Assessment of Oxidative DNA Damage by Alkaline Comet Assay in Human Essential Hypertension

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Abstract The objective of the present study was to investigate the antioxidant status and the extent of oxidative DNA damage in lymphocytes and their relation with essential hypertension (EHT). A total of 100 South Indian subjects aged 30–65 were included for the study. Of these 50 were normotensive controls (group-1) with blood pressure $\geq 120/80$ mm Hg, 50 were newly diagnosed (group-2) and were not on any antihypertensive drugs, but had systolic blood pressure ranging between 140 and 160 mmHg and diastolic blood pressure 95–100 mmHg and 50 newly diagnosed essential hypertensive patients underwent drug therapy for 1 year was considered as group-3. Enzymatic and non-enzymatic antioxidants significantly decreased and lymphocyte DNA damage was significantly increased in newly diagnosed hypertensive patients compared with control group. The major decrease in DNA damage and significant improvement in enzymatic and non-enzymatic antioxidants were observed after 1 year of antihypertensive therapy in treated group compared with newly diagnosed hypertensive patients. Total antioxidant status and lymphocyte DNA damage showed a strong negative correlation in all the three groups. Essential hypertension associated with oxidative stress which in turn causes genotoxic susceptibility to variety of disease including cancer. In the absence of DNA repair process and DNA checkpoint mechanisms, the genomic integrity is susceptible to extensive damage. In our study, increased oxidative DNA damage and decreased antioxidant levels were frequently observed in the newly diagnosed essential hypertensive patients, suggesting that oxidative

stress is important in the pathogenesis of EHT. Therefore, the present study has additional clinical implication. Further investigations with large number of patients along with antioxidant supplement are highly warranted.

Keywords Antioxidants · Oxidative stress · Lymphocyte DNA damage · Comet assay · Essential hypertension

Introduction

Hypertension is a major health problem in developed as well as developing countries with a common end result of elevated blood pressure (BP). Hypertension is present in 60–70 % of the population over 60 years of age and may result in cardiovascular complication such as stroke, coronary heart disease (CHD), and heart failure.

Hypertension (HT) is considered as a state of oxidative stress that can contribute to the development of atherosclerosis and other HT-induced organ damage. Lymphocyte DNA damage is caused by various factors including oxidative stress mainly.

Essential hypertension (EHT) or “pre-hypertensive” refers to the high blood pressure with no identifiable causes it can be diagnosed when the systolic pressure (SBP) greater than 140 mmHg and/or diastolic pressure (DBP) of 80–90 mmHg at 3 random checks and need medical monitoring and lifestyle changes [1]. EHT is a common trait of multifactorial determination, imparting an increased risk of cardiovascular, cerebrovascular and renal disease. Several lines of evidence suggest that patients with essential hypertension have impaired balance between oxidant and antioxidant. However the mechanism underlying these abnormalities and its effect on DNA level remains unclear.

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It has been proposed that release of oxygen free radicals (OFR) would be stimulated by acute hypertension and this increase in OFR might mediate the vascular damage, which can be seen in hypertensive subjects. OFR are highly reactive and can damage cellular membranes through lipid peroxidation [2, 3]. Recent experimental evidence suggests that oxidative stress plays an important role in the development of high BP in some animal model [4].

The alkaline comet assay has been extensively used for human biomonitoring studies. Lymphocytes are the preferred cell type as they are an internationally accepted indicator tissue to assess the consequence of human exposure to genotoxic hazard [5, 6].

Comet assay is a simple, sensitive, rapid and non-invasive technique for the assessment of DNA damage in an individual cell. Lymphocyte DNA damage is caused by multiple factors including, oxidative stress mainly [7, 8] and the other causes such as coronary artery diseases [9] and inflammation [10–12]. It has been suggested that oxidative stress and the generation of reactive oxygen species may play an important role in the induction of DNA damage in atherosclerosis [13].

During therapy the entire patients received different antihypertensive drug with various combination. In this group we are not looking for single drug and its effect. On the whole, the complete status of antihypertensive drug effect over oxidative stress in essential hypertensive patients was seen. The following drugs were administered in Dr. K. M. Cherian Heart Foundation, Natrilix SR (diuretics), Telmisartan (Angiotensin II receptor antagonist), Amlodipine and Nifedipine (calcium channel blocker), Doxazosin (alpha blocker), Prolomet XL (beta blocker), Carvedilol (both alpha and beta blocker) and Captopril (ACE inhibitor).

