Possible Augmentive Effect of Antioxidant Vitamin C in Patients with Essential Hypertension Treated with Amlodipin or Enalapril

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Abstract

Hypertension is identified as one of the most significant risk factors for cardiovascular diseases (CVDs). There is growing evidence showing that oxidative stress plays a major role in hypertension. Increased production of reactive oxygen species and decrease bioavailability of antioxidant have been demonstrated in experimental and human hypertension. The present study was directed to determine the beneficial effect of the antioxidant vitamin C in patients with essential hypertension treated with the calcium channel blocker (amlodipine) or with the angiotensin converting enzyme inhibitor (enalapril). Ninety six patients (50 females and 46 males) with essential hypertension and treated with amlodipine or enalapril, age (50.43 ± 0.55 year) were participated in this study and divided in to: Twenty five patients received amlodipine 5mg tablet once daily (group 1), twenty five patients received the same dose of amlodipine plus 500mg of vitamin C tablet twice daily (group 2), twenty three patients received enalapril 5 mg tablet once daily (group 3), and twenty three patients received the same dose of enalapril plus 500mg of vitamin C tablet twice daily (group 4). Twenty apparently healthy subjects (11 females and 9 males) also participated in this study as control group (group 5). All patients were treated and followed up for one month. The mean blood pressures were measured for each patient before treatment and one, two, three and four weeks after treatment. Serum malondialdehyde (MDA), lipid profile, electrocardiogram (ECG) parameters and heart rate were measured for each patient before treatment and two and four weeks after treatment. The addition of vitamin C to amlodipine or enalapril treated patients had resulted in a significant ($p<0.05$) lowering effect on mean blood pressure after two, three and four weeks. On the other hand, the addition of vitamin C to amlodipine or enalapril treated patients had resulted in a significant lowering of serum MDA after two and four weeks of treatment. While there was no significant change in lipid profile or ECG parameters and heart rate of patients in group two and four after two and four weeks of vitamin C treatment as compared to their values at zero time and to patients in group one and three respectively. The study indicates that oxidative stress may have a significant role in essential hypertension, and addition of the antioxidant vitamin C to antihypertensive drugs used in this study can give a good blood pressure lowering effect and good protection against lipid peroxidation.

Key words: Antioxidant ,Vitamin C, Essential Hypertension, Amlodipin , Enalapril

الخلاصة

ارتفاع ضغط الدم واحد من أكثرالعوامل للاصابة بأمراض القلب والاوعية . هناك دلائل متنامية تشير الى أن الجهد التأكسدي يلعب دورا رئيسيا في مرض فرط ضغط الدم. الدراسة الحالية وجهت نحو تحديد التأثير المفيد المتوقع لمضاد التأكسد فيتامين ث لدى المرضى المصابين بفرط ضغط الدم الجوهري والذين يتم معالجتهم باستخدام عقاقير مضادة لارتفاع ضغط الدم هي الساد لقناة الكالسيوم (املودبين) والمثبط للانزيم المحول للانجيوتنسين (انالبريل). ست وتسعون شخص مصاب بفرط ضغط الدم الجوهري (٥٠)اناث و (٤٦) ذكور يعالجون باستخدام الاملودبين او الانالبريل لكن لم يصلوا الى الهدف بضغط الدم وبمعدل أعمار (٥٥.٠ ± ٥٠.٤٣) سنة شاركوا في هذه الدراسة وقسموا الى : (٢٥) مريض استلموا (٥) ملغلرام يوميا من (املودبين) المجموعة الاولى و (٢٥) مريض استلموا نفس الجرعة من (املودبين) و (٥٠٠) ملغرام مرتين يوميا من فيتامين ث المجموعة الثانية و (٢٣) مريض استلموا (٥) ملغرام يوميا من (انالبريل) المجموعة الثالثة و (٢٣)مريض استلموا نفس الجرعة من (انالبريل) و (٥٠٠) ملغرام يوميا من فيتامين ث المجموعة الرابعة. (٢٠) شخص مصح مجموعة السيطرة (١١) اناث و (٩) ذكور المجموعة الخامسة شاركوا في الدراسة. حيث استمر العلاج ومراقبة المرضى لمدة شهر واحد ومعدل ضغط الدم تم قياسه لكل مريض قبل العلاج وبعد (٤،٣،٢،١) اسابيع من المعالجة ، مستوى المالونداليديهايد ومستوى الدهون، بيانات تخطيط القلب ومعدل نبضات القلب تم قياسها لكل مريض قبل البدء بالمعالجة وبعد (٤،٢) اسابيع من المعالجة. إن إضافة مضاد التأكسد فيتامين ث للمرضى الذين يعالجون باستخدام املودبين او الانالبريل نتج عنه انخفاضا معنويا في معدل الضغط بعد مرور(٤،٣،٢) اسابيع من بدء المعالجة. ومن ناحية اخرى إن إضافة مضاد التأكسد فيتامين ث للمرضى في المجموعة الثانية أو الرابعة نتج عنه انخفاضا معنويا في مستوى المالونداليديهايد بعد مرور(٤،٢) اسابيع من بدء المعالجة بينما لم يحدث اي تغير معنوي في مستوى الدهون، بيانات تخطيط القلب ومعدل نبضات القلب للمرضى في المجموعة الثانية والرابعة بعد مرور (٤،٢) اسابيع من بدء المعالجة بفيتامين ث عند مقارنتهم بالقيم وقت الصفر والاشخاص في المجموعة الاولى والثالثة تتابعا. ختاماً إن هذه الدراسة أظهرت إن للجهد التأكسدي دورا معنويا في مرض فرط ضغط الدم الجوهري و إن اضافة مانع التأكسد فيتامين ث للعقاقير المستخدمة (املودبين أو انالبريل) لعلاج المرضى قد أعطى انخفاضاً معنوياً جيداً في ضغط الدم ووقاية جيدة ضد أكسدة الدهون..

