A clinical study showing altered antioxidants profile in patients with hypertension

Abad Khan[1] and Zafar Iqbal[2]
[1]Department of Pharmacy, University of Swabi, Peshawar, Pakistan
[2]Department of Pharmacy, University of Peshawar, Peshawar, Pakistan

Abstract: We compared the plasma antioxidants level of normal control group with that of hypertensive patients in order to test the hypothesis "that antioxidants level has been diminished in hypertensive patients and that antioxidants are interconnected with each other making a network. The plasma and red blood cells antioxidants level of newly diagnosed hypertensive patients [(n=30), (mean age 53 years), (mean systolic BP 158 mmHg, mean diastolic BP 100 mmHg)] were compared to those of the control subjects [(n=30), (mean age 50 years), (mean systolic BP 126 mmHg, mean diastolic BP 90 mmHg)] using liquid chromatography linked with electrochemical detector (HPLC-ECD). The data was analyzed by Minitab software at a 95% confidence interval ($p \leq 0.05$) as significant. The comparison between the two groups was made applying 2-sample and paired *t*-test. The individual concentration of antioxidants in both plasma and red blood cells of hypertensive patients was lower in comparison with that of control group while the oxidized/ reduced ratios of these antioxidants were higher in hypertensive patients in comparison with that of control group. It is concluded that antioxidants level had been diminished in the hypertensive patients when compared with control group. The overall concentration of all antioxidants has been diminished in the oxidative stress induced pathological conditions which confirm that the studied antioxidants are working in a network. This study may be helpful for the recommendation of antioxidants intervention.

Keywords: Antioxidants, oxidative stress, hypertension, plasma, control group, pathological conditions, HPLC

INTRODUCTION

Oxygen plays a vital role in the life of aerobic organisms via performing different metabolic functions inside the cells under normal conditions. Reactive oxygen and nitrogen species (ROS/RNS) are generated in human body constantly via two processes mainly. Primarily these are generated accidently through leakage of electrons from mitochondrial electron-transport chain, nuclear membrane, endoplasmic reticulum (prostaglandin synthesis, xenobiotic metabolism), and via hepatocytes while the secondary cause of free radicals generation is the biological processes. Endogenous sources that generate oxygen and nitrogen oxidants in biological systems are enzymes such as xanthine oxidase, D-amino acid oxidase, D-glucose oxidase, cytochrome P-450 enzymes, nitric oxide synthases (NOSs), and myeloperoxidase, free transition metals along with mitochondrial electron-transport chain that leaks electrons during normal metabolic processes and different pathological conditions (Maeda, 2009; Gutteridge, 1993; Kaul *et al*., 2001; Levy, 1996; Venditti *et al*., 2002). Exogenous sources include direct exposure to oxygen molecule (Lee *et al*., 2001), various ionizing and non-ionizing irradiations, smoke (Rao and Davis, 2001; Koren, 1995), drugs and anesthetic agents; (Victorin, 1994; Naito et., 1998) and xenobiotics such as toxins, insecticides and herbicides and the by-products of metabolism of different chemicals like alcohol and mustard gas are the main sources of ROS (Victorin, 1994; Naito et., 1998; Ray *et al*., 2001; Elsayed *et al*., 1992).

Aerobes including humans are capable of tolerating oxygen via electron-transport chain, and body's antioxidant system comprised of both enzymatic and non-enzymatic antioxidants (Kanner and Lapidot, 2001). The system is comprised of two main groups i.e., antioxidant enzymes and low-molecular-weight antioxidants (LMWA). Major antioxidant enzymes are SOD, catalase and glutathione peroxidase. Non-enzymatic antioxidants include various fat-soluble vitamins and water soluble antioxidants that protect cell membrane of human body from peroxidation (Kohen and Gati, 2000; Gul *et al*., 2000). Thiol compounds such as cysteine (Cys), cystine (CySS), methionine (Met), N-acetylcysteine (NAC), homocysteine (Hcy), reduced (GSH) and oxidized glutathione (GSSG) have a pivotal role in the human body, e.g. protection against reactive oxygen and nitrogen species, control of gene expression, heavy metal detoxification, markers of various health disorders and signal transduction (Semeraro *et al*., 2009). *In vitro* studies have shown that both hydrophilic and lipophilic antioxidants work together, recycling each other making a network and effective body defense system ameliorating oxidative damage induced by free radicals (Potesil *et al*., 2005). It has been well established that increased oxidative stress and low antioxidants level are occurring in hypertension and diabetes mellitus (Tan *et al*., 2005; Borcea *et al*., 1999; Choi *et al*., 2008). There is a need to

**Corresponding author:* e-mail: drabadkhan@uoswabi.edu.pk

evaluate the complete antioxidant profile of patients with respect to normal subjects and highlight the basic mechanisms of antioxidants biochemical action that will lead to an optimal antioxidant therapy in oxidative stress associated pathologies. This research will mainly focus on the accurate and precise HPLC quantification of various antioxidants in patients having hypertension that will evaluate effects of oxidative stress on the body antioxidants level as well as correlation of oxidative stress to hypertension.

