Original Research Paper

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ROLE OF OXIDATIVE STRESS AND EFFECT ON ENDOGENOUS ANTIOXIDANTS IN CARDIOVASCULAR DISEASE

Biochemistry

KEYWORDS: Cardiovascular disease, albumin, uric acid, bilirubin, FRAP (ferric reducing ability of plasma) and oxidative stress.

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ABSTRACT

Cardiovascular diseases (CVD's) is one of the leading cause of deaths in India & worldwide. Potential cardiovascular risk factor could be hypertension, diabetes, stroke etc. The increased production of reactive oxygen species may be a unifying mechanism in CVD progression, and antioxidants may have therapeutic value in this setting. In this study we have analyzed oxidative stress marker (FRAP), endogenous antioxidants level (albumin, uric acid and Bilirubin), Lipid profile, cardiac marker (CK-MB, LDH & AST) in patients with CVDs. FRAP & HDL cholesterol and albumin were significantly decreased in study group. The lipid profile and cardiac markers were significantly increased in study group. No significant changes were observed in uric acid and bilirubin levels. It was concluded that increased oxidative stress, abnormal cardiac markers and lipid profile were the risk factors in CVD's.

INTRODUCTION

Cardiovascular disease (CVD's) is one of the leading causes of deaths in India & Worldwide. Approximately 32 million heart attacks and strokes are estimated every year[1]. It is responsible for 10% of DALYs (disability adjusted life years) lost in low and middle-income countries and 18% in high income countries[2].

Oxidative stress is defined as a disturbance in the balance between the production of reactive oxygen species (free radicals) and antioxidant defences. All living organisms are constantly exposed to oxidant agents deriving from both endogenous and exogenous sources capable to modify biomolecules and induce damages. Free radicals generated by oxidative stress exert an important role in the development of tissue damage and aging. Reactive species (RS) derived from oxygen (ROS) and nitrogen (RNS) pertain to free radicals family and are constituted by various forms of activated oxygen or nitrogen. RS are continuously produced during normal physiological events but can be removed by antioxidant defiance mechanism: the imbalance between RS and antioxidant defense mechanism leads to modifications in cellular membrane or intracellular molecules.[3]

Oxidative stress is increased in heart failure and may contribute to many of the structural and functional changes that characterize disease progression. There are both indirect and direct evidences of increased oxidative stress in humans with heart failure.[4] It causes injury to cells in cardiac and vascular myocytes by increased formation of ROS (Reactive Oxygen Species) and decreased antioxidant reserve.[5]

Antioxidant compounds protect against oxidative damage using four main mechanisms: (i) sequestration of transition metal ions into complexes, (ii) scavenging or quenching free radicals and other ROS and RNS, (iii) breaking chain reactions initiated by free radicals, and (iv) repairing damaged molecules.

Albumin represents the major and predominant antioxidant in plasma. During its long lifetime (more than 20 days), an albumin molecule makes about 15000 passes through the circulation. Albumin incurs some damage that affects its antioxidant properties. After showing that albumin antioxidant property was modified following in vitro glycation by methylglyoxal. Favier et al, showed impairment of the antioxidant properties of serum albumin in patients with diabetes.[6]

Recent finding have shown important role of bilirubin in the prevention of ischemic injury in isolated hearts.[7]

Uric acid is a nitrogenous waste product of purine metabolism and is ubiquitous in body fluids. It is also very important as a free radical scavenger. In patients with Heart Failure (HF), raised UA levels may be considered as a marker of hyperinsulinemia, inflammatory cytokine activation, endothelial dysfunction, cardiac cachexia, exercise intolerance, and worse cardiac function. Among echocardiographic parameters, patients with raised UA have elevated filling pressures both in acute and chronic heart failure.[8]

MATERIALS AND METHODS:

This case control study was conducted in Biochemistry department in collaboration with the Department of Cardiology at NIMS Medical College & Hospital, Jaipur. We assessed 100 subjects which included 50 patients with CVD's taken as study group and 50 normal healthy subjects taken as control group regardless of sex with age between 25 and 60 years submitted to cardiac marker analysis. Inclusion criteria were: patients with Cardiovascular Disease of both the genders with the age criteria of 25 - 60 years were taken in the study along with those cardiovascular patients with or without treatment either with dietary control or through anti - hyperlipidemic drugs. Exclusion Criteria were : Subjects having age <25 and >60 years , the patients with any concurrent sickness like chronic liver disease, hypothyroidism etc, pregnant or lactating females, patients on drugs like diuretics, oral contraceptives (in women) were not considered in the study.