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Received : 13/3/2010
Accepted : 5/4/2011

Introduction

Hypertension is a common disease that is defined simply as persistently elevated blood pressure (B.P). Hypertension is identified as one of the most significant risk factors for cardiovascular diseases (CVDs)[1]. Hypertension adversely affects many organ systems throughout the body. Target organ damage includes Brain (Stroke or transient ischemic attack), Eyes (Retinopathy), Heart (Left ventricular hypertrophy, angina, prior myocardial infarction, prior coronary revascularization and heart failure). Kidney (Chronic kidney disease) and peripheral vasculature (peripheral arterial disease) [2]. The overall goal of treating hypertension is to reduce hypertension-associated cardiovascular and renal morbidity and mortality[3]. Pharmacological therapy involves prescribing of different antihypertensive drug classes. These agents can be given alone or in combination to treat the majority of hypertensive patients depending on B.P stage[4]. The role of oxidative stress in genesis and maintenance of hypertension is supported by amelioration of hypertension with antioxidant administration[5] . Vitamin C (ascorbic acid) is an essential micronutrient required for normal metabolic function of the human body which is considered a very efficient water soluble antioxidant[6].Several studies have been shown that human with essential hypertension have a decreased antioxidant capacity[7,8] .A number of epidemiological studies have been shown a negative correlation between B.P. and vitamin C level[9,10].Vitamin C might influence B.P. by acting as a free radical scavenging properly preventing prostacyclin synthetase inhibition. It has been found a lower levels of vitamin C in sustained hypertensive than normotensives human, this could be a result of greater antioxidant consumption, either for direct reactions or for regeneration of vitamin E to its reduce form, in response to an increased oxidant load associated with sustained hypertension[11] . The present study was directed to determine the beneficial effect of the antioxidant vitamin C in patients with essential hypertension treated with the calcium channel blocker (amlodipine) or with the angiotensin converting enzyme inhibitor (enalapril).

Patients and Methods

This study was carried out over nine months from November 2006 till July 2007. Many patients were interviewed according to patient's information sheet. Patients who enrolled in our study were diagnosed as they having essential hypertension and treated by specialist physicians while attending AL-Sadr Teaching Hospital in Basrah city. Ninety six patients (50 females and 46 males) with essential hypertension treated with calcium channel blocker (amlodipine), or with angiotensin converting enzyme inhibitor (enalapril), but did not reach the target blood pressure, and twenty apparently healthy subjects (11 females and 9 males) participated in this study.