MATERIALS AND METHODS

Selection of Subjects

Age- and gender-matched subjects including both normal control group and newly diagnosed hypertensive patients have been selected randomly from general population and out-patients departments (OPDs), of cardiology ward of Hayatabad Medical Complex (HMC), respectively under the supervision of qualified staff from for this clinical study in a period of one year from September 2010 to September 2011 after detailed interviews including questions related to their social life, medical history, and nutritional history. A written informed *consent letter* has been signed from each participant at the beginning of this study.

Inclusion & exclusion criteria

Inclusion in this study was based on the normal physical and biochemical evaluation of laboratory tests including, blood pressure (BP), fasting blood glucose (FBG), blood cholesterol, serum creatinine, liver function tests (LFTs), lipid profile, serum electrolytes profile, routine urinalysis, complete blood count (CBC) and blood Hb. The tests for both normal control group and patients were carried out in pathology laboratory of Hayatabad Medical Complex (HMC), Peshawar. The information regarding the patient history, laboratory evaluations, and diagnosis were recorded on *patient history record form***.** The normal control subjects having neither any type disease nor smokers or taking multivitamins, antioxidants, alcohol, and any other medicines were included in this study. The patients having hypertension and diagnosed for the first time as hypertensive were selected from the clinical setup. Only those patients who were diagnosed for the first time as hypertensive and who having neither any type disease nor smokers or taking multivitamins, antioxidants, alcohol, and any other medicines were included in this study. Normal control subjects having history of any disease, smoking, alcohol consumption, taking multivitamins/antioxidants or any other therapy and patients having any other pathology except hypertension were excluded from the study.

Measurement of blood pressure

Blood pressure in normal control group was measured for a week in the sitting position with a mercury sphygmomanometer following the standard recommend-dations of the British Hypertension Society. The mean blood pressure (MBP) was taken as the average of three readings. Participants whose blood pressure was in the range of 80-139 mm Hg were included in the study. The data of the hypertensive patients was obtained from the Hayatabad Medical Complex (HMC), Peshawar (Pakistan).

Biological samples collection

Blood samples (≈4mL) were collected in the morning after overnight fasting from the veins of both patients and normal control groups (age- and gender-matched) in Gel and clot activator tubes (≈5mL), and ethylene diamintetraacetic acid (EDTA) tubes (≈5mL) and borosilicate glass tubes (≈5mL) for serum, plasma and whole blood samples, respectively. The study protocol was approved by the ethical committee of Department of Pharmacy, University of Peshawar and ethical committee of Hayatabad Medical Complex (HMC), Peshawar (Pakistan). Whole blood, plasma and erythrocyte samples were added an equal volume of 10% MPA aqueous solution immediately after separation and vortexed.

Serum samples preparation for the determination of Vitamins A & E

Serum samples were prepared as reported by khan *et al*., 2010 (Khan *et al*., 2010). Since both vitamins are light and heat sensitive, antioxidants such as ascorbic acid and BHT were incorporated into the samples, during sample preparation as stabilizers, and the process of sample preparation and handling was carried out in dim light and at room temperature (25°C) (Khan *et al*., 2010).

Plasma samples preparation for the quantification of LA & DHLA

Samples preparation was carried out as reported by Khan *et al*., 2011. The clear solution was separated and transferred to autosampler vial and 20 µL sample was injected into HPLC system (Khan *et al*., 2011).

Plasma & erythrocytes (RBCs) samples preparation for the quantification of ascorbic acid and aminothiols

Plasma and RBCs samples were prepared as reported by khan *et al*., 2011. Liquid-liquid extraction from plasma and erythrocytes was carried out as reported by khan *et al*., 2011. After centrifugation the clear supernatant was separated, transferred to auto sampler vial, followed by injection (5 µL) into HPLC system (Khan *et al*., 2011).

Methods of analysis

Various HPLC methods including HPLC-UV/Vis and HPLC-ECD have been developed for the determination of these antioxidants. These methods were optimized and validated in accordance with standard guidelines (Khan *et al*., 2010; Khan *et al*., 2011; Khan *et al*., 2011).

Table 1: Plasma levels of various antioxidants in control group and hypertensive patients.

