

Role of Oxidants and Anti Oxidants in Patients with Cardiovascular Diseases

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Abstract: Over the last two decades, it has become increasingly clear that reactive oxygen species (ROS), including free radicals are involved in cardiovascular diseases. In recent years, there has been a growing interest in the clinical implications of these oxidants. The ROS are common by-products of many oxidative biochemical and physiological processes. Therefore the present study was carried out to evaluate the total antioxidant capacity, lipid peroxidation and status of superoxide dismutase in patients with cardiovascular diseases. Total 185 patients of both sexes were included in the study and further classified into 3 groups as hypertensive, Ischemic heart disease and cerebrovascular disease/stroke. The 60 healthy subjects who were not on any kind of prescribed medication or dietary restrictions were included in the control group. MDA is estimated as a marker of lipid peroxidation, levels were significantly increased in all groups than controls ($p < 0.001$). Superoxide dismutase and glutathione reductase activities were significantly lower in all groups than control ($p < 0.001$), GPX levels were decreased in all groups except hypertension ($p < 0.001$), NO level were decreased in all the groups except cerebrovascular disease / stroke when compared to the control. Significantly lower level of total antioxidant capacity and prominent scavenger of superoxide anion radicals suggests that failure of antioxidant defense mechanism against oxidative stress may be an important factor in the pathogenesis of cardiovascular diseases.

Key words: Glutathione peroxidase, glutathione reductase, lipid peroxidation, Malonyldialdehyde, nitric oxide, oxidative stress, superoxide dismutase

INTRODUCTION

Cardiovascular Diseases (CVD) refer to any disorder affecting the heart (that is cardio and blood vessels of the body). It includes hypertension, ischemic heart diseases, cerebrovascular disease, congestive cardiac failure, rheumatic heart disease, non-rheumatic valvular disease, aortic aneurysm, and peripheral arterial diseases.

Out of this hypertension, ischemic heart disease and stroke are the major forms of CVD, which leads to morbidity and mortality (World Health Report, 2002).

The condition of oxidative stress is an imbalance in the rate at which the intracellular content of reactive oxygen species (free radicals) increases relative to the capacity of the cell to eliminate free radicals. (Harrison, 1997) Oxidative stress alters normal endothelial function, supporting proinflammatory, prothrombotic, proliferative and vasoconstrictor mechanism that support the atherogenic process.

The immune system uses the lethal effects of oxidants by making production of oxidizing species a central part of its mechanism of killing pathogens; with activated phagocytes producing both Reactive Oxygen Species (ROS) and reactive nitrogen species. These include superoxide ($\bullet\text{O}_2^-$), nitric oxide ($\text{NO}\bullet$) and their

particularly reactive product. Impaired Nitric Oxide (NO) bioactivity and increased oxidative stress are common features of disease states associated with atherosclerosis. (Nathan and Shiloh, 2000). During lipid peroxidation, unstable hydroperoxides resulting from peroxy radical-dependent chain reactions involving unsaturated fatty acyl moieties. Later break down to smaller and more stable products like malonyldialdehyde (MDA) or Thiobarbituric Acid-Reactive Substances (TBARS), which are considered to be oxidative stress markers (Locatelli *et al.*, 2003).

Natural antioxidant mechanisms of the human body includes enzymes like Superoxide Dismutase (SOD), Glutathione Peroxidase (GPX), Glutathione Reductase (GR) which can react with various free radicals and detoxify them.

Oxidative stress plays a major role in initiation, propagation and rupture of atherosclerotic plaque. Thus measuring oxidative stress by various markers can predict the development of various future catastrophic events like myocardial infarction, stroke etc. Since cardiovascular diseases are emerging as the major cause of morbidity and mortality and their relation with the oxidative stress is the near emerging concept.

Table 1: Value of nitric oxide and antioxidant enzymes in various groups

Parameters	Group-I (Hypertension)	Group-II (Ischemic Heart)	Group-III (Cerebrovascular stroke)	Control	p-value
NO ($\mu\text{mol/L}$)	32.28 \pm 5.87	38.24 \pm 6.17	68.39 \pm 3.68	51.00 \pm 5.81	<0.001
GPX (U/gHb)	40.91 \pm 5.88	20.01 \pm 3.38	23.07 \pm 2.91	33.31 \pm 5.25	<0.001
GR(U/gHb)	3.92 \pm 1.82	4.12 \pm 1.32	3.89 \pm 1.33	9.68 \pm 4.68	<0.001
SOD (U/gHb)	491.39 \pm 200.46	401.46 \pm 101.46	498.13 \pm 158.33	728.91 \pm 208.86	<0.001
MDA (nmol/mg Protein)	4.39 \pm 0.98	4.64 \pm 0.88	4.79 \pm 0.61	3.89 \pm 0.94	<0.001

p<0.001 = highly significant value compare to control group

Table 2: Comparison of NO, GPX, GR, SOD, MDA

Parameters	r-value				
	NO	GPX	GR	SOD	MDA
GR	0.213	0.218	-----	0.331	- 0.162
SOD	0.175	0.190	0.338	-----	- 0.191
GPX	- 0.059	-----	0.219	0.196	- 0.118
MDA	- 0.056	- 0.118	- 0.161	- 0.192	-----
NO	-----	- 0.060	0.214	0.175	- 0.058

The ROS are common by-products of many oxidative biochemical and physiological processes. Therefore the present study was carried out to evaluate the total antioxidant capacity, lipid peroxidation and status of superoxide dismutase in patients with cardiovascular diseases.