Inclusion and Exclusion Criteria

The inclusion and exclusion criteria of all patients in this study are summarized as follow:

Inclusion and exclusion criteria of hypertensive patients enrolled in the study

Inclusion criteria

Patients with mild to moderate essential hypertension treated with calcium channel blocker (5mg daily of amlodipine), or with angiotensin converting enzyme inhibitor (5 mg daily of enalapril), but did not reach the target blood pressure.

Exclusion criteria

Secondary hypertension. Smoking or alcohol consumption. History of myocardial infraction (Ml), angina pectoris, heart failure (HF), thyrotoxicosis, pregnant women, diabetes mellitus, malabsorption, uncontrolled arrhythmias and significant renal, hepatic or hematological diseases.Use of nonsteroid anti-inflammatory drugs, steroids, oestrogen containing preparation (like oral contraceptive pills and hormonal replacement therapy), cyclosporine, erythropoietin and sympathomimetic containing drugs or other medications that effect blood pressure,lipid profile, ECG or antioxidant status.

Patients were divided as follows:

Group 1: twenty five patients with essential hypertension treated with amlodipine 5 mg tablet once daily.

Group 2: twenty five patients with essential hypertension received the same dose of amlodipine plus 500 mg vitamin C twice daily.

Group 3: twenty patients with essential hypertension treated with enalapril 5 mg tablet once daily.

Group 4: twenty three patients with essential hypertension received the same dose of enalapril plus 500 mg vitamin C twice daily.

Group 5: twenty apparently healthy subjects.

All patients in each group were followed up for four weeks, and mean blood pressure had

been taken for each patient before and one, two, three and four weeks after starting treatment[1]. Serum MDA, lipid profile (TC, TG, HDL-C, LDL-C and VDL-C), ECG parameters (PR, QRS, and P- wave), and heart rate have been taken for each patient before and two and four weeks after starting treatment. Twenty apparently healthy subjects have also participated in this study. The thiobarbituric acid essay of Buege and Aust (1978) was used to measure serum MDA levels [12]. The estimation of total cholesterol depends on enzymatic colorimetric method of Richmond and Allain et al.,(1974) [13,14]. The estimation of Triglycerides depends on the enzymatic colorimetric method of fossati and Principe[15]. The estimation of High Density Lipoprotein-Cholesterol depends on the enzymatic method of Burestien et al, (1970) [16]. The LDL-C was calculated by the Friedwald formula as follows[17]:
LDL-C= total cholesterol - [(HDL-C) + (triglyceride/5)].

The VLDL-C is calculated from the formula:
VLDL – C = triglyceride/5

All values are expressed as mean ± standard error of the mean. Data are entered in to computer system using Microsoft office Excel 2003 software for all mathematics and statistical analysis. the student's test was used to determine the significant difference in means of groups. $P < 0.05$ was considered to be the lowest limit of significance.

Results

Table (1) showed the serum levels of MDA in patients with essential hypertension treated with amlodipine (group 1) or with enalapril (group 3) at zero time, these values were significantly higher than their values in control group. Table (2) showed that patients received amlodipine and vitamin C (group two) or enalapril and vitamin C (group four) showed significant lowering in average mean blood pressure after two, three and four weeks of treatment as compared with their values at zero time and their values in group one or three. Table (3) showed that the addition of vitamin C caused a significant lowering in serum MDA after two and four weeks of treatment in amlodipine and vitamin C treated group (group two) or enalapril and vitamin C treated group (group four), as compared with zero time values and their values in group one or three. The lowering in serum MDA was more prominent after four weeks of amlodipine or enalapril and vitamin C treatment as compared with their values after two weeks of treatment. The serum level of MDA in patients treated with enalapril and vitamin C (group four) were significantly lower than their values in those patients treated with amlodipine and vitamin C (group two) after two and four weeks of treatment.

Table 1: Serum malondialde(µmol/I) of control group, patients treated with amoldipine (group one), or with enalapril (group three) at zero time. Values are presented as mean± standard error; p<0.05 indicated significant changes from control group.