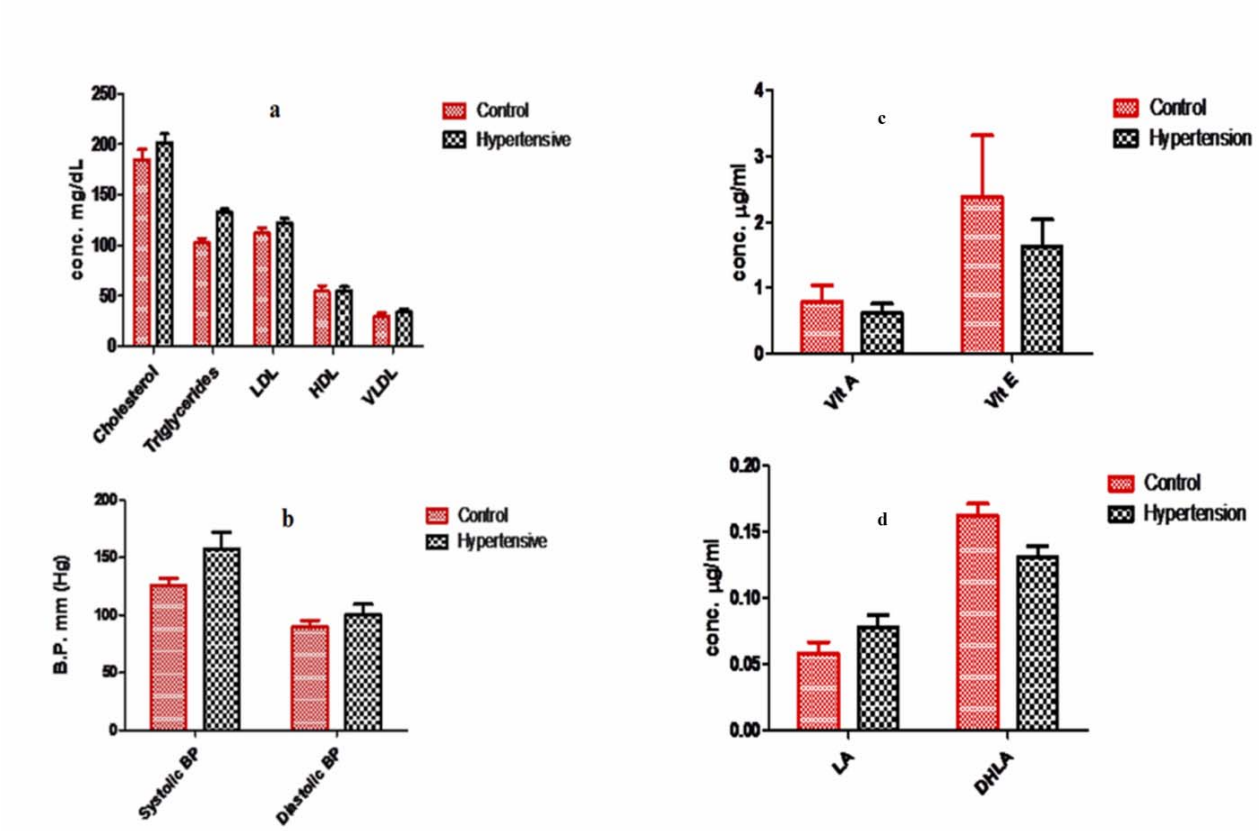
Parameters	Control Subjects (n=30) (µg/mL)		Control Subjects (n=30 (µmol/L)		Hypertensive Patients (n=30) (µg/mL)		Hypertensive Patients (n=30) (µmol/L)		*p*-value
Conc.	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD	
Vitamin A	0.7970	0.2472	2.782	0.862	0.6179	0.143	2.157	0.499	p <0.0013
Vitamin E	2.386	0.937	5.549	2.175	1.6380	0.399	3.803	0.926	p<0.0003
Lipoic Acid (LA)	0.05845	0.0082	0.283	0.039	0.0779	0.0094	0.377	0.045	p<0.29
Dehydrolipoic Acid (DHLA)	0.16281	0.0088	0.782	0.042	0.13145	0.0081	0.629	0.039	p<0.0000
Ascorbic Acid (AA)	5.646	1.519	32.060	8.620	3.385	1.000	19.22	5.68	p<0.0000
Dehydroascorbic acid (DHA)	0.7678	0.0935	4.413	0.537	1.1723	0.2826	6.73	1.625	p<0.0000
Cystine (CySS)	7.628	2.349	31.74	9.78	6.396	2.191	26.62	9.11	p<0.040
Cysteine (Cys)	24.585	3.406	203.18	28.11	14.422	3.338	119.04	27.55	p<0.0000
Homocysteine (Hcy)	1.2567	0.4335	9.298	3.20	1.6444	0.324	12.16	2.396	p<0.0003
Methionine (Met)	3.744	1.015	25.092	6.802	3.060	0.640	20.508	4.289	p<0.0031
N-acetylcysteine (NAC)	0.7902	0.277	4.840	1.697	0.5412	0.249	3.316	1.525	p<0.0006
Glutathione Reduced (GSH)	1.8021	0.4221	5.863	1.373	1.2813	0.331	4.168	1.077	p<0.0000
Glutathione Oxidized (GSSG)	0.9422	0.245	1.537	0.399	1.1123	0.223	1.815	0.364	p<0.0067

Table 2: RBCs profile of antioxidants and aminothiols in control and hypertension groups.

Parameters	Control Group (n=30) (µg/mL)	Control Group (n=30) (µmol/L)	Hypertensive Patients (n=30) (µg/mL)	Hypertensive Patients (n=30) (µmol/L)	*p*-value
	Mean ± SD		Mean ± SD		
Ascorbic acid (AA)	5.878±1.460	33.375±8.289	3.886±1.013	22.065±5.752	p□0.0001
Dehydroascorbic acid (DHAA)	0.730±0.085	4.193±0.488	0.999±0.184	5.738±1.057	p□0.0001
Cysteine (Cys)	1.188±0.196	9.806±1.618	1.016±0.189	8.386±1.560	p□0.0001
Cystine (CySS)	4.107±0.894	17.091±3.720	4.337±0.907	18.048±3.774	p=0.0005
Homocysteine (Hcy)	0.532±0.061	3.935±0.451	0.738±0.101	5.459±0.747	p□0.0001
Methionine (Met)	3.627±0.554	24.308±3.713	3.331±0.555	22.324±3.719	p=0.001
N-acetylecysteine (NAC)	0.178±0.026	1.090±0.159	0.131±0.031	0.803±0.189	p□0.0001
Glutathione Reduced (GSH)	701.52±16.37	2282.702±53.267	607.0±55.90	1975.139±181.895	p□0.0001
Glutathione Oxidized (GSSG)	17.485±3.291	28.541±5.372	29.812±4.526	48.662±7.388	p□0.0001

Table 3: Ratio of various anti-oxidants in Plasma and RBCs of Control group and Hypertensive patients.