MATERIALS AND METHODS

Patients who were attending cardiovascular OPD as well as Patients admitted in cardiac wards at Dr. Ulhas Patil Medical College, Jalgaon Maharashtra, India during a period of Dec. 2008-2009 were taken for evaluation of oxidants, antioxidants and nitric oxide content in their blood. Total 184 subjects were included for study out of which 128 were males and 56 were females. 60 age sex matched healthy human volunteers, were not on any kind of prescribed medication or dietary restrictions were included in the control group.

The subjects were classified into 3 groups:

- Group I: Subjects with hypertension. Total patients 60 out of which 34 were males and 26 were female
- Group II: Subjects with Ischemic heart disease. Total patients 68 out of which 48 were males and 20 were female
- Group III: Subjects with cerebrovascular diseases. Total patients 56 out of which 46 were males and 10 were female
- Group IV: Healthy age sex matched controls groups. Total patients 60 out of which 36 were males and 24 were female

The diagnosis of cardiovascular diseases was based on Echocardiogram, stress test and angiography as required for the disease.

Informed consent was taken from all subjects involved in the study and the study was approved by the Institutional Ethical Committee. Blood samples (5mL)

were drawn into plain vacutainers from the antecubital veins of healthy controls and patients.

Concentration of serum nitric oxide (Cortas *et al.*, 1990), Glutathione peroxidase (GPX) in RBCs (Pagila *et al.*, 1967), Glutathione Reductase (GR) in RBCs (Mannervik, 1985), Superoxide dismutase (SOD) in RBCs (Marklund *et al.*, 1974) and serum Malonyldialdehyde (MDA) in serum (Byrne *et al.*, 2003) were analyzed. The RA- 50 Chemistry analyzer was used to carry out all analysis.

Patients having diseases other than cardiovascular diseases and patients taking antioxidant therapy that interferes with study parameters like oxidative stress are excluded from study.

RESULTS

The data of antioxidant enzymes and nitric oxide are given in Table 1. There is significant difference found in antioxidant enzymes and nitric oxide level between the different groups of cardiovascular diseases when compared with control groups. The significant difference was found in nitric oxide, it was decreased in group I and group II except group III that was cerebrovascular diseases/stroke (p<0.001). The glutathione peroxidase levels were significantly decreased in II and III group except group I that is hypertension (p<0.001). Glutathione reductase and superoxide dismutase activities was significantly lower in all groups when compared with control (p<0.001).

The MDA level was found to be significantly increased in all three groups (p<0.001). On applying Pearson's correlation, (Table 2) nitric oxide was found to be positively correlated with GR (r = 0.213) and SOD (r = 0.175) where as it was negatively correlated with GPX (r = -0.059) and MDA (r = -0.056).

GPX was found to be positively correlated with GR (r = 0.0218) and SOD (r = 0.0190) where as it is negatively correlated with MDA (r = -0.0118) and NO (r = -0.060).

GR was found to be positively correlated with GPX ($r = 0.219$) and SOD ($r = 0.338$), NO ($r = 0.214$) and negatively correlated with MDA ($r = -0.161$).

SOD was found to be positively correlated with GPX ($r = 0.196$) and GR ($r = 0.331$) NO ($r = 0.175$) and negatively correlated with MDA ($r = -0.192$).

MDA was found to be negatively correlated with GPX ($R = -0.118$) and GR ($R = -0.162$), SOD ($R = -0.191$) and NO ($R = -0.058$).

DISCUSSION

In this study we assessed the relationship between oxidative stress, Nitric oxide in various forms of CVD. Nitric Oxide (NO) is an endothelium-derived vasodilator produced in the human body by the enzyme Nitric Oxide Synthase (NOS). Antioxidant mechanism of NO is carried out by blocking lipid peroxidation. NO can inhibit the oxidation of free fatty acids and lipoprotein molecules and thus this mechanism bears a clinical importance. There are three types of NOs in human body - endothelial nitric oxide synthase (eNOs), Neuronal Nitric Oxide synthase (nNOs) and inducible Nitric Oxide synthase (iNOs). The eNOs and nNOs are normally synthesized in the body, whereas iNOs production is induced in response to inflammation thus leading to increased levels of NO and free radicals. The superoxide ($\bullet\text{O}_2^-$) may react with excess NO in a rapid diffusion, limited reaction to generate peroxynitrite (ONOO^\bullet), which oxidize thiol groups favoring lipid peroxidation and reducing protein damage (Byrne *et al.*, 2003).

