

Research Article

Antioxidant status and level of oxidants in patients of coronary heart disease

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Abstract

Objectives: The aim of our study was to investigate the predictive value of an oxidative stress and free radical scavenger enzyme superoxide dismutase (SOD) in patients having coronary heart disease (CHD).

Material & Methods: The study population contained 200 subjects divided into two groups, 150 patients with CHD & 50, age & sex matched healthy control subjects. All biochemical parameters MDA, SOD & Lipid profile were analyzed by spectrophotometer and autoanalyser. P value <0.05 was taken as statistically significant.

Results: In our study we have found that lipid profile level (Mean \pm SD) of CHD patients & controls i.e. total cholesterol (TC) level 241.24 ± 45.83 in CHD patients & in control subjects were 169.6 ± 22.24 , triglycerides (TG) levels 191.15 ± 52.54 in CHD patients & in control subjects were 113.32 ± 22.82 , low density lipoprotein cholesterol (LDL-C) 167.69 ± 39.5 in CHD patients & in control were 97.03 ± 21.51 , very low density lipoprotein cholesterol (VLDL) levels 38.43 ± 10.51 in CHD patients & 22.67 ± 4.56 in controls. We have also found that the level of MDA in CHD patients & in control (Mean \pm SD) were 5.68 ± 1.20 & 3.73 ± 1.17 & the level of SOD in CHD patients & in control (Mean \pm SD) were 2.50 ± 0.18 & 3.31 ± 0.49 .

Conclusion: Our study indicates an imbalance between lipid peroxidation, status of antioxidants & free radical scavenger enzyme in CHD. Therefore these biomarkers may be useful in diagnosis of coronary heart disease.

Keywords: Oxidative stress, Coronary heart disease, Superoxide dismutase, Malondialdehyde, Lipid peroxidation

1. Introduction

Coronary heart disease is one of the major causes of mortality and morbidity in the world. The most common cause of coronary heart disease is atherosclerosis with erosion or rupture of a plaque causing transient, partial or complete arterial occlusion. Heart cannot continue to function without adequate blood flow, and if it is severely compromised, death is inevitable. Several risk factors for coronary heart disease have been well documented, including hypertension, hyperlipidemia, diabetes, a positive family history of CHD, smoking, obesity and physical inactivity.¹ However; these factors explain only part of attributable cardiovascular disease. Evidence suggests that reactive oxygen species (ROS) may play important role in the pathogenesis of coronary heart disease.² ROS are capable of reacting with unsaturated lipids and initiating the self-perpetuating chain reactions of lipid peroxidation in the membranes.³ Free radicals can also cause oxidation of sulphhydryl groups in proteins and strand scission in nucleic acids is also possible.⁴ Myocardial antioxidants inhibit or delay the oxidative damage to sub cellular proteins, carbohydrates, lipids and DNA. There is evidence that antioxidants can protect against free radical defense, which is responsible for reperfusion-induced damage and lipid

peroxidation, and may thereby inhibit thrombosis, myocardial damage and arrhythmias during coronary heart disease. Antioxidant status is a critical tool for assessing redox status.⁵

Free radicals are highly reactive molecules generated by biochemical redox reactions that occur as a part of normal cell metabolism. The human body has an inherent synergistic defense mechanism, which comprises cellular protection against ROS.⁶ Free radical scavenger enzyme namely SOD, that have the ability to inhibit oxidative stress by scavenging the highly destructive free radicals.⁷ The deleterious effects of the free radicals are kept under check by a delicate balance between the rate of their production and the rate of their elimination by these defense systems. When there is an excessive addition of free radicals from exogenous sources added to the endogenous production, the available tissue defense system becomes overwhelmed resulting in oxidative damage to the tissues.⁸

In the present communication, we assessed the extent of oxidative stress and the activities of sod in patients with coronary heart disease with age and sex matched healthy subjects.