	PLASMA SAMPLES					RBCs SAMPLES				
Parameters ratio	Control Group (n=30)		Hypertension Group (n=30)			Control Group (n=30)		Hypertension Group (n=30)		
	Mean	SD	Mean	SD	*p-value*	Mean	± SD	Mean	± SD	*p*-value
VA/VE	0.360	0.134	0.394	0.119	0.31	------	------	-------	--------	-------
VA/AA	0.146	0.051	0.198	0.077	0.004	------	------	-------	--------	-------
VE/AA	0.448	0.193	0.518	0.176	0.15	------	------	-------	-------	-------
LA/DHLA	0.358	0.042	0.604	0.808	0.0001	------	------	------	-------	-------
LA/AA	0.011	0.003	0.024	0.024	0.0076	------	------	-------	-------	-------
AA/DHA	7.411	2.041	3.172	1.476	0.00001	8.182	2.390	4.100	1.450	p□0.0001
Cys/CySS	3.223	1.449	2.255	1.524	0.01	0.289	0.022	0.234	0.0208	p□0.001
AA/CySS	0.836	0.422	0.600	0.258	0.012	1.495	0.496	0.910	0.209	p□0.0001
GSH/GSSG	1.971	0.462	1.1707	0.301	0.00001	41.58	8.250	20.59	1.780	p□0.0001
AA /GSH	3.136	0.117	3.005	0.127	0.060	0.008	0.002	0.006	0.001	p□0.0001
Hcy/Met	0.336	0.043	0.537	0.051	0.01	0.147	0.011	0.222	0.018	p□0.0001
AA+DHA	6.413	1.542	4.557	0.850	0.00001	6.609	1.457	4.885	0.946	p□0.0001



Note: Mean ± SD values are shown by graphs and standard bars, respectively.

Fig. 1: a. Plasma level (Mean ± SD values) of various biochemical parameters in control group and hypertensive patients. b. Graphical presentation (Mean ± SD values) of SBP and DBP of control group with respect to hypertensive patients. c. Mean serum level of vitamin A and vitamin E in control group and diabetic patients with standard bars showing (SD). d. Mean plasma level of Lipoic acid (LA) and Dihydrolipoic acid (DHLA) in control group and hypertensive patients with standard bars showing SD.

Statistical interpretation and correlations of data

Various statistical tools such as mean (X) ± standard deviation (SD), standard error of mean (SEM), and relation standard deviation (%RSD) were applied for the quantification of different antioxidants in human serum, plasma, erythrocytes and whole blood samples. The differences and correlation among different antioxidants in the same group and different groups were established applying unpaired and paired student's *t*-tests and one-way analysis of variance (ANOVA), considering $p < 0.05$ as significant.

RESULTS

Subject parameters

There was no significant difference between normal control and hypertensive patients in terms of age, body weight, hemoglobin, hematocrit, creatinine, protein, FBG, and HDL values. However hypertensive patients had significantly higher values (p℟0.001) of systolic blood pressure (SPB), diastolic blood pressure (DPB), total cholesterol, triglyceride, low density lipoprotein (LDL), and very low density lipoprotein (VLDL) when compared with age and sex-matched normal control group (Supplemental Data table 1). The data has graphically presented in fig. 1a. SBP had high degree variation in comparison with DBP between the normal control and hypertensive groups, respectively as shown in fig. 1b.

Quantification of vitamins A (All-trans-retinol) & E (α-Tocopherol) in serum samples of control group and hypertensive patients

The concentration of all-trans-retinol and α-tocopherol in control subjects and patients was determined considering 95% confidence interval (p□0.05) as significant. The values of vitamins A and E in hypertensive patients with respect to control group are given in table 1 and graphically represented by fig. 1c.