This study explored that, NO levels were decreased in all the groups except cerebrovascular disease / stroke when compared to the control. These results are in agreement with the findings of Chandra *et al.* (2003), Bulent *et al.* (1998) Dogan *et al.* (2006). A significant increase in Nitric oxide levels, were found in stroke patient, which is concurrent with EI-Kossi and Zakhary (2000): A positive correlation of NO was found with glutathione reductase ($r = 0.213$) and SOD ($r = 0.175$) where as a negative correlation of NO is found with GPX ($r = -0.059$), MDA ($r = -0.056$).

Human body has various antioxidant mechanisms out of which glutathione related mechanism is most important in tissue antioxidant production. There are three forms of glutathione dependent antioxidant enzyme: Glutathione Peroxidase (GPX), Glutathione Reductase (GR) Glutathione transferase, out of which assessed the activities of GPX and GR.

Glutathione Peroxidase (GPX) catalyses the reduction of H_2O_2 and hydroperoxides formed from fatty acids, there by effectively removing toxic peroxides from living cells. In our study GPX levels were decreased in all groups

except hypertension. These results are concurrent of Domenico *et al.* (1998). It might be due to increased production of GPX induced by the high concentration of lipoperoxides, the natural substrate for this enzyme. A positive correlation of GPX is observed with GR ($r = 0.218$), SOD ($r = 0.190$) and negative correlation with MDA ($r = -0.118$), NO ($r = -0.060$)

Glutathione reductase catalyses the reduction of oxidized glutathione (GSSG) to reduced glutathione (GSH). Cysteiny residue of GSH offers a nucleophilic thiol, which is important in the detoxification of electrophilic mobilities and metabolically produced oxidizing agents and GSH is a substrate for the enzyme GPX and Glutathione S-transferase. GSH is converted to GSSG is reduced back to GSH by an NADPH dependent enzyme glutathione reductase. In present study glutathione reductase levels were found to be significantly decreased in all the groups when compared with normal, which is concurrent with Domenico *et al.* (1998). A positive correlation is observed with GPX ($r = 0.219$), SOD ($r = 0.338$), NO ($r = 0.214$) and is negatively correlated with MDA ($r = -0.161$).

Super oxide dismutase (SOD) catalyses the breakdown of the superoxide radical ($\bullet\text{O}_2^-$). The present study demonstrates significantly lower activity of SOD in all groups as compared to normal. These findings are correlated with Bulent *et al.* (1998). A negative correlation of SOD is found with MDA ($r = -0.192$), while positive correlation is seen with GPX ($r = 0.196$), GR ($r = 0.331$) and NO ($r = 0.175$).

Super oxide dismutase plays a major role in the first line of the antioxidant defense system by catalyzing the dismutation of superoxide radicals to form hydrogen peroxide and molecular oxygen. Mechanism of catalase provided by SOD suggests, that this enzyme is incomplete antioxidant, which prevent the superoxide anion and produces the other. Its biological action is connected with catalase via H_2O_2 . Catalase is a ubiquitous enzyme present in cells of aerobic organism. Catalase converts two molecules of the strong oxidant, hydrogen peroxide to molecular oxygen and two molecules of water (Laszlo *et al.*, 1991). Kono *et al.* (1982) found that superoxide anion inhibited these catalase action and the presence of hydrogen peroxide inhibited the action of dismutase.

All the biomolecules like lipids, proteins and nucleic acids may be attacked by free radicals, but lipids are probably the most susceptible. The oxidative destruction of lipids (lipid peroxidation) is a destructive, self-perpetuating chain reaction, releasing Malonyldialdehyde (MDA) as the end product. Significant increase of MDA concentration in serum is found in all groups when compared with normal, which correlated with

Bulent *et al.* (1998) and Russo. A negative correlation of MDA is found with GPX ($r = -0.118$), GR ($r = -0.162$), SOD ($r = -0.191$) and NO ($r = -0.058$).

CONCLUSION

With drastic changes in life style pattern, increasing number of subjects are at risk of vascular disease. There is preponderance of evidence for the association of increased oxidative stress with various vascular diseases. It results in premature death from angina, heart attack, stroke, peripheral artery disease, hypertension, ischemia and thrombosis.

The Reactive Oxygen Species (ROS), including free radicals are involved in cardiovascular disease. The ROS are common by-products of many oxidative biochemical and physiological processes.

Antioxidant mechanism of NO is carried out by blocking lipid peroxidation. NO can inhibit the oxidation of free fatty acids and lipoprotein molecules and thus this mechanism bears a clinical importance. Glutathione related mechanism is most important in tissue antioxidant production. Super oxide dismutase plays a major role in the first line of the antioxidant defense system by catalyzing the dismutation of superoxide radicals to form hydrogen peroxide and molecular oxygen. The oxidative destruction of lipids (lipid peroxidation) is a destructive, self perpetuating chain reaction, releasing Malonyldialdehyde (MDA) as the end product. The present study concludes the importance of assessing the oxidants and anti oxidants in patients with cardiovascular diseases. The existing evidence support the view that oxidative stress may play a crucial role in cardiac and vascular abnormalities in different types of cardiovascular diseases and that the antioxidant therapy may prove beneficial in combating these problems.

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