The purpose of the present study was to explore the antioxidants activities and oxidative DNA damage in essential hypertensive patients before and after antihypertensive drug therapy and to examine the possible relationship between the oxidative stress and blood pressure levels.

Materials and Methods

Subjects

Controls were fifty normotensive healthy subjects without any family history of hypertension (group-1). Fifty essential hypertensive patients age range from 30 to 65 years visiting Dr.K.M.Cherian Heart Foundation, Chennai were selected for the study. Hypertension was diagnosed when

the systolic and diastolic pressure read 140 and 90 mmHg respectively at 3 random checks. 50 newly diagnosed essential hypertensive patients without any previous history of antihypertensive medication (group-2) and 50 newly diagnosed essential hypertensive patients underwent drug therapy for 1 year was considered as group-3.

All the participants underwent a detailed clinical examination and their BP and ECG were recorded. A fully informed consent was obtained from these subjects prior to participation in the study. The study was approved by both research and ethics committee of our institute.

All procedures performed in this study were in accordance with the ethical standards of the institutional research committee.

Subject Selection Criteria

Inclusion Criteria

- All volunteers enrolled in this study were aged between 30 and 65 years.
- Subjects of both sexes were included. Women, being on a regular menstrual cycle were included in the study.
- Newly diagnosed EHT patients without any previous history of antihypertensive medication were included in group 2.

Exclusion Criteria

Subjects with secondary hypertension, past history of stroke, coronary artery disease (CAD), myocardial infarction, and peripheral vascular disease with evidence of tissue injury or loss, renal diseases, diabetes mellitus, after infections, smokers, alcoholic and who with vitamin supplement, known to cause changes in antioxidant and lipid status, were excluded from the study.

Blood Sample Collection

5 ml of peripheral venous blood samples were drawn from all the subjects in the fasting state and placed in heparinized tubes. 1 ml of heparinized blood was pipetted into another tube immediately for the analysis of lymphocyte DNA damage by comet assay. The remaining blood was centrifuged at 3000 rpm × 10 min for plasma separation. Plasma samples were used for the analysis of TAS, vitamin C, vitamin E, protein thiols, Catalase, fasting blood glucose and lipid profile. The Hemolysate samples were used for the analysis of Reduced glutathione, Superoxide dismutase, Glutathione peroxidase, Glutathione-S-transferase, and Malondialdehyde.

Measurement of Biochemical Markers

Plasma triglyceride, total cholesterol, LDL, HDL and fasting glucose concentration were measured with an auto analyzer (Randox Daytona, clinical biochemistry analyzer) by using commercial kits.

Non-enzymatic antioxidant such as GSH, vitamin C, vitamin E, protein thiols and TAS were assayed by the method of Beutlar et al. (1963), Omaye et al. (1979), Desai (1984), Sedlak and Lindsay (1968) and Ferric reducing antioxidant power (FRAP) respectively [14–18].

Enzymatic antioxidant such as SOD, CAT, GPX and GST were assayed by the method of Winterbourn et al. (1975), Aebi (1984), Flohe and Gunzler (1984) and Coombes B and Stakelum GS (1961) respectively [19–22].

Measurement of Oxidative Markers

Lipid peroxidation product, MDA was determined by Ohkawa [23] et al. (1979).

Lymphocyte DNA damage by comet assay was determined by the method of Singh [24] et al.

Comet Assay: Oxidative DNA Damage Determination

The endogenous lymphocytes DNA damage was analyzed by alkaline comet assay by the method of Singh et al. with minor modifications.

Separation of Lymphocytes

Lymphocyte isolation for the comet assay was performed by using the Histopaque 1077 (Sigma). An amount of 1 ml heparinized blood was carefully layered over 1 mL Histopaque and centrifuged for 35 min at $500\times g$ at 25 °C. The interface band containing lymphocyte was washed with phosphate buffer saline (PBS) and then collected by 15 min centrifugation at $400\times g$. The resulting pellets were resuspended in PBS to obtain 20,000 cells in 10 μ L.

Cell Viability Test

Membrane integrity of lymphocyte cells was assessed by using Trypan Blue exclusion method.

- (1) 10 μ L of sample and 5 μ L of trypan blue dye were taken in a micro centrifuge tube.
- (2) It was allowed to stand for 2 min and placed on a slide and then with cover slip.
- (3) 100 cells were scored and the number of viable cells (shiny) and dead cells (blue) were recorded.

Ten micro liter of fresh lymphocyte cell suspension (around 20,000 cells) was mixed with 80 μ L of 0.7 % low melting point agarose (LMA) (Sigma) in PBS at 37 °C. Subsequently, 80 μ L of this mixture was layered onto slides that had previously been coated with 1.0 % hot normal melting agarose (NMA), covered with a cover slip at 4 °C for at least 5 min to allow the agarose to solidify.

After removing the cover-slips, the slides were submerged in freshly prepared cold (4 °C) lysing solution (2.5 M NaCl, 100 mM EDTA-2Na; 10 mM Tris-HCl, pH 10–10.5; 1 % Triton X-100 and 10 % DMSO added just before use) for at least 1 h. Slides were then immersed in freshly prepared alkaline electrophoresis buffer (0.3 mol/L NaOH and 1 mmol/L Na₂EDTA, pH > 13) at 4 °C for unwinding (30 min) and then electrophoresis was performed (400 mA/24 V, 25 min).

All of the above steps were conducted under red light or dim light in order to prevent additional DNA damage. After electrophoresis, the slides were stained with ethidium bromide (2 μ mL in distilled water; 80 μ L/slide), covered with a cover slip and analyzed by using a fluorescence microscope (Olympus).

Scoring of Slides

The images of 100 randomly chosen nuclei (50 Cells from each of two replicate slides) were analyzed visually by manual scoring [25]. As shown in Fig. 1 each image was classified according to the intensity of the fluorescence in the comet tail and was given a value of either of 0, 1, 2, 3 or 4 (from undamaged class 0 to maximally damaged class 4), so that the total scores of slide could be between 0 and 400 Arbitrary Units (AU). All procedures were completed and the extent of DNA damage was detected by a single observer.

Measurement of Total Antioxidant Status (TAS)

Total antioxidant status of plasma was determined by ferric reducing antioxidant power assay [26, 27], whereby at low pH, reduction of a ferric tripyridyl triazine (Fe³⁺-TPTZ) complex to a ferrous form, which had an intense blue color, that can be monitored by measuring the absorbance at 593 nm using spectrophotometer. It was directly related to the combined or total reducing power of the electron donating antioxidants present in the reaction mixture. The results were expressed as micro mole per liter.

Statistical Analysis

Results are expressed as mean $\pm$ S.D or frequency expressed as a percent, categorical variables were compared by using χ^2 -test. We assessed normality of all study

variables through Kolmogorov–Smirnov one sample test (K-S test). Comparison among multiple groups was performed by one-way analysis of variance (ANOVA) with LSD post hoc test for continuous variables. Correlation of lymphocyte DNA damage with total antioxidant status was assessed by Pearson correlation coefficient. Values of probability less than 0.05 was considered statistically significant. The statistical analysis was performed with SPSS 12 for windows.

Results

The base line characteristics and lipid profile of EHT patients and control groups are summarized in Table 1. Total cholesterol, TAG, LDL ($p < 0.05$) was significantly increased in EHT patients when compared with controls, whereas HDL ($p < 0.05$) was significantly decreased in EHT patients when compared with control group.

Reduced glutathione, vitamin C, protein thiols and TAS was significantly decreased in newly diagnosed EHT patients when compared with control and EHT-during therapy group ($p < 0.05$). After 1 year of treatment there was significant increase in non-enzymatic antioxidants when compared with EHT newly diagnosed group ($p < 0.05$). Vitamin E does not show significant change between EHT-Newly diagnosed and during therapy (Table 2).

As shown in Table 3, Enzymatic antioxidants like SOD, Catalase, GPx, GST was significantly decreased in newly diagnosed EHT patients when compared with control and EHT-during therapy group ($p < 0.05$). After 1 year of treatment there was significant increase in enzymatic

antioxidants when compared with EHT newly diagnosed group ($p < 0.05$).

Mean MDA and lymphocyte DNA damage of EHT patient group was found at high level compared with control group ($p < 0.05$) and Mean lymphocyte DNA damage of EHT-Newly diagnosed group was found significantly high compared with EHT patient-During therapy ($p < 0.05$, Table 4).

Mean total antioxidant status and lymphocyte DNA damage was illustrated in Figs. 2 and 3. Lymphocyte DNA damage was negatively correlated with TAS and it is highly significant ($p < 0.0001$). The correlation of lymphocyte DNA damage with TAS of all the three groups was illustrated in Fig. 4.

Discussion

To the best of our knowledge, the present study is the first report in South Indian Population to evaluate the association between lymphocyte DNA damage and antioxidants in both newly diagnosed and during drug therapy for 1 year. Our findings showed that oxidative DNA damage occurs in essential hypertensive patients more than in normal blood pressure subjects and that the oxidative DNA damage is significantly reduced by the administration of antihypertensive drugs for the period of 1 year.

We used lymphocytes as a cell line for detecting oxidative DNA damage due to the fact that it is readily available and widely used as a sentinel cell type to provide early warning signals for adverse effects, an attempt was made to see whether oxidative stress in essential

Table 1 The inter comparison of baseline characteristics of the control and EH patients

Parameters	Control	Essential hypertension		<i>p</i> -Value
		Newly diagnosed	During therapy	
Number of subjects	50	50	50	–
Gender (male/female) ^a	37/13	35/15	35/15	NS
Age (years)	42 ± 13	45 ± 12	45 ± 12	–
SBP (mmHg)	121.2 ± 9.4	159.6 ± 12.3*	126.2 ± 11.6 [‡]	$p < 0.01$
DBP (mmHg)	80.4 ± 2.4	100.6 ± 5.2*	85.7 ± 6.3 [‡]	$p < 0.01$
Glucose (mg %)	95.1 ± 7.8	93.2 ± 6.8	94.2 ± 6.5	NS
Total cholesterol (mg %)	148.1 ± 13.7	170.5 ± 19.5*	163.8 ± 12.4 [‡]	$p < 0.01$
TAG (mg %)	96.4 ± 11.2	135.2 ± 22.4*	126.6 ± 25.3 [‡]	$p < 0.01$
HDL cholesterol (mg %)	40.6 ± 7.2	31.2 ± 8.5*	37.4 ± 6.2 [‡]	$p < 0.01$
LDL cholesterol (mg %)	96.4 ± 12.1	111.2 ± 24.2*	106.0 ± 25.6 [‡]	$p < 0.01$

Results are expressed as mean ± SD. One-way ANOVA and LSD post hoc were used to derive the *p*-value
 SBP systolic blood pressure, DBP diastolic blood pressure, TAG Triacylglycerol

* *p*-value significant compared with control ($p < 0.05$), [‡] *p*-value significant compared with control ($p < 0.05$), [‡] *p*-value significant compared with EHT newly diagnosed ($p < 0.05$)

^a χ^2 test

Table 2 Comparison of non-enzymatic antioxidants between control and essential hypertensive subjects

Parameters	Control	Essential hypertension		<i>p</i> -Value
		Newly diagnosed	During therapy	
GSH (mg/g Hb)	3.6 ± 0.9	2.3 ± 0.4*	2.9 ± 0.4 ^{‡‡}	<i>p</i> < 0.001
Vitamin C (mg %)	1.5 ± 0.2	0.6 ± 0.2*	1.0 ± 0.1 ^{‡‡}	<i>p</i> < 0.001
Vitamin E (mg %)	1.3 ± 0.2	1.0 ± 0.1*	1.0 ± 0.1 [‡]	<i>p</i> < 0.05
Protein thiols (μM/L)	296.3 ± 40.7	139.8 ± 34.7*	159.4 ± 21.2 ^{‡‡}	<i>p</i> < 0.001
TAS (μM/L)	904 ± 185	674 ± 105*	811 ± 152 ^{‡‡}	<i>p</i> < 0.001

Results are expressed as mean ± SD for all the parameters. One-way ANOVA and LSD post hoc were used to derive the *p*-value

GSH reduced glutathione, TAS total antioxidant status

* *p*-value significant compared with control (*p* < 0.05), [‡] *p*-value significant compared with control (*p* < 0.05), ^{‡‡} *p*-value significant compared with EHT newly diagnosed (*p* < 0.05)

Table 3 Comparison of enzymatic antioxidants between control and essential hypertensive subjects

Parameