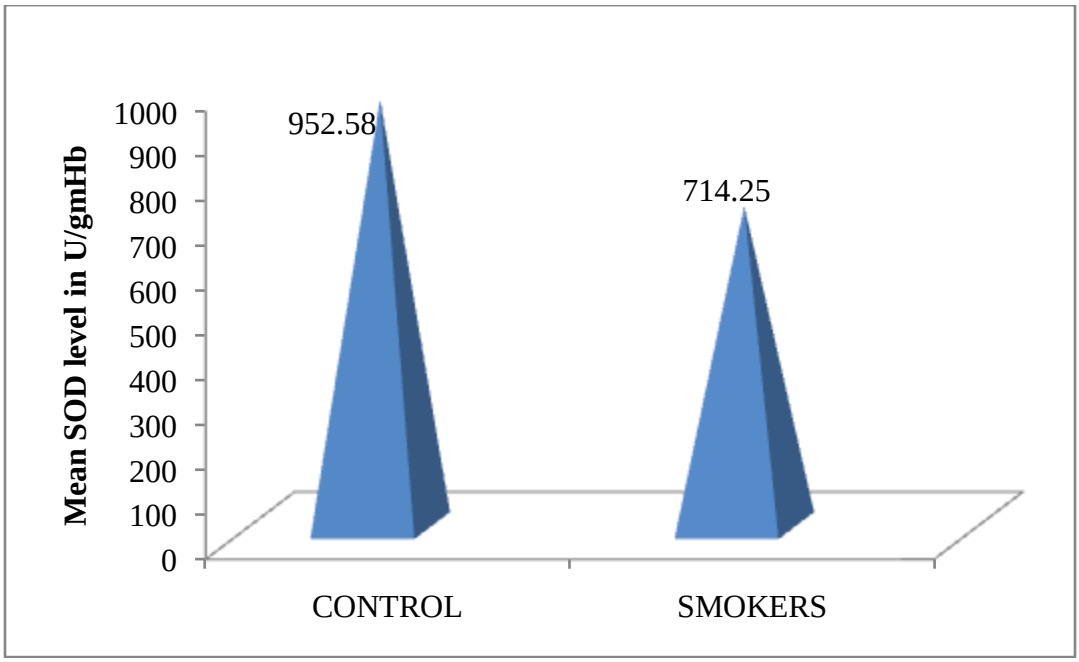
	CONTROL	SMOKERS
No of cases	40	40
Serum Lipid peroxide (n moles /ml) Mean± SD	3.28± 0.54	5.47± 0.76
P value	P<0.001	
Significance	Statistically significant	



Graph 1: Comparison of mean serum lipid peroxide in controls & Smokers

Table 2: Showing comparison of Erythrocyte Superoxide Dismutase levels in control and smokers

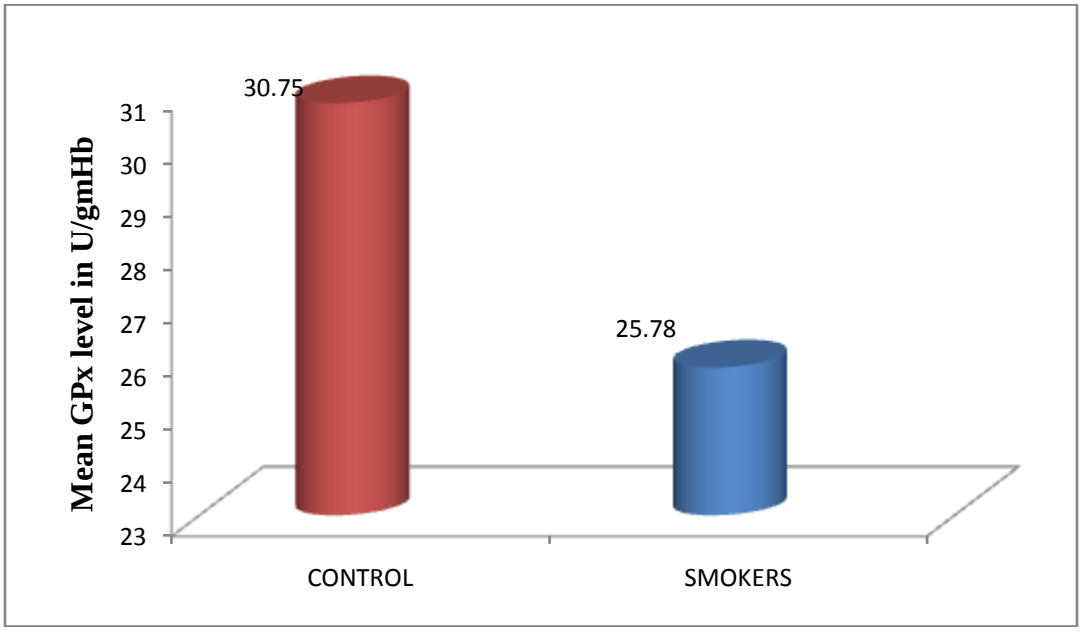
	CONTROL	SMOKERS
No of cases	40	40
Superoxide Dismutase (U / gm Hb.) Mean± SD	952.58± 92.25	714.25±72.42
P value	P<0.001	
Significance	Statistically significant	



Graph 2: Comparison of mean erythrocyte SOD level in controls & smokers

Table 3: Showing comparison of Glutathione Peroxidase levels in control and smokers

	CONTROL	SMOKERS
No of cases	40	40
Glutathione Peroxidase (U / gm Hb.) Mean± SD	30.75± 4.12	25.78± 4.36
P value	P<0.001	
Significance	Statistically significant	



Graph 3: Comparison of mean GPx level in controls & smokers

We also observed the significantly decreased level of GPx in smokers than controls. Glutathione peroxidase appears to have a major role in the prevention of oxidative stress; it may also be an important antiatherogenic enzyme. Several studies demonstrated that erythrocyte GPx activity is inversely associated with future fatal and non fatal cardiovascular events. This enzyme is responsible for the removal of hydrogen peroxide and other organic hydroperoxides formed during cellular oxidative metabolism. Zhou *et al.,* (2000) reported gravely aggravated series of free radical chain reactions in smokers leading to serious disruption of dynamic balance between oxidation and antioxidation with resultant exacerbation of oxidative stress which in turn is closely related to occurrence of coronary artery disease in smokers (Zhou *et al.,* 2000). The association between low levels of GPx activity and high cardiovascular risk was also observed in studies done by Nia *et al.,* (1984) and Kocyigit *et al.,* (2001). Therefore measurement of glutathione peroxidase should identify smokers who are at highest risk for cardiovascular events.

Conclusion

To conclude, there is an imbalance between oxidants and antioxidants in smokers which is the basic cause for CAD. Present study showed significantly elevated levels of lipid peroxide (MDA) in cases who are at risk for CAD. This increase in lipid peroxide may be due to the increased activity of the free radical formation. A deficiency of the antioxidant activity of superoxide dismutase and glutathione peroxidase has been related to higher concentration of lipid peroxide. There may be imbalance between production and scavenging free radical produced due to lack of antioxidant system. The estimation of lipid peroxide along with superoxide dismutase & glutathione peroxidase in smokers is very useful as it may serve as a useful monitor to judge the prognosis of the patients. The detection of risk factors in the early stage of the disease will help improve morbidity rate. Thus we propose that along with clinical examination, oxidative stress will be supportive additional biochemical markers for early diagnosis and therapeutic intervention. Therapy of supplementation of antioxidants may prevent the risk of CAD in smokers.

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