Lipid peroxidation and the levels of antioxidant enzymes in hypertension

Mohammad Amanullah,[1] Gaffar Sarwar Zaman,[2]* Jalelur Rahman,[3] Sajida Sultana Rahman[4]

[1]Department of Clinical Biochemistry, College of Medicine, King Khalid University, Abha, Kingdom of Saudi Arabia
[2]Department of Clinical Biochemistry, College of Medicine, King Khalid University, Abha, Kingdom of Saudi Arabia
[3]Department of ENT, Prathima Instiute of Medical Science, Karimnagar, Hyderabad, India
[4]Department of Clinical Biochemistry, Prathima Instiute of Medical Science, Karimnagar, Hyderabad, India

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ABSTRACT

Background and Aim: There is substantial evidence that oxidative stress plays a major role in hypertension and subsequently the atherosclerotic process. The aim of the present study was to investigate the presence of oxidative stress in hypertension. **Materials and Methods:** The study included 46 hypertensive and 48 control subjects. Superoxide dismutase, glutathione, glutathione peroxidase activity and malondialdehyde level were measured in serum samples of the participants along with fasting lipid levels. **Statistical Analysis Used:** All data was entered into an Excel spreadsheet and analyzed using standard statistical software like Statistical Package for the Social Sciences (SPSS). Chi square test was used for categorical variables. **Results:** Serum malondialdehyde level was significantly raised in the study group as compared to control group ($p < 0.05$). Superoxide dismutase activity, whole blood glutathione levels and glutathione peroxidase activity were significantly decreased in all the subgroups of study group as compared to control group ($p < 0.05$). Cholesterol, low density lipoproteins and triglycerides showed significant rise, whereas high density lipoprotein was decreased as compared to normal. **Conclusions:** The higher malondialdehyde level and lower activity levels of other antioxidant molecules measured in this study could have resulted from increased free radical generation, which may confirm the presence of oxidative stress in hypertension. However, further elaborate clinical studies are required to evaluate the role of such antioxidant enzymes.

Keywords: antioxidant, oxidative stress, malondialdehyde, glutathione, superoxide dismutase.

INTRODUCTION

Several risk factors for coronary heart disease (CHD) has been documented, one of them is hypertension (HT).[1] Evidence suggests that reactive oxygen species (ROS) may play important roles in the pathogenesis in HT and subsequently in myocardial infarction too.[2]

***Corresponding address:**
Dr. Gaffar Sarwar Zaman
Assistant Professor, Department of Clinical Biochemistry,
College of Medicine, King Khalid University,
Abha, Kingdom Of Saudi Arabia
Phone number: + 966509228143
E-mail address: gffrzaman@gmail.com
gaffarzaman@rediffmail.com

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The adverse effects of ROS on biological systems have become a major focus of current biomedical research.[3] Oxygen free radicals (OFRs) and lipid peroxides have been implicated in the pathogenesis of many diseases, including diabetes mellitus (DM), cancer, rheumatoid arthritis, systemic lupus erythematosus, Behcet's disease, infectious diseases, atherosclerosis and aging.[4-9] Oxidative stress may contribute to the generation and/or maintenance of hypertension via a number of possible mechanisms. These include quenching of the vasodilator nitric oxide (NO) by ROS such as superoxide;[10] generation of vasoconstrictor lipid peroxidation products, such as F2-isoprostanes;[11] depletion of tetrahydrobiopterin (BH4), an important NO synthase cofactor;[12] and structural and functional alterations within the vasculature.[13] These vascular changes may be mediated