Group	Number of patients	Serum malondialdehyde. (µmol/I)
Control group	20	0.5 ±0.03
Group one	25	1.39 ±0.04*
Group three	23	1.13 ±0.03*

* Significant at p<0.05 as compared with control group.

Table 2 : Mean blood pressure (mmHg) of patients in group one, two, three and four.

Mean blood pressure in (mmHg)				
Time	Group 1 Amlodipine n=25	Group 2 Amlodipine +vit.C n=25	Group 3 Enalapril n=23	Group 4 Enalalpril + vit.C n=23
Zero time	119.06 ±0.68	118.8 ± 0.82	117.48 ± 1.06	117.97 ± 1.02
1st week	118.62 ± 0.64	117.2 ± 0.78	117.01 ± 0.95	116.37 ± 1.02
2nd week	117.82 ± 0.61	114.4 ± 0.83 * a	116.52 ± 0.93	114.03± 0.97 *b
3rd week	117.7 ± 0.62	111.57 ± 0.85* a	116.08 ± 0.97	114.36 ± 0.94*b
4th week	117.28 ± 0.52	108.08 ± 0.78* a	115.16 ± 0.85	110.66 ± 0.93*b

*significant at p<0.05 as compared with zero time value.
a significant at p<0.05 as compared with group one.
b significant at p<0.05 as compared with group three.

Table 3 : Serum malondialdehyde (µmol/l) of patients in group one, two, three and group four.

Serum malondialdehyde (µmol/l)				
Time	Group 1 Amlodipine	Group 2 Amlodipine + vit.C	Group 3 Enalapril	Group 4 Enalalpril + vit.C
Zero time	1.39 ± 0.05	1.37 ± 0.4	1.13 ± 0.03	1.06 ±0.03
2nd week	1.34 ± 0.5	1.16 ± 0.4 * a	1.13 ± 0.03	0.97± 0.03 *bc
4th week	1.34 ± 0.04	0.83 ± 0.03* a	1.11 ± 0.03	0.77±0.03*bc

a significant at p<0.05 as compared with group one.
b significant at p<0.05 as compared with group three.
c significant at p<0.05 as compared with group two.

There was a significant change in lipid profile in hypertensive patients treated with amlodipine or enalapril at zero time as compared to their values in control group (Table 4). This change includes an elevation in serum level of (TC,TG, LDL-C and VLDL-C), while there was slight, but not significant reduction in serum level of HDL-C in hypertensive patients as compared to their values in control group. There were no significant changes in lipid profile, ECG parameters and heart rate of patients treated with amlodipine pulse vitamin C (group two) or patients treated with enalapril plus vitamin C (group four) compared to their values at zero time or to patients received amlodipine or enalapril alone (group one and two). (Table 5and 6).

Table 4 : Lipid profile parameters (TC, TG, LDL-CHDL-C and VLDL-C) of control group, patients in group one and in group three at zero time.

Lipid profile parameters	Control group n=20	Group 1 n=25	Group 3 n=23
TC (mmol/L)	4.18±0.15	5.0 ± 0.09*	4.87 ± 0.1*
TG (mmol/L)	1.94 ±0.09	2.51± 0.04*	2.43 ± 0.04*
HDL-C (mmol/L)	1.22± 0.02	1.1 ±0.02	1.08 ±0.03
LDL-C (mmol/L)	2.57 ±0.14	3.41 ± 0.09*	3.36 ±0.09*
VLDL-C (mmol/L)	0.39± 0.02	0.5 ±0.01*	0.49 ± 0.01*

* Significant at p<0.05 as compared with control group.
TC: Total cholesterol; TG: Triglyceride; HDL-C: High density lipoprotein cholesterol; LDL-C: Low density lipoprotein cholesterol; VLDL-C: Very low density lipoprotein cholesterol; N: number of patients.

Table 5 : lipid profile (TC, TG,HDL-C, LDL-C and VLDL-C) in patients treated with amlodipine (group one), patients treated with amlodipine and vitamin C (group two), patients treated with enalapril (group three), and patients treated with enalapril and vitamin C (group four).

