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ASSESSMENT OF OXIDATIVE STRESS, ANTIOXIDANT ENZYMES AND LIPID PROFILE IN THE SUBJECTS OF CORONARY ARTERY DISEASE (CAD)

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ABSTRACT: Coronary artery disease (CAD) is the major cause of mortality and morbidity worldwide. The incident of CAD is rising and they are predicted to be the biggest causes of death by 2020 in India. Therefore, the aim of the present study was to assess the association of oxidative stress, antioxidant enzyme and lipid status parameters in CAD patients and to compare the results age- sex matched healthy control in our community. Three hundred participants were enrolled for the present study, with their ages ranging from 40 to 60 years from Era's Lucknow Medical College & Hospital, Lucknow. Out of which one hundred fifty were clinically new diagnosed case of coronary artery disease like angina pectoris and Myocardial infarction (MI), remaining one hundred fifty were healthy controls. These participants were investigated for serum lipid profile i.e. total cholesterol (TC), triglyceride (TG), low density lipoprotein (LDL), very low density lipoprotein (VLDL). High density lipoprotein (HDL) along with blood levels of TBARS and some antioxidant enzyme namely Catalase (CAT), Superoxide dismutase (SOD), Glutathione peroxidase (GPx), Glutathione reductase (GSSH-reductase) level. There was significant rise in serum levels of TC, TG, LDL-cholesterol and significant decrease in HDL- Cholesterol in CAD patients as compared to healthy controls. There was a significant increase in serum MDA, and significant decrease in CAT, SOD, GPx, GSSH-Reductase in CAD patients as compared to healthy controls. These two independent risk factors can results in oxidative modifications of LDL that could lead to atherosclerotic lesions which is underlying cause of CAD. The CAD patients are consistently associated with disorder of lipid metabolism and imbalance in oxidative- anti- oxidative status in them.

INTRODUCTION: Coronary artery diseases (CAD) are the most alarming of the health prediction for the new millennium worldwide. According to world health report of 2002, CVD will be the largest death causing disease in India. In India by 2020AD, 2.6 million Indian are predicted to die due to CAD, which constitutes 54.1% of all CVD death[1].

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Nearly half of these deaths are likely to occur in young and middle aged individuals (30-69 years). There are number of risk factors for CAD like age, sex, hypertension, positive family history, dyslipidaemia, diabetes, overweight or obesity, physical inactivity, tobacco use, alcohol and stress. It is an area where major health gain can be made through the implementation of primary care interventions and basic public health measures by targeting the diet, lifestyle and environment. CAD, the most common form of heart disease is characterized by atherosclerosis and the development of fibro-fatty plaques, which is followed by the formation of occlusive thrombii and the precipitation of acute events that interrupts

the blood flow [2]. This condition leads to an imbalance between oxygen supply and demand, if this imbalance is exceeds, it results in myocardial infarction (MI) [3]. Growing evidence supports the involvement of oxidative stress due to the disturbance in the balance between the production of reactive oxygen spices (ROS) and antioxidant defense play an important role in the pathogenesis of coronary atherosclerosis and its complications.

Increase oxidative stress and the generation of free radicals may affect four fundamental mechanisms that contribute to atherogenesis namely, oxidation of low density lipoprotein (LDL), endothelial dysfunction, vascular smooth muscle cells growth, and monocytes migration[4]. Antioxidants may protect against the development of atherosclerosis and its clinical manifestations such as myocardial infarction (MI) and a verify of cardiovascular disease (CVD). Hence blood SOD, CAT, GPx, GSSH-Reductase, the anti- oxidative enzymes in our body may be protective against atherosclerosis. Therefore, present study was design to assess the level of altered lipid profile and to correlate with deficiencies of antioxidant factors (i.e. SOD, CAT, GPx, GSSH-Reductase) in CAD patients and healthy subjects following the measurement of oxidative status as the levels of blood lipid peroxide in them.

MATERIALS AND METHODS:

The present study was carried out in the Department of Biochemistry in collaboration with department of Medicine, Era's Lucknow Medical college and Hospital (ELMC & H), Lucknow (U.P). Three hundred, participants were enrolled for the present study, with their ages ranging from 40 to 60, out of these, 150 were clinically newly diagnosed case of CAD like angina pectoris, hypertension and myocardial infarction along with remaining control group consist of 150 healthy individual with no known history of any disease.

Some patients were also selected from those admitted in the department of Medicine of the hospital. Written Informed consent form was taken from all the individuals subjects inducted into the study. The study proposal was approved by the Institutional Ethics Committed E L M C & H Lucknow. All the patients were examined clinically and information pertaining to age, gender, habits and health status was recorded in a special case performa. Clinical examination was followed by series laboratory investigations to carry out biochemical studies.