Blood samples were collected in the morning in a plain tube with aseptic conditions for estimating lipid profile, endogenous antioxidant, and cardiac marker. Blood sample for the estimation of FRAP was collected in EDTA vial. Ferrous tripyridyltriazine complex reactive substances were estimated in plasma, described by Benzie and Strain,[9] using FRAP as reference standard, expressed as μ moles of $FeSO_4$ equivalent/L of plasma. Total cholesterol (CHOD-PAP-Method),[10]HDL (PEG/CHOD-PAP method), LDL (Friedwald formula), triacylglycerol (TAG) (GPO/ADPS Method), Total protein (Biuret Method),[11] albumin (BCG Method),[12] SGOT/AST (Modified UV IFCC, Kinetic Method),[13]TROP T (Card Method),[14] Uric acid (Uricase Method),[15] LDH(Lactate Dehydrogenase) By Pyruvate kinetic Method,[16] Bilirubin (DMSO Method),[17] CK-MB (Modified IFCC Method),[18]were measured by Trivitron Nano Lab 150 fullyautoanalyzer in all the subjects.

STATISTICAL ANALYSIS: Statistical analysis was done, using the SPSS 20. mean SD were calculated. The control and study group was analyzed using unpaired "t"-test.

RESULTS

Table -1: Comparison of Oxidative stress and endogenous Antioxidant activity in Control & Study group.

Parameters	Control Group (n=50)		Study Group	
Ferric Reducing Ability of Plasma (FRAP). (μ moles of $FeSO_4$ equivalent/ L of plasma)	Mean 902.28	SD 132.10	Mean 577.74	SD 58.89 *
Albumin (gm/dl)	4.57	0.37	3.35	0.56 *
Total Bilirubin (mg/dl)	0.75	0.15	0.76	0.13 NS
Uric Acid (mg/dl)	4.40	0.60	4.59	0.90 NS

*P<0.001; NS not significant.

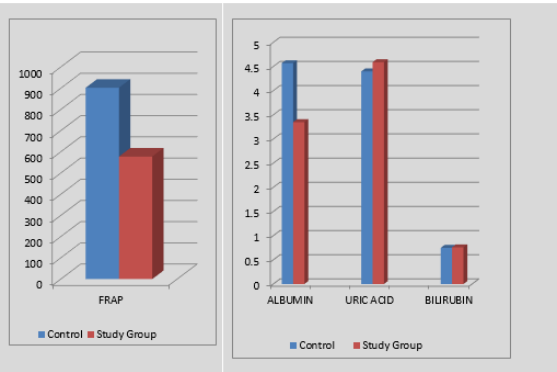

Figure. No – 1 Comparison of Oxidative stress and endogenous Antioxidant activity in Control & Study group

Table - 2: Comparison of degree of Oxidative stress and Level of dyslipidemia in Control & Study group.

Parameters	Control Group (n=50)		Study Group (n=50)	
Ferric Reducing Ability of Plasma (FRAP) (μ moles of $FeSO_4$ equivalent / L of plasma)	Mean 902.28	± SD 132.10	Mean 577.74	± SD 58.89 *
Total Cholesterol (mg/dl)	155.78	10.21	245.42	56.08 *
HDL Cholesterol (mg/dl)	43.56	3.00	37.36	5.37 *
LDLCholesterol (mg/dl)	90.22	8.93	176.97	59.84 *
VLDL Cholesterol (mg/dl)	22.0	4.13	31.08	8.43*
Triglyceride (mg/dl)	110.0	20.7	155.44	42.18 *

*=p<0.001 (statistically significant)

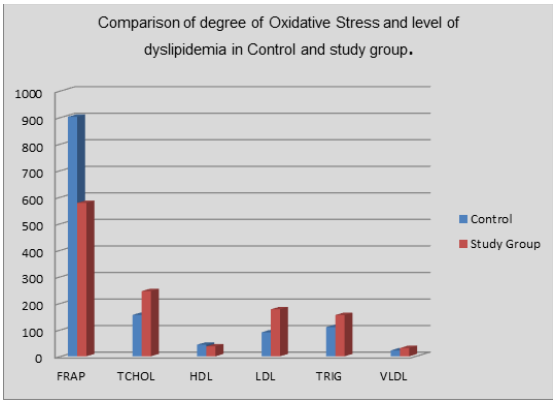

Figure. No -2. Comparison of degree of Oxidative stress and Level of dyslipidemia in Control & Study group

Table – 3: Comparison of Oxidative stress with Level of Cardiac Markers in Control & Study group.