Lipid profil parameters	Group 1 Amlodipine		Group 2 Amlodipine + vit C		Group 3 Enalapril		Group 4 Enalapril + vit C	
	Zero time	4th week	Zero time	4th week	Zero time	4th week	Zero time	4th week
TC (mmol/L)	5.02 ± 0.09	5 ± 0.09	5.03 ± 0.11	4.87 ± 0.12	4.87 ± 0.1	4.82 ± 0.1	4.89 ± 0.1	4.84 ± 0.1
TG (mmol/L)	2.51 ± 0.04	2.49±0.04	2.46 ± 0.04	2.39 ± 0.04	2.43 ± 0.04	2.39 ± 0.04	2.42 ± 0.05	2.38 ± 0.05
HDL-C (mmol/L)	1.1 ± 0.02	1.13±0.02	1.08 ± 0.02	1.15 ± 0.02	1.08 ± 0.03	1.12 ± 0.02	1.11 ± 0.01	1.18 ± 0.02
LDL-C (mmol/L)	3.41 ± 0.09	3.38±0.04	3.43 ± 0.12	3.35 ± 0.12	3.31 ± 0.09	3.25 ± 0.1	3.29 ± 0.1	3.23 ± 0.1
VLDL-C (mmol/L)	0.53 ± 0.01	0.51±0.01	0.5 ± 0.01	0.46 ± 0.01	0.5 ± 0.01	0.51 ± 0.01	0.49 ± 0.01	0.46 ± 0.01

TC: Total cholesterol; TG: Triglyceride; HDL-C: High density lipoprotein cholesterol; LDL-C: Low density lipoprotein cholesterol; VLDL-C: Very low density lipoprotein cholesterol

Table 6 : ECG parameters (PR,P-wave, and QRS) and heart rate in patients treated with amlodipine (group one), patients treated with amlodipine and vitamin C (group two), patients treated with enalapril (group three), and patients treated with enalapril and vitamin C (group four). Values are presented as mean ± standard error.

ECG parameters	Group 1 Amlodipine		Group 2 Amlodipine + vit C		Group 3 Enalapril		Group 4 Enalapril + vit C	
	Zero time	4th week	Zero time	4th week	Zero time	4th week	Zero time	4th week
HR(bpm)	68±0.16	69.28±0.92	66.64±1.18	71.64±1.11	69.04±1.03	71.2±0.93	68.78±1.07	72.12±0.98
PR(ms)	167.12±3.33	167.84±3.4	167.52±2.74	162.44±2.28	167.04±2.59	163.57±2.43	169.39±2.18	163.83±1.96
P-wave(ms)	95.84±1.81	98.12±1.64	94.62±2.33	100.16±1.86	95.91±1.83	101.39±1.67	98.39±1.92	103.83±1.69
QRS (ms)	103.32±2.66	105.84±2.73	102.8±2.89	108.2±2.41	104.09±2.26	107.48±2.19	103.83±2.14	108±2.1