Quantification of Plasma Lipoic acid (LA) and Dihydrolipoic acid (DHLA) in Control Group and Hypertensive Patients

Lipoic acid (LA) and dihydrolipoic acid (DHLA) plasma level were measured in control group and hypertensive

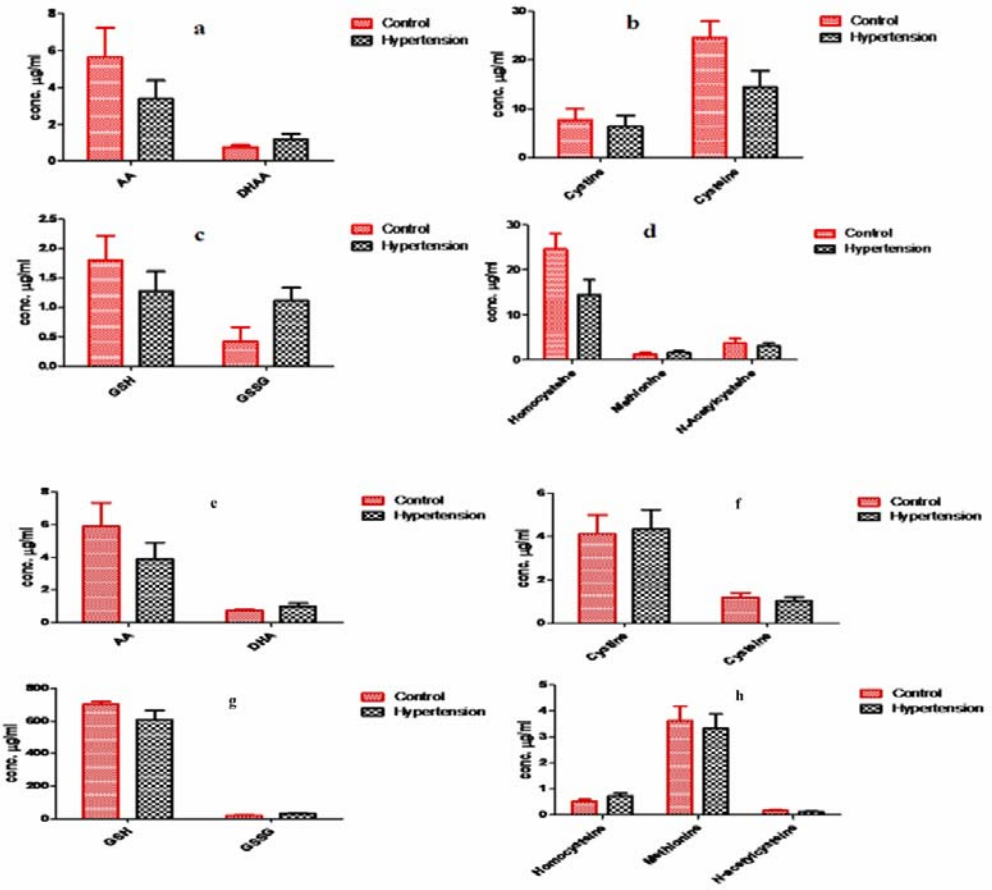


Fig. 2: a. Mean plasma level of ascorbic acid (AA) and dehydroascorbic acid (DHA) in control group and hypertensive patients with bars showing SD. b. Mean plasma level of cystine (CySS) and cysteine (Cys) in control group and hypertensive patients with standard bars showing SD. c. Mean plasma level of glutathione reduced (GSH) and glutathione oxidized (GSSG) in control group and hypertensive patients with standard bars showing SD. d. Mean plasma level of homocysteine (Hcy), methionine (Met), and N-Acetylcysteine (NAC) in control group and hypertensive patients with standard bars showing SD of mean values. **e.** Mean RBCs level of Ascorbic acid (AA) and Dehydroascorbic acid (DHA) in control group and hypertensive patients with standard bars showing SD of mean values. f. Mean RBCs level of cystine (CySS) and cysteine (Cys) in control group and hyperyensive patients with standard bars showing SD of mean values.g. Mean RBCs level of GSH and GSSG in control group and hypertensive patients with standard bars showing SD of mean values. h. Mean RBCs level of Homocysteine, methionine and N-acetylcysteine in control group and hypertensive patients with standard bars showing SD of mean values.

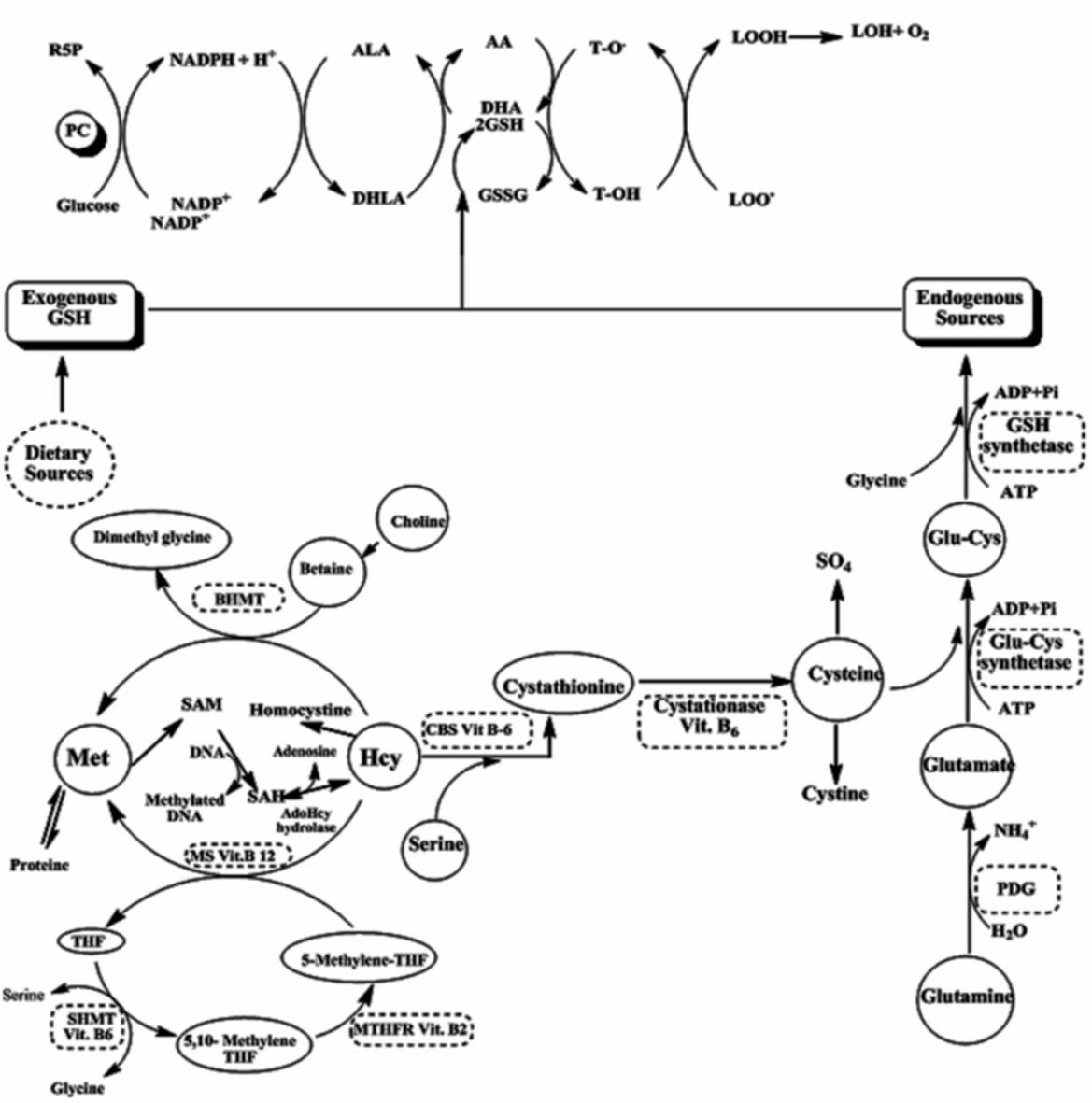
patients. The LA and DHLA values of control group and hypertensive patients are given as mean (±SD) in table 1 and graphically presented by fig. 1d.

Quantification of Plasma Ascorbic acid (AA), Dehydroascorbic acid (DHA), and Aminothiols in Control Group and Hypertensive Patients

Plasma AA, DHA, and aminothiols values were determined in control group and hypertensive patients. The respective values expressed as mean (±SD) are given in table 1 and represented graphically in fig. 2, respectively. The respective plasma values of AA and DHA are given in table 1 and represented by fig. 2a. The mean (±SD) plasma Cys, and CySS are given in table 1 and represented by fig. 2b. Similarly obtained plasma GSH and GSSG are given in table 1 and represented by fig. 2c while that of Hcy, Met, and NAC are given in table 1 and represented by fig. 2d.

Quantification of Vitamin C and Aminothiols in RBCs of Control Group and Hypertensive Patients

Ascorbic acid (AA), dedydroasorbic acid (DHA), cystine (CySS), cysteine (Cys), methionine (Met), homocysteine (Hcy), N-acetylcysteine (NAC), GSH, and GSSG were determined in the erythrocytes (RBCs) of control group and hypertensive patients using HPLC-ECD methods. The concentrations of the analytes were expressed as mean (±SD) as given in table 2. The mean (±SD) values of these analytes are graphically represented in fig. 2 e, f, g, and h, respectively. The overlays of chromatograms



THF; Tetrahydrofolate. SAM; S-adenosylmethionine. SAH; S-adenosylhomocysteine. MS; Methionine Synthase. CBC; Cystathionine beta synthase. MTHFR; Methylenetetrahydrofolate reductase. Glut-Cys; glutamylcysteine. Cys-Glu; cysteinylglycine. GSH-P_X; Glutathione Peroxidase. GSH-R; Glutathione reductase. GSH-T; Glutathione S-Transferase. $LOO^{\bullet}$; Lipid peroxyl radical; LOOH; Lipid hydroperoxid.

Fig. 3: Antioxidants network and their inter-correlation

showing the serum concentration of vitamins A/E, plasma profile of LA/DHLA, plasma profile of AA/DHA and aminothiols, and RBCs profile of AA/DHA and aminothiols in control group and patients are given (See Supplemental Data fig. 1 a, b, c, and d), respectively.

Correlation Studies of Various Antioxidants in plasma and RBCs of Control Group and Hypertension Group

The correlation between plasma and RBCs concentration of various antioxidants between control group and hypertension group was determined. The correlation of various important antioxidants plasma and RBCs ratios in control group and hypertensive patients have been shown in table 3 and represented graphically (See Supplemental Data fig. 2 and 3), respectively.

DISCUSSION

The data was analyzed by Manitab software at a 95% significance level. The comparison among the control group and hypertensive patients was made applying 2 sample t-test and paired t-test using Minitab Software.