in several ways, including direct damage to endothelial and vascular smooth muscle cells, effects on endothelial cell eicosanoid metabolism, altered redox state, increases in intracellular free calcium concentrations, and stimulation of inflammatory and growth-signaling events.[13-15] Thus, oxidative stress promotes vascular smooth muscle cell proliferation and hypertrophy and collagen deposition, leading to thickening of the vascular media and narrowing of the vascular lumen. In addition, increased oxidative stress may damage the endothelium and impair endothelium-dependent vascular relaxation and increases vascular contractile activity.[10] Oxygen radicals may also induce endothelial permeability, with extravasation of plasma proteins and other macromolecules and recruitment of inflammatory proteins and cells, which could further impair endothelial function and aggravate vascular damage. All these effects on the vasculature may explain how oxidative stress can cause hypertension. Reactive oxygen species are capable of reacting with unsaturated lipids and of initiating the self-perpetuating chain reactions of LP in the membranes.[16] Studies using nonspecific markers of oxidative damage have observed higher superoxide and hydrogen peroxide production in hypertensive subjects, which returned to levels observed for control subjects after blood pressure reduction.[17] A reduction in superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activity have been observed in newly diagnosed and untreated hypertensive subjects, compared with control subjects, with superoxide dismutase activity being inversely correlated with blood pressure within the hypertensive group, but not the control group.[18] Higher production of hydrogen peroxide has also been observed in treated and untreated hypertensive subjects compared with normotensive subjects, with a significant correlation between hydrogen peroxide levels and systolic blood pressure.[19] Free radicals can also cause oxidation of sulphydryl groups in proteins and strand scission in nucleic acids is also possible.[20] Antioxidant enzyme represents an important defense mechanism against oxidative stress. Antioxidants are compounds that dispose, scavenge and suppress the formation of free radicals or oppose their actions[21] and two main categories of antioxidants are those whose role is to prevent the generation of free radicals and those that intercept any free radicals that are generated.[22] Mammalian cells have a complex network of antioxidants like catalase, SOD, reduced glutathione etc. to scavenge reactive oxygen species.[23] Lipids, especially polyunsaturated fatty acids, are sensitive to oxidation, leading to the term LP, of which, malondialdehyde (MDA) is the most abundant.[24] The accumulation of MDA in tissues or biological fluids is indicative of the extent of free radical generation, oxidative stress and tissue damage.[25] Oxidative stress ensues when ROS evade or overwhelm antioxidants.[26] Due to their highly reactive and non-specific nature, ROS can attack almost all biomolecules including lipid membranes.[27] Lipid peroxides are derived from the oxidation of polyunsaturated fatty acids of membranes and are capable of further LP by a free radical chain reaction.[28] Malondialdehyde (MDA) is a breakdown product of peroxidation of long chain fatty acids which accumulates when LP increases.[29] Glutathione (GSH) reduces disulfide bonds formed within cytoplasmic proteins to cysteines by serving as an electron donor. In the process, GSH is converted to its oxidized form glutathione disulfide (GSSG), also called L(-)-Glutathione. So, GSH is an intracellular tripeptide that directly quenches the ROS and protects against deleterious effects of free radicals. GSH-Px is the general name of an enzyme family with peroxidase activity whose main biological role is to protect the organism from oxidative damage. The biochemical function of GSH-Px is to reduce lipid hydroperoxides to their corresponding alcohols and to reduce free hydrogen peroxide to water. GSH-Px is a selenoprotein that reduces lipidic or nonlipidic hydroperoxides as well as H_2O_2 while oxidizing GSH. Oxidized glutathione (GSSG) is reduced back to GSH by glutathione reductase.[30-33] Reduced glutathione (GSH) and its redox enzymes are the most important cellular antioxidants and play a major role in protecting cells against oxidative stress caused by ROS.[34] Superoxide dismutases (SOD) are a class of enzymes that catalyze the dismutation of superoxide into oxygen and hydrogen peroxide. As such, they are an important antioxidant defense in nearly all cells exposed to oxygen. Superoxide dismutase exists in three isoenzyme forms, all of which suppress oxidative stress under normal conditions and cause catalytic removal of superoxide anions. The superoxide anion radical (O_2^-) spontaneously dismutes to O_2 and hydrogen peroxide (H_2O_2) quite rapidly ($\sim 10^5\ M^{-1}s^{-1}$ at pH 7). Superoxide dismutase is necessary because superoxide reacts with sensitive and critical cellular targets. For example, it reacts with NO radical and produces toxic peroxynitrite. It is still uncertain whether hypertensive patients without obesity or any organ damage, have substantial oxidative stress. This hospital-based study was undertaken with the following objectives:

1. To find out the magnitude of oxidative stress in hypertensive patients.
2. To find out the status of lipid profile in hypertension.
3. To find out if any relation exists between lipid profile and oxidative stress

MATERIALS AND METHODS

A total of 46 hypertensives in the age group of 30–70 years were recruited. The study was designed as a case control study.

Inclusion criteria

The hypertensive patients recruited had a negative coronary artery disease (CAD) history and a history of HT based on several separate documented blood pressure (BP) measurements above 140/90 mmHg and the use of antihypertensive medications for at least six months duration. The controls consisted of 48 normotensive subjects who were free of any disease and had negative family histories for early atherosclerosis and hypertension. They had normal BP after three measurements.

Exclusion criteria

The patients who had total cholesterol (TC) level > 220 mg/dL or triglyceride (TG) concentration > 200 mg/dL, or receiving lipid lowering drugs were excluded. Exclusion criteria also included cardiovascular disease and was diagnosed by ECG, echocardiography or self reported use of a β-blocker, angiotensin I converting enzyme (ACE) inhibitor and/diuretic drug. Patients with infection, inflammatory disease, malignancy, congenital malformations of the heart or vessels or history of acute myocardial infarction (AMI) were excluded. People with DM, obesity and nephritic range of proteinuria were excluded. Other exclusion criteria were HIV-positive status, overt liver and renal disease. Alcohol consumption and the use of hormonal substitutive treatment (in women) were assessed by questionnaire and excluded. The study was approved by the ethics committee of the hospital and all subjects gave written informed consent. Prior to the study, participants were informed that their confidentiality would be maintained and consent was obtained. Data was collected for age, sex, personal past history of DM, hypertension, waist circumference, weight and height to calculate the body mass index (BMI), history of smoking and cerebrovascular accidents. Smoking habits and a familial history of ischemic heart disease (IHD) or cardio vascular disease (CVD) were assessed by a questionnaire. Then BP measurement was done using standard mercury sphygmomanometer. An average of three readings measured thrice at an interval of 15 minutes was taken with subjects in a sitting position. The average of three measurements of Korotkoff phase I was considered as systolic blood pressure (SBP), and the average of three values of phase IV was recorded for diastolic blood pressure (DBP).[35]

Blood samples were collected from the subjects after a 12 hour fast. Samples were centrifuged; serum was collected and stored at 20°C until analyzed. Lipid profiles comprising total cholesterol (TC), high density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C) and triglyceride (TG) concentrations were measured at fasting. Sample serum concentrations of TC, HDL-C and TG were measured by enzymatic colorimetric methods using Star Plus 21 semi-autoanalyser of Rapid Diagnostic Company. Calculation of LDL-C concentrations was based on the Friedewald equation.[36] Estimation of fasting and post prandial plasma glucose (FPG and PPPG) levels was carried out using the Star Plus 21 semi-autoanalyser of Rapid Diagnostic Company to exclude DM. Kidney diseases were excluded by measuring serum creatinine and blood urea levels. The diagnosis of DM was based on WHO criteria[37] i.e. a FPG level ≥ 7.0 mmol/L or ≥ 126 mg/dL, or a 2-hour PPPG level ≥ 11.1 mmol/L or ≥ 200 mg/dL on more than one occasion, with symptoms of diabetes. Liver disease and nephritic range of proteinuria were excluded with the help of liver function tests and HIV test was done to exclude HIV positives. MDA was determined as the measure of thio barbituric acid reactive substances (TBARS).[38] Spectrophotometric analysis of SOD and GSH (plasma glutathione) in erythrocytes and of GSH-Px in plasma was performed as described previously.[39,40] All data were entered into an Excel spreadsheet and were analyzed using standard statistical software like SPSS. Chi square test was used for categorical variables. All numerical data was presented as mean ± standard deviation. A p value of less than 0.05 was considered statistically significant.

RESULTS

The anthropometric measurements of the groups of patients and the control subjects are summarized in Table 1. Hypertensive (n = 46) and control subjects (n = 48) were matched for age, BMI (mean ± SD = 25.9 ± 3.2 vs. 25.5 ± 2.7 kg/m^2 respectively), waist circumference (mean ± SD = 93.7 ± 9.6 vs. 94.5 ± 8.3 cm respectively) and waist hip ratio (WHR) (mean ± SD = 0.92 ± 0.06 vs. 0.91 ± 0.07 respectively). The mean ± SD SBP in the hypertensive was 157 ± 17 mm of Hg, while it was only 114 ± 8 mm of Hg in the controls. The mean ± SD DBP recorded in hypertensives was 85 ± 14 mm of Hg while in controls it was 79 ± 14 mm of Hg.

As expected the hypertensive had higher TC (228.36 ± 67.07 mg/dL vs. 168.26 ± 29.41 mg/dL), LDL-C (128.09 ± 37.16 mg/dL vs. 92.56 ± 21.22 mg/dL) and

Table 1 Anthropometric characteristics of control and hypertensive patients

Parameter	Control subjects (Mean ± SD)	Hypertensive patients (Mean ± SD)
Total no. of participants	48	46
Body mass index (kg/m^2)	25.5 ± 2.7	25.9 ± 3.2
Waist Circumference (cm.)	94.5 ± 8.3	93.7 ± 9.6
Waist hip ratio (WHR)	0.91 ± 0.07	0.92 ± 0.06
Systolic blood pressure (mm of Hg)	114 ± 8	157 ± 17
Diastolic blood pressure (mm of Hg)	79 ± 14	85 ± 14

TG (181 ± 49.09 mg/dL vs. 104.49 ± 27.22 mg/dL) but lower HDL-C (44.46 ± 5.28 mg/dL vs. 48.59 ± 8.76 mg/dL) levels than the healthy controls ($p < 0.05$, $p < 0.05$, $p < 0.05$, and $p < 0.05$, respectively). There was insignificant difference in VLDL-cholesterol levels between the patients and healthy controls.

Plasma activities of antioxidant enzymes are shown in Table 2. Superoxide dismutase activities in the hypertensive group when compared to the control group (5.181 ± 1.143 U/mg protein vs. 6.254 ± 0.93 U/mg protein, $p < 0.05$), blood GSH (8.8 ± 3.6 µmol/L vs. 11.4 ± 2.8 µmol/L, $p < 0.05$) and plasma GSH-Px activities compared with the same (183.8 ± 34.6 nmol/min per ml vs. 231.5 ± 18.7 nmol/min per ml, $p < 0.05$, respectively) were significantly lower in the hypertensive group. Plasma MDA levels were significantly higher in the hypertensive group (7.9 ± 1.1 µmol/L versus 5.7 ± 1.4 µmol/L) than controls. This was significantly elevated over the controls ($p < 0.05$).

DISCUSSION

LP is a free radical chain reaction, which arises from the oxidative conversion of polyunsaturated fatty acids by HO to lipid peroxides, which in turn can damage biological membranes.[41] Oxidative stress is caused by imbalance between the production of reactive oxygen and biological systems ability to readily detoxify the reactive intermediate or easily repair the resulting damage.[42-46] As is well known, ROS produced in excess may cause toxic effects by oxidative damage of molecules, membranes, and tissues. Free radicals can attack almost any component of the cell, but lipids, proteins, and nucleic acids are particularly important targets. Lipids of cell membranes and organelles are frequently damaged, resulting in LP.[47] Above all, the oxidation of membrane lipids has been implicated as one of the primary events in oxidative cellular damage. Antioxidants constitute the foremost defense system that limit the toxicity associated with free radicals. It is known that plasma antioxidant capacity decreases and oxidative/antioxidative balance shifts to the oxidative side in patients with HT. A reason for increased LP in plasma of patients with HT may be a poor enzymatic and non-enzymatic antioxidant defense system.

Vascular oxidative stress has been demonstrated in spontaneous (genetic) and experimental models of hypertension.[48-58] In aortas from hypertensive rats, p22phox mRNA expression and NADH/NADPH oxidase activity are increased.[56] Increased oxidative stress has been demonstrated in aortic and mesenteric vessels of stroke-prone spontaneously hypertensive rats.[57] Enhanced superoxide anion formation was described in vascular tissues from spontaneously hypertensive and desoxycorticosterone acetate-salt hypertensive rats.[58] Vascular oxidative stress has also been demonstrated in many forms of

Table 2 Level or activity of some parameters of antioxidants and MDA concentration in serum in patients with hypertension and controls

Parameter	Control group	Hypertensive group	p-value
Serum MDA (µmol/L)	5.7 ± 1.4	7.9 ± 1.1	p < 0.05
Blood GSH (µmol/L)	11.4 ± 2.8	8.8 ± 3.6	p < 0.05
SOD (U/mg protein)	6.254 ± 0.93	5.181 ± 1.143	p < 0.05
GPX (nmol/min per ml)	231.5 ± 18.7	183.8 ± 34.6	p < 0.05

Table 3 Lipid profiles of the controls and hypertensive patients

Parameters in mg/dL	Controls mean	Hypertensives mean	p-value
HDL-C	48.59 ± 8.76	44.46 ± 5.28	p < 0.05
LDL-C	92.56 ± 21.22	128.09 ± 37.16	p < 0.05
Total cholesterol	168.26 ± 29.41	228.36 ± 67.07	p < 0.05
TG	104.49 ± 27.22	181 ± 49.09	p < 0.05

experimentally induced hypertension, such as angiotensin (Ang) II–mediated hypertension, salt–sensitive hypertension, lead-induced hypertension, obesity-associated hypertension, aldosterone-provoked hypertension, and nitric oxide synthase inhibitor–induced hypertension.[53-60] Unlike the findings in animal models, the association between oxidative stress and HT in humans is less consistent, and results vary depending on the marker of oxidative damage being investigated.[59] Studies using nonspecific markers of oxidative damage have observed higher superoxide and hydrogen peroxide production in hypertensive subjects, which returned to levels observed for control subjects after blood pressure reduction.[60] Russo et al.[61] showed that essential HT is associated with greater than normal lipoperoxidation and an imbalance in antioxidant status, suggesting that oxidative stress is important in the pathogenesis of essential hypertension or in arterial damage related to essential hypertension. Reductions in SOD and GSH-Px activity have been observed in newly diagnosed untreated hypertensive subjects compared with control subjects, with SOD activity being inversely correlated with blood pressure within the hypertensive group, but not control subjects.[62] Higher production of hydrogen peroxide has also been observed in treated and untreated hypertensive subjects compared with normotensive subjects, with a significant correlation between hydrogen peroxide levels and SBP.[63]

Mammalian cells have a complex network of antioxidants like catalase, SOD, reduced GSH etc. to scavenge reactive oxygen species.[64] Oxidative stress ensues when ROS evade or overwhelm antioxidants.[65] Due to their highly reactive and non-specific nature, ROS can attack almost all biomolecules including lipid membranes.[66] Lipid peroxides are derived from the oxidation of polyunsaturated fatty acids of membranes and are capable of further LP by a free radical chain reaction.[67] MDA is a breakdown product of peroxidation of long chain fatty acids which accumulates when LP increases.[68] In the current study, MDA level in the serum of the hypertensive patient group were higher than those in the control group, which confirmed previously published data. The high concentration of MDA in all the patients indicates increased membrane LP. Enhanced LP may occur as a result of the fact that naturally occurring scavenging mechanisms are suppressed and the free radical generation processes are enhanced.[69] It has also been suggested that hyperlipidemia, specially hypercholesterolemia, can cause an increase in LP.[70] Superoxide dismutase along with CAT and GPx, the preventive antioxidants, plays a very important role in protection against LP. In this study, SOD, CAT and GPx activities were significantly lower in hypertensive patients than in control subjects, thus making those individuals more vulnerable to oxidative stress. Decrease in SOD activity may be attributed to hypoxia (due to ischemia) and reperfusion. There is an enhanced production of superoxide anions by ischaemic cells. Also increased concentration of LDL-C causes uncoupling of endothelial nitric oxide synthase and consequently increased production of superoxide anions in vessel wall.[71] GSH is one of the most important endogenous antioxidants. It plays the role of a sulfhydryl (SH) group provider for direct scavenging reactions. GPx, an oxidative stress inducible enzyme, plays a significant role in the peroxyl-scavenging membranes.[72] We have observed a decrease in the GPx activity in the hypertensive patients. Decreased GSH concentration may also contribute to decreased GPx activity because GSH is one of the substrates for GPx. Ischaemia induces metabolic alterations capable of reducing the defense mechanisms of heart against oxygen toxicity, depending on the severity of ischaemic damage. Ischaemia shifts the redox state of cell towards oxidation, GSH and protein-SH content being significantly reduced.[73] The significantly decreased levels in our study could be secondary to increased oxidative stress. In the present study, the decreased activity or level of antioxidant molecules measured could have resulted from increased free radical generation. The results of our study support the higher oxidative stress hypothesis in HT. The increased activities of antioxidant enzymes may be a compensatory regulation in response to increased oxidative stress. Lipid peroxides could be a part of the cytotoxic mechanisms leading to the endothelial injury. The decreased concentrations of the GSH, support the hypothesis that LP is an important causative factor in the pathogenesis of hypertension. Therefore, the treatment with antioxidants in the initial stages of HT may be useful as secondary therapy to prevent the oxidative damage.

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