Discussion

Results in this study showed that patients with essential hypertension treated with amlodipine or enalapril had significantly higher levels of malondialdehyde as compared to control group. This is in agreement with other worker who demonstrated an increase in ROS production in patients with essential hypertension, based in general, on increased levels of serum thiobarbituric acid-reactive substance and other biomarkers of lipid peroxidation and oxidative stress[11]. Animals and human studies demonstrated that reactive oxygen species play an important pathophysiological role in the development of hypertension, this is due to excessive superoxide formation, decreased nitric oxide bioavailability in the vasculature and kidney, and ROS- mediate cardiovascular remodeling[18] . In both endothelial and vascular smooth muscle cells mechanical stretch has been shown to activate the NAD(P)H oxides which is an important source for ROS production[19]. Our results showed that patients with essential hypertension treated with amoldipine or enalapril showed a significant change in lipid profile as compared with control group. This is consistent with Sarkar et al (2007), who showed that TC, TG, and LDL-C in forty hypertensive patients were significant higher than their values in thirty normotensive and healthy subjects[20] .The addition of vitamin C in a dose of 1000 mg for one month for patients with essential hypertension treated with amlodipine or enalapril produced significant lowering in serum levels of MDA after two and four weeks of treatment, this lowering could be due to the activity of vitamin C as free radicals scavenger, that helps to terminate the free radicals damaging effect and to prevent lipid per- oxidation[21] .The lowering of serum level of MDA in patients treated with enalapril plus vitamin C was significantly greater than that observed in amlodipine plus vitamin C treated patients after two and four weeks of treatment. This could be due to the different mechanism of action of angiotensin converting enzyme inhibitor (enalapril) and calcium channel blockers (amlodipine). Where the activation of rennin-angiotensin system has-been proposed as mediator of NAD(P)H oxidase activation and ROS production[22] . In fact, some mechanism of action of angiotensin - converting enzyme inhibitors have been attributed to NAD(P)H oxidase inhibition and decreased in ROS production[23] . Likewise the addition of vitamin C in the same dose and for the same period had result in significant loweing effect in mean blood pressure after two, three and four weeks of treatment. This is consistent with Hajjar et al (2002), who demonstrated that both systolic and diastolic blood pressure decreased during the vitamin C supplementation phase in thirty one patients treated with antihypertensive drugs and vitamin c[24] and with Jennifer (2004) who showed that the antioxidant supplementation might reduce dependence on blood pressure lowering effect of calcium channel blocker nifedipine in 58 adults with essential hypertension [25] .The role of vitamin C in reducing blood pressure has different ways including, its direct free radicals scavenging ability and termination their damaging effect on blood vessels and prevent lipid peroxidation[26] ,the second way is its ability to regulate nitric oxide synthase that generate nitric oxide a potents vasodilator that play a key role in controlling the cardiovascular system, the third way is its ability to regulate the NAD(P)H oxidase activity and ROS production, the fourth way is its ability to regulate antioxidant enzyme including superoxide dismutase and glutathione, in addition to their ability to reduce vitamin E to its active form; the fifth way is that ascorbic acid increase tetrahydrobiopterin an important cofactor of nitric oxide synthase enzyme by

preventing its oxidation (chemical stability)[27]. Finally it has been demonstrated that oxidative stress and hypertension are closely associated with higher sympathetic activity[28] . Thus, it could be argued that vitamin C induce change in blood pressure observed in the present study may be in part attributed to its inhibitory effect on the sympathetic activity or antioxidant induced change in the release of norepinephrine from the nerve terminal[29] .In the first week of treatment with vitamin C there was no significant lowering effect on the mean blood pressure, this would indicate that the blood pressure lowering effect of vitamin C was in long-term, where long period required to restore nitric oxide cofactor (tetrahydrobiopterin) level by preventing its oxidation[26] .In conclusion the lowering in mean blood pressures were not differ significantly when comparing patients treated with enalapril plus vitamin C to those treated with amlodipine plus vitamin C.