The vitamins A and E concentration (Mean ± SD) were found in normal ranges in control group as reported in other research studies (Maeda, 2009; Gutteridge, 1993; Kaul *et al*., 2001; Thibeault *et al*., 2009). The slight differences found may be due to certain parameters such as body mass index, diet, gender, and sampling time that directly or indirectly affects the vitamin A concentration as shown in table 1 and fig. 1c. The obtained plasma values of LA and DHLA are parallel with the reported values (Teichert and Preiss, 1992). The concentration of the DHLA in hypertensive patients was significantly lower when compared with the control group as given in table 1 and fig. 1d. In contrast to LA plasma level small variations were observed for DHLA plasma values in hypertensive patients. The plasma AA, DHA and thiols compounds were quantified in control group and hypertensive patients as given in table 1 and fig. 2. The greater variation in AA concentration was found in control group. However these values are within the normal range as reported by others (Karlsen *et al*., 2005; Melnyk *et al*., 1999; May *et al*., 1995; Nolin *et al*., 2007; Jacobsen *et al*., 1994; Ivanov *et al*., 2001; Pastore *et al*., 1998) (fig. 2a). Plasma Cys and CySS profile showed the highest values of both CySS and Cys in control group in comparison with their respective values in hypertensive patients (Karlsen *et al*., 2005; Melnyk *et al*., 1999; May *et al*., 1995; Nolin *et al*., 2007; Jacobsen *et al*., 1994; Ivanov *et al*., 2001; Pastore *et al*., 1998) (fig. 2b). Plasma concentration of glutathione (GSH) was in the normal range as reported by other studies (Karlsen *et al*., 2005; Melnyk *et al*., 1999; May *et al*., 1995; Nolin *et al*., 2007; Jacobsen *et al*., 1994; Ivanov *et al*., 2001; Pastore *et al*., 1998) (fig 2c). The higher plasma level of GSH was reported for control group with greater variations in the individual concentration in comparison with patients where smaller plasma level was found with little variations in the individual plasma GSH concentration ($p<0.0001$). The plasma values of homocysteine in hypertensive patients having a significant difference when compared with its plasma values of control group ($p<0.0003$). The significant differences of plasma Met were there for hypertensive patients when compared with control group ($p<0.0031$) (Melnyk *et al*., 1999; May *et al*., 1995; Nolin *et al*., 2007; Jacobsen *et al*., 1994; Ivanov *et al*., 2001; Pastore *et al*., 1998) (fig. 2d). Similarly erythrocytes (RBCs) antioxidants profile of control group and hypertensive patients were in normal range and parallel to the reported values, (Michaelsen *et al*., 2009; Cereser *et al*,., 2001; Unt *et al*., 2008; Giustarini *et al*., 2008; Mercier *et al*., 2006; Johnson *et al*., 1951). The higher RBCs level of AA was found in control group with greater variations in individual concentration in comparison with hypertensive patients ($p<0.0001$) (table 2 and fig. 2e). The total ascorbic acid (AA + DHA) concentration in control group was higher than hypertensive patients, however; the DHA concentration of RBCs was higher in patients than control group. The highly significant difference was found in Cys concentration between control group and hypertensive patients ($p<0.0001$). The difference in RBCs, CySS concentration between control group and hypertension group was highly significant ($p= 0.0005$). The higher Cys values were observed in control group while CySS values were higher in hypertensive group. The observed RBCs values of these analytes were in normal range and parallel to the reported values (Giustarini *et al*., 2003) (table 2 and fig. 2f). The highly significant differences in RBCs, GSH concentration were found between control group and hypertension group ($p<0.0001$). The GSH values of control group were higher than hypertension group. The highly significant GSSG concentration variations in RBCs were found between control group and patients ($p<0.0001$). The higher GSSG values were reported in hypertension group. The GSH level is higher in control group in comparison with patients, while GSSG values are higher in patients than its respective values in control group; however the total glutathione level (GSH + GSSG) was higher in control group with respect to patients (tables 2 and fig. 2g). The differences in Hcy values were highly significant in hypertensive patients when compared with control group ($p<0.0001$). The higher Hcy values were reported in RBCs of hypertension group with respect to control group. The differences in RBCs, Met concentration were highly significant for hypertensive patients ($p=0.001$) when compared with RBCs, Met values of control group ($p=0.043$). The higher Met values were reported for control group in comparison with hypertensive patients (table 2 and fig. 2h). The overlays of chromatograms showing the serum concentration of vitamin A and vitamin E, plasma values of LA and DHLA, plasma profile of AA, DHA and thiols, and RBCs profile of AA, DHA and thiols obtained from control group and hypertensive patients are given (See Supplemental Data fig. 1).