Parameters	Control Group (n=50)		Study Group (n=50)	
Ferric Reducing Ability of Plasma (FRAP) (μ moles of $FeSO_4$ equivalent / L of plasma)	Mean 902.28	SD 132.10	Mean 577.74	SD 58.89 *
CK-MB (IU/L)	14.72	3.85	80.3	43.95 *
LDH (IU/L)	101.32	17.88	188.72	48.56 *
SGOT (IU/L)	22.54	7.06	66.50	80.14 *

*p<0.001 (statistically significant)

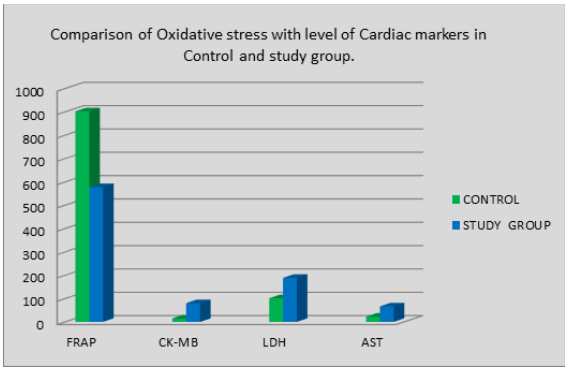

Figure. No -3. Comparison of Oxidative stress with Level of Cardiac Markers in Control & Study group

Table No. 1 and Figure no 1. shows comparison of oxidative stress, endogenous antioxidant serum albumin, serum uric acid, serum bilirubin of control and study group. These figures show that values of FRAP and serum albumin were decreased in study group compared to control group ($p < 0.001$). The value of serum uric acid & serum bilirubin in study group did not show any significant change when compared to control group.

Table No. 2 and Figure no 2. shows comparison of degree of oxidative stress (FRAP) with lipid profile. In this study, values of FRAP, TCHOL, HDL, LDL, VLDL and TRIG were compared between control and study group. The levels of lipids were found to be significantly increased in study group as compared to control ($p < 0.001$). The value of FRAP and HDL in study group was significantly decreased as compared to control group ($p < 0.001$).

Table No. 3and figure no3. shows comparison of oxidative stress with level of Cardiac markers. In these studies, value of CK-MB, LDH and AST were found to be significantly increased in study group as compared to control ($P < 0.001$). The value of FRAP in study group was significantly decreased as compared to control group ($p < 0.001$).

DISCUSSION:

FRAP (ferric reducing ability of plasma) altered lipid parameters and level of endogenous antioxidants in CVD's. The inherent oxidative stress can lead to more profound and deleterious consequences and so, antioxidant therapy can be an answer to the problem.In our studies value of FRAP in study group was significantly decreased as compared to control group. The same observation was found by Benzie et al[19]

Albumin binds many types of molecules and was called a "sponge" or a "tramp steamer" of the circulation. Many antioxidant activities of albumin result from its ligand-binding capacities. Our study concluded that the mean values of serum albumin were significantly decreased in cardiovascular disease as compared to control. Kuller et al. found that there was a highly significant inverse relation between

serum albumin level and risk of coronary heart disease. The lower albumin level may be a marker of persistent injury to arteries and progression of atherosclerosis and thrombosis.[20]

Bilirubin helps in the attenuation of oxidative damage in cultured cells and modulation of airway smooth muscle contractility.[21] We found that the value of bilirubin is markedly similar in case of cardiovascular patient as compared to control. According to Yavuz et al. it may be said that there is a relationship between bilirubin and CVD, compatible with previous studies, but we have not observed any relationship between bilirubin and lipid.[22]

Ames et al, proposed that uric acid may be an important antioxidant in humans.[23] This hypothesis is supported by the ability of uric acid to scavenge hydroxyl radicals, singlet oxygen, and oxo-heme oxidants. Further support stems from the ability of uric acid to form stable co-ordination complexes with ferrous and ferric ions and, in so doing, protect ascorbate against iron-ion-catalyzed oxidation in human blood.[24] We found that, the value of uric acid is also similar in case of cardiovascular disease patient as compared to the control and within in normal range. This controversy caused uric acid to be no longer regarded as a true CVD risk factor.[25]

The mean level of HDL in cardiac patients was significantly decreased as compared to control. These findings are consistent with those of Vanessa et al.[26]

Mohd et al. also found that the level of lipid profile in oxidative stress status is increased in coronary artery disease patients. The lipid profile revealed significantly higher serum TRIG and lower HDL-C levels in patients. Total cholesterol, LDL-C and VLDL-C levels were also significantly higher in patients than control.[27]

CONCLUSION : The values of FRAP and serum albumin were decreased in study groups. The value of serum uric acid & serum bilirubin in study group did not show any significant change when compared to control group. value of CK-MB, LDH and AST was increased in study group. The levels of lipids were found to be significantly increased in study group. The value of FRAP and HDL levels was significantly decreased in study group when compare to control.

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