Inclusion Criteria

The patients were diagnosed as having CAD by clinical cardiologists on the basis of clinical symptoms, a positive stress test with chest pain and echocardiography results. ECG and Trade mill test.

Exclusion Criteria

Patients with Rheumatoid Arthritis, Renal disease, DM, and who have been taken up steroidal hormone (oral contraceptive drug and other medication contradictive to CVD), Pregnant women, Hepatic failure and Sepsis, the Patients not giving consent were excluded from the study.

Sample Collections

After obtaining the consent, 12 hours fasting blood samples were withdrawn from healthy groups and CAD patients by venipuncture in plane vials, allowed to clot and then carefully centrifuges at 1500 X g for 15 minutes at room temperature. Clear serum was separated out. The sediment containing RBC and serum samples were kept at -20^{o}C till analysis. The samples which were obtained were used for the analysis of the lipid profile, lipid peroxide and antioxidant enzymes.

- Estimation of serum malondialdehyde (MDA)[5]
- Estimation of serum Catalase (CAT)[6]
- Estimation of serum Superoxide dismutase (SOD)[7]
- Estimation of serum Glutathione peroxidase(GPx)[8]
- Glutathione reductase (GSSH-reductase)[9]
- Reduced Glutathione(GSH)[10]

Serum levels of Total cholesterol (TC), triglyceride (TG) and HDL-Cholesterol were determined on a semi-automated clinical chemistry analyzer (Erba Chem-7) using commercially available kits (erba). Serum LDL-Cholesterol and VLDL levels were deduced according to Friedewald's Formula[11].

LDL-C = TC- TG/ 5 - HDL-cholesterol

VLDL-C = TG/ 5

The values were expressed as mg/dl serum.

Statistical Analysis

The data from the controls and patients were compared by using the Student's t- test. The values were expressed as mean ± standard deviation (S.D). Microsoft Excel for Windows 2007 was used for statistical analysis. P-values <0.01 (<0.05) were consider to indicate statistical significance.

RESULTS AND DISCUSSION:

The finding of the current study based on CAD patients, are summarized in **Table 1**. When lipid profile variables were compared, the results were highly significant rise in serum triglyceride, total cholesterol and LDL-C and a significant decrease in HDL-C levels in CAD patients as compared to controls. The study also observed higher levels of lipid per oxidation product, MDA in the CAD patients as compared to the controls. The antioxidant enzyme like CAT, SOD, GSH-Reduced, GPx and GSSH-Reductase activity in CAD patients was also observed to be significantly decreased as compared to controls. The results are shown in **Table 2**.

TABLE 1: CASE STUDY OF SUBJECTS SELECTED FOR THE STUDY

Variables	Category	Control groups(n=150)	Patient groups(n=150)
Age (years)	40-48	60	47
	48-60	90	103
Gender	Male	105	122
	Female	45	28
BMI(Kg/m^2)	Underweight<18.0	0	2
	Normal 18-22.9	8	11
	Overweight 23-24.9	69	59
	Obese> 25	75	78
Blood pressure	Pre-hypertension <120-129/80-84	55	29
	Hypertension >140/90	-	56
	Stage 1 >140-159/90-99	-	42
	Stage 11 160-179/110-109 >180/110	-	23
Socioeconomic status	Upper 1	68	70
	Upper 11	45	27
	Upper 111	37	53
Alcohol drinking	Yes	50	85
	No	100	65

TABLE 2: PARAMETERS EXAMINED FOR THE STUDY

Parameters	Control(n= 150) (Mean± S.D.)	CAD Patients(n=150) (Mean± S.D.)
SERUM TC(Mg/dl)	180.95±96.62	194.83±46.49**
TG(Mg/dl)	141±58.1	180±35.6**
HDL(Mg/dl)	45.69± 10.20	40.85± 7.37*
LDL(Mg/dl)	107.26±38.5	147.98±29.8**
VLDL(Mg/dl)	28.0± 19.41	36.0± 9.29**
Lipid peroxide (nmole MDA/ml serum)	5.4±32.11	10.2±95.89**
RBC LYSATE CAT (unit/mg protein RBC lysate)	18.03±6.82	12.45±12.36*
SOD (nmole of NBTreduced/min/mg of protein)	4.86±1.86	3.04±1.07*
GPX (nmole NADPHoxidase/min./mgofprotein)	384.87±192.61	287.47±101.06*
GSSH-REDUCTASE (nmole NADPHoxidase/min./mgofprotein)	214±30.0	149.95±89.45*
GSH-Reduced (µmole/mg protein)	58.66±48.67	28.37±35.67*

**P<0.001 --highly significant, *P>0.01------less significant

Coronary artery disease is a multi-factorial disease. Significant elevated levels of serum, TC and LDL cholesterol and low levels of HDL cholesterol have been reported as most important risk factors for CAD. The Framingham Heart study demonstrated the concept of low HDL-C as a major risk factor for CAD. In our study, significantly lower levels of HDL-C were found in CAD patients when we compared to controls. The present study observed a significant decrease in HDL levels and a significant increase in TC, LDL-C, and TG levels in CAD patients. Our results are correlated with the previous findings[12].

Our study showed a significance increase in the oxidative stress in CAD patients than controls. Oxidative stress generated by reactive oxygen species may play a causative role in the pathogenesis of coronary artery disease[13]. Antioxidant defense in our body comprise of enzymatic and non-enzymatic moieties can inactivate or remove the reactive species. Serum MDA, a biomarker end product of lipid peroxidation, has been extensively used to estimate oxidative stress in CAD patients[14].

The increase MDA also occurs as a consequence of oxidative stress when balance pro-oxidant and antioxidant status is impaired. MDA is a product of auto-oxidation of polyunsaturated fatty acid and is used as an oxidative damage[15]. While other antioxidant enzymes such as CAT, SOD, GSH-reduced, GPx, GSSH-Reductase was decrease in CAD patients as compared to healthy controls. In the present, abnormal lipid profile and MDA levels were significantly higher in CAD patients compared to healthy controls indicating increase oxidative stress.

CONCLUSION: Our study indicates the pivotal role oxidative stress in pathogenesis and progression of CAD. This study shows a significant increase in lipid peroxidation in patients with coronary artery disease. A significant increase in total oxidant status and oxidative stress index and significant decrease in antioxidant status were also observed in these patients. This indicates an imbalance between oxidant and antioxidant molecules in CAD requiring rectification as it has ramification in terms of causing other co morbidities. This clearly suggests that increased oxidative stress and abnormal lipid profile are two independent risk factors in the patho-mechanism of arthrosclerosis.

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REFERENCES:

1. Kumar A, Nagtilak S, Sivakanesan R, Gunasekera S : Cardiovascular Risk Factors in Elderly Normolipidemic Acute Myocardial Infarct Patients- A Case Controlled Study from India. Southeast Asian J Trop Med Public Health 2009; 40(3): 581-592.
2. Kulkarni Jyoti, Phalak radnya : Study of oxidative stress and lipid profile in coronary artery disease. International Journal of research in pharmaceticual and biomedical science 2013; 4(2): 624-627.
3. Surekha R H, Srikanth B B M V, Jhaena P, ramachandra R V, Dayasagar R V, Jyothy A : oxidative stress and total antioxidant status in myocardial infarction. Singapore Med J 2007; 48(2): 137-142.
4. Ohkawa H., Ohishi N and Yagi K: Assay for lipid peroxides in animal tissue by thio-barbituric acid reaction. Anal Biochem 1979; 95: 357-358.
5. Aebi Hugo, Sonja R. Wyss, Bernhard Scherz, and Frantisek Skvaril: Heterogeneity of Erythrocyte Catalase I1, Isolation and Characterization of Normal and Variant Erythrocyte Catalase and Their Subunits. Eur. J. Biochem 1974; 48: 137-145.
6. McCord JM. and Fridovich I: SOD enzyme function for erythrocuprein. J. Biol. Chem. 1969; 224: 6049-6055.
7. Paglia D.E. and Valentine W.N: Studies on the qualitative and quantitative characterization of erythrocyte GPX. J. Lab. Clin. Med. 1967; 20: 150-168.
8. Hazelton, G.A. and Lang, C.A: GSH content of tissue in aging mouse, Biochem. J. 1985; 188: 25-30.
9. Beuteler, E: The glutathione instability of drug sensitivity red cell, A new method for the in vitro detection of drug sensitivity. J Lab Clin Med 1957; 49: 84-95.
10. Friedwald W T et al: Estimation of the concentration of low density lipoprotein cholesterol in Plasma, without use of preparative ultracentrifuge. Clinical chemistry 1972, 18: 499-502.
11. Mohd. Akbar Bhat et al: oxidative stress status in coronary disease patients. Int. J. Life Sc. Bt & Pharma, 2012, 1: 2.
12. Chisolm G M and Steinberg D: the oxidative modification hypothesis of atherogensis, an overview, frees radical Biology and medicine 2000, 28: 1815-1826.
13. Sedar Z et al: lipid and protein oxidation and antioxidant status in patients with angiographically proven coronary artery disease. Clinically biochemistry 2006; 39:794-803.
14. Cavalca et al: oxidative stress and homocysteine in coronary artery disease. Clinical chemistry 2001; 47: 887-892.
15. WHO: Appropriate body mass index for asian populations and its implication for policy and intervation strategies, Lancent 2004; 363: 157-163.

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