2. Material and Methods

The population for study consisted of 200 subjects divided into two groups, 150 subjects (mean age 31 to 70 years) had coronary heart disease and the other 50 subjects age and sex matched healthy control. Study subjects were taken from the outdoor and indoor department of Medicine of our institute. Healthy control subjects were selected from the institution. The diagnosis of coronary heart disease was confirmed by clinical presentation and other investigations like characteristic electrocardiogram (ECG) changes, positive treadmill test and positive Echocardiographic findings. The patients who had total cholesterol level of >250 mg/dL or triglycerides concentration >200 mg/dL, or receiving lipid lowering drugs were defined as having hyperlipidemia.

2.1 Exclusion Criteria

Confounding factors which could interfere in the biochemical analyses of study subjects and alter the results were smoking, diabetes, active inflammatory diseases, nutritional deficiencies, estrogen therapy, and collagen disease arthritis. Patients with these diseases were excluded from the study. The same exclusion criteria utilized for cases were applied for control selection. No subject (patients or controls) was taking antioxidant or vitamin supplements, or other drugs known as affecting serum lipid peroxidation and antioxidant values. All the above exclusion factors were confirmed from the patient's personal physician report and history.

2.2 Collection and analysis of sample

Blood samples were drawn from patients and controls. Five ml blood was collected in plain vials (without any anticoagulant) for estimation of serum lipids (Total cholesterol, triglycerides, and high density lipoprotein cholesterol), superoxide dismutase and malondialdehyde.

2.3 Biochemical analysis

2.3.1 Assay of lipid peroxidation products malondialdehyde (MDA)

As a measure of lipid peroxidation malondialdehyde formation (MDA) was estimated using the level of thiobarbituric acid reactive substances (TBARS) according to the method of Satoh, (1978)⁹. MDA read as nmol/ml.

2.3.2 Assay of superoxide dismutase activity (SOD)

SOD was assayed utilizing the technique of Nandi, *et al* (1988)¹⁰. This method utilizes the inhibition of auto-oxidation of pyrogallol by superoxide dismutase enzyme. SOD read as unit/ml of serum sample.

2.3.3 Estimation of lipid parameter

Lipid profile was estimated by commercially available kits. Serum total cholesterol (TC) estimated by enzymatic Cholesterol Oxidase Peroxidase (CHOD-POD), end point method Allain *et al* (1974)¹¹ at 510 nm. Serum triglyceride (TG) estimated by enzymatic Glycerol Phosphate Oxidase Peroxidase GPO/POD, endpoint method Bucolo *et al* (1973)¹² at 510 nm. Serum high density lipoprotein cholesterol (HDL-C) estimated by Phosphotungstic Acid, End Point method Assmann *et al*, (1983)¹³ at 510 nm. Serum very low density cholesterol (VLDL-C) and low density lipoprotein cholesterol (LDL-C) were calculated from the Friedwald's formula (Friedwald *et al*, 1972)¹⁴. All parameter read on spectrophotometer.

2.4 Statistical Methods

Statistical analysis was done, using the statistical package for social science (SPSS 16) for Windows Software, Microsoft Excel 2007 and scientific calculator. The results were expressed as mean± standard deviation (S.D). The statistical significance was evaluated by t- test. P value <0.05 was taken as statistically significant.

3. Results

The sex distribution, mean age and lipid parameters are shown in table 1. The mean age was 44.02 ± 12.8 years in control subjects and 53.73 ± 9.27 years in CHD subjects. Significant differences were observed between CHD and control group as shown (Table 1).

Table -1: Mean Age and levels of lipid parameter in coronary heart disease (CHD) patients and control subjects

Parameters	CHD Subjects (n = 150)	Control Subjects (n = 50)	P
Sex (M/F)	94/56	30/20	-
Age (years)	53.73 ± 9.27	44.02 ± 12.8	-
Total cholesterol (mg/dl)	241.24 ± 45.83	169.6 ± 22.24	<.001
Triglycerides (mg/dl)	191.15 ± 52.54	113.32 ± 22.82	<.001
HDL-Cholesterol (mg/dl)	40.99 ± 8.30	51.86 ± 7.08	<.001
LDL-Cholesterol (mg/dl)	167.69 ± 39.5	97.03 ± 21.51	<.001
VLDL-Cholesterol (mg/dl)	38.43 ± 10.51	22.67 ± 4.56	<.001

Mean value of MDA and SOD are shown in (Table 2). Mean value of MDA in CHD subjects was 5.68 ± 1.20 nmol/ml and 3.73 ± 1.17 nmol/ml in control subjects. MDA levels in CHD subjects was significantly higher (<.001) as compared to control subjects. The mean value of SOD in CHD subjects was 2.50 ± 0.18 U/ml and 3.31 ± 0.49 in control subjects. SOD levels in CHD subjects was significantly lower (<.001) as compared to control subjects.

Table -2: Mean levels of SOD and MDA in CHD subjects and Control subjects

Parameters	CHD Subjects (n = 150)	Control Subjects (n = 50)	P
MDA (nmol/ml)	5.68 ± 1.20	3.73 ± 1.17	<.001
SOD (units/ml)	2.50 ± 0.18	3.31 ± 0.49	<.001

4. Discussion

There is growing evidence that increased free radical production and impaired antioxidant protection is relevant to plaque activation. In addition to traditional risk factors, oxidative stress has been regarded as one of the most important contributors to the progression of atherosclerosis. Increased lipid peroxidation is thought to be a consequence of oxidative stress, which occurs when the dynamic balance between prooxidant and antioxidant mechanism is impaired. In ischemia, the ATP is drastically reduced and is converted to hypoxanthine and then to uric acid by xanthine oxidase upon reperfusion. During this process, enormous amounts of superoxide radicals formed which can simulate Haber-Weiss reaction for further generation of ROS, initiating lipid peroxidation.¹⁵ We observed that increased concentrations of MDA in CHD patients indicating increased lipid peroxidation. Our results are in accordance with previous reports.¹⁶

Changes in the concentration of plasma lipids including cholesterol are complications frequently observed in CHD patients and certainly contribute to the development of vascular disease. Cholesterol has been singled out as the primary factor in the development of atherosclerosis. HDL is regarded as one of the most important protective factors against arteriosclerosis. HDL's protective function has been attributed to its active participation in the reverse transport of cholesterol. Numerous cohort studies and clinical trials have confirmed the association between a low HDL and an increased risk of coronary heart disease. The concentration of LDL correlates positively whereas HDL correlates inversely to the development of coronary heart disease. We observed that increased concentration of total cholesterol, triglycerides, LDL cholesterol and VLDL cholesterol but decreased concentration of HDL cholesterol in CHD patients when compared to control subjects. Our results are in accordance with previous reports.¹⁷

Antioxidants constitute the foremost defense system that limit the toxicity associated with free radicals. It is known that plasma antioxidant capacity decreases and oxidative/ant oxidative balance shifts to the oxidative side in CHD patients. A reason for increased lipid peroxidation in plasma of CHD patients may be a poor enzymatic and non-enzymatic antioxidant defense system. SOD is the preventive antioxidant plays a very important role in protection against lipid peroxidation. Free radical scavenging enzyme such as SOD is the first line of cellular defense against oxidative injury, decomposing O_2^- and H_2O_2 before interacting to form the more reactive hydroxyl radical (OH). It was found that mean levels of SOD were lower in CHD patients when compared control subjects. Our results are in accordance with previous

reports.^{18,19} Bhat *et al*, reported in their study a significant increase in total lipid peroxidation in patients with coronary artery disease they also found a significant increase in total oxidant status and oxidative stress index and significant decrease in total antioxidant status.²⁰

5. Conclusion

Our study shows a significant increase in lipid peroxidation and lipid profile in the circulation of patients with CHD. A significant decreased in free radical scavenger enzyme SOD was observed CHD patients. Thus, our study indicates an imbalance between oxidant and antioxidant molecules in CHD. Therefore these biomarkers may be useful diagnosis of CHD patients.

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