Reference

1. Chobanian AV, Bakris GL, Black HR, et al. Seventh Report of The Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. Hypertension 2003; 42: 1206-1252.
2. Joseph J S, Barry L C (Eds); Cardiac and vascular disorders, essential hypertension, in: Applied therapeutics: the clinical use of drugs. Mary A, Lloyd Y, Wayne A, Joseph B, 8th edition, Philadelphia, Baltimore, New York, London, 2005; (1): 1-39(13), 1-31(20).
3. Wright J M, Lee C H, Chambers G.K. Systematic review of antihypertensive therapies: dose the evidence assist in choosing a first- line drug. Can. Med. Assoc. J. 1999; 161:25-32.
4. Wright J M Chossing a first- line drug in the management of elevated blood pressure: what is the evidence 3: Angiotensin- converting enzyme inhibitors? CMAJ.2000; 163: 293-296.
5. Schnackenberg CG, Welch WJ, Wlicox CS. Normalization of blood pressure and renal vascular resistance in SHR with amembrane-permeable superoxide dismutase mimetic: role of nitric oxide.Hypertension 1998; 32: 59-64.
6. Grievink L, Smit HA, Ocke Mc, et al. Dietary intake of antioxidant (pb)-Vitamins, respiratory symptoms and pulmonary function: the MORGEN study. Thorax. 1998; 53: 166-171.
7. Galley HF, Thornton J, Hovvdle PD., etal. Combination oral antioxidant supplementation reduces blood pressure. Clin. Sci. 1997; 92: 361-365.
8. Russo C, Olivier O, Girelli D, etal. Antioxidant status and lipid peroxidation in patients with essential hypertension. J. Hypertens. 1998; 16: 1267-1271.
9. Moran JP,Cohen L, Greene JM, et al. Plasma ascorbic acid concentrations relate inversely to blood pressure in human subjects. Am. J. Clin. Nutr. 1993; 57: 213-217.
10. Ness AR, Khaw KT, Bingham S, et al. Vitamin C status and blood pressure. J. Hypertens. 1996; 14: 503-508.
11. Redon J, Olive MR, Tomos C, et al. Antioxidant activities and oxidative stress by products in human hypertension. Hypertension 2003; 41: 1096-1101.
12. Bueg J A, Aust SD. Microsomal lipid peroxidation. Method enzymol 1978; 52: 302-303.
13. Richmond W. Preparation and properties of a cholesterol oxidase from Nocardia SP and application to the enzymatic assay of total cholesterol in serum. Clin. Chem. 1973; 33: 195-201.
14. Allain C C, Poon L S, Chan C S, et al. Enzymatic determination of total serum cholesterol. FUPC. 1974; 20: 470- 475.
15. Fossati P, Prencipe L. Serum triglyceride determined colorimetrically with an enzyme that produce H202. Clin. Chem. 1982; 28(10): 2077-80.
16. Burstein M, Scholnick H R, and Morfin R. Rapid method for the isolation of lipoproteins from human serum by precipitation with poly anions. J. Lipid Res. 1970; 11: 583-595
17. Rifai N, Bachorik P S, Alberts J J (Eds); Lipids, Lipoproteins, and Apolipoproteins, in:Titz textbook of clinical chemistry. Burits C A, Ashwood ER, 3rd edition, W B Sounders Co, London, 1999; pp: 809-30, 837-47.
18. Christopher S. Oxidative stress and nitric oxide deficiency in the kidney: a critical link to hypertension. Am. J. Physiol. Regul. Integr. Comp. physiol. 2005; 10: R913-R935.
19. Majid D S, et al. Inhibition of nitric oxide synthase enhances superoxide activity in canine kidney. Am. J. Physiol. Regul. Integre. Comp. Physiol. 2004; 287: R27-R32.
20. Sarkar D, Latif SA, Uddin MM. Studies on serum lipid profile in hypertensive patient. Mymensingh Med. J. 2007 Jan; 16(l):70-6.
21. Morikawa K, Shimokawa H, Matoba T, et al. Pivotal role of Cu, Zn-superoxide dismutase in endothelium-dependent

hyperpolarization. J. Clin. Invest. 2005; 112: 1871-1879.

22. Vito M, Zhong H, Shaohua Ye. Oxidative stress Mediates angiotensin II-dependent stimulation of sympathetic nerve activity. Hypertension 2005; 46: 533.

23. Rodney J, Melissa C, Luis A, et al. Effects of captopril on the renin angiotensin system, oxidative stress, and endothelin in normal and hypertensive rats. Hypertension. 2005; 46(4): 943-947.

24. Hajjar IM, George V, Sasse EA, et al-Arondomized, double-blind, controlled trial of vitamin C in the management of hypertension and lipids. Am. J. Ther. 2002; 9(4): 289-93.

25. Jennifer W. Antioxidant may help lower blood pressure. Liu x. Life. Science 2004; 74: 855- 862.

26. Sibel 0, Pascal P, Ulvi B. Vitamins reverse endothelial dysfunction through regulation of eNOS and NAD (P)H oxidase activities. Hypertension 2003; 41: 534.

27. Livius V, Sheldon M, Darcy R, et al. Long-term vitamin C treatment increase vascular tetrahydrobiopterin levels and nitric oxide synthase activity. Circulation Research. 2003; 92: 88.

28. Fink G D Tempol lowers blood pressure and sympathetic nerve activity but not vascular Õ2 in DOCA-salt rats. Hypertension 2004; 43: 329-334.

29. Jou S- B, Cheng J T The role of free radicals in the release noradrenalin from mysentric nerve terminals of guinea-pig ileum. J. auto. Merve. syst. 1997; 66: 126-130.