The ratios of various antioxidants concentration in serum/plasma are given in table 3 and graphically represented (See Supplemental Data fig. 2). The variation in the ratios of vitamin A with respect to vitamin C was highly significant ($p=0.0037$), showing the greater decrease in vitamin C values with respect to vitamin A concentration in hypertensive patients when compared with their respective values in control group. The increase in LA plasma values while decrease in AA plasma values occurred in hypertensive patients. Similarly decrease in AA to DHA plasma concentration ratio from control group to hypertensive group has shown the highly significant decrease in AA values and highly significant increase in DHA values in hypertensive patients with respect to control group ($p=0.0001$). The plasma concentration ratio of Cys/CySS has been decreased from control group to hypertensive patients showing a significant decrease in plasma Cys values and an increase in plasma CySS values in hypertensive patients when

compared with their respective plasma values of control group as given in table 3. It has been observed that GSH level decreased while GSSG level increased in plasma of hypertensive patients when compared with their respective plasma values of control group. The observed values are parallel to the reported values (Klemm *et al*., 2001; Michaelsen *et al*., 2009; Cereser *et al*., 2001; Unt *et al*., 2008). Since the total ascorbic acid plasma concentration (AA+DHA), in control group was higher than its plasma concentration of hypertensive group (p□0.0001), therefore there was greater decrease in plasma AA values in comparison with GSH values in hypertensive patients as given table 3. Hcy/Met ratios in plasma of control group and hypertensive patients showing the increase in Hcy and decrease in Met level in plasma of hypertensive patients with respect to their plasma values in control group as shown in table 3. The higher Hcy/Met ratio will be used as a good biomarker of oxidative stress (Melnyk *et al*., 1999).

The decrease in RBCs, AA to DHA concentration (Mean ± SD) ratio from control group to hypertensive group, showing significant decrease ($p<0.0001$) in AA values in hypertensive patients with respect to their RBCs values in control group. The RBCs concentration ratio of Cys/CySS has been decreased from control group to hypertensive group showing a significant decrease (p□0.001), in RBCs Cys values and an increase in RBCs CySS values in hypertensive patients as shown in table 3. The RBCs, AA to CySS concentration (Mean ± SD), ratio decreased from control group to hypertensive patients ($p<0.0001$). The total ascorbic acid and total cysteine content have been decreased in RBCs of hypertensive patients with respect to their RBCs level in control group. Highly significant increase ($p<0.0001$), in GSSG was occurred in RBCs of hypertensive patients in comparison with control group. The RBCs, AA/GSH concentration (Mean ± SD) ratio decreased from control group to hypertensive patients showing highly significant decrease ($p<0.0001$) in AA/GSH ratio in hypertensive patients with respect to control group. The decrease in AA values was greater than the corresponding decrease of GSH values in RBCs of hypertensive patients with respect to their RBCs values in control group. Hcy/Met ratios in RBCs of control group and hypertensive patients showing the increase in Hcy and decrease in Met level in RBCs of hypertensive patients with respect to its RBCs values in control group as shown in table 3. The higher Hcy/Met ratio will be used as a good biomarker of oxidative stress (Gutteridge, 1993; Levy, 1996; Pastore *et al*., 1998). The total ascorbic acid (AA + DHA) values in control group and hypertensive patients show that total ascorbic acid level greatly decreased ($p<0.0001$), in hypertensive patients with respect to their values in control group as given in table 3. RBCs ratio graphs of various antioxidants have been given (See Supplemental Data fig. 3) which shows their mutual relationship.

Overall interpretation of data and test hypothesis

From the molar concentration of various antioxidants in plasma (table 1) as well as in RBCs (table 2) it is concluded that there is an overall decrease in the molar concentration of reduced from of various antioxidants in hypertensive patients with respect to their corresponding molar concentration in control group. The data was correlated to the test hypothesis keeping in view the mutual relationship of these antioxidants within the body and it was concluded that different antioxidants can work together in a network to recycle each other and complete the cycle. Therefore a decrease in the concentration of one antioxidant would result in the corresponding decrease of another antioxidant concentration and since overall decrease in the total concentration of these antioxidant might occur. It may also be concluded that for working of this network each antioxidant will be available at its appropriate concentration otherwise the corresponding antioxidant will not be recycled and may present at its pro oxidant state that may leads to provoking or initiation of oxidative stress conditions and consequently oxidative stress induced pathological conditions including cardiovascular abnormalities like hypertension. The antioxidant network and their mutual correlation are shown in fig. 3.

CONCLUSION

The present work was carried out to evaluate the antioxidants profile in control and hypertensive groups to assess the involvement of oxidative stress in the initiation and progression of hypertension. Our laboratory findings showed that the antioxidant profile of patients was decreased with respect to that of control group which might prove the potential role of oxidative stress in the pathogenesis of hypertension. The low antioxidant profile and higher biomarker index of patients with respect to normal group further confirm the hypothesis that oxidative stress might be the possible cause of these diseases or it may play a crucial role in the initiation and progression of these diseases. Since large variations were found in the individual antioxidant profile of control group as well as patients the other multifactorial causes such as gender, age, sex differentials, demographic variations, dietary changes, and life-style may not be overruled. In the perspective of our work where there were several limitations regarding to dietary data, individual genotyping, and small number of participating volunteers, it is not the ultimate landmark. However, new ideas will be put forward in the light of these findings and a mechanistic approach will be adopted to evaluate further the possible role of oxidative stress in these pathologies and explore mechanistic links between diet, health status, and disease. For the recommendation of antioxidants intervention in oxidative stress induced pathological conditions further investigations and clinical trials on large scale would be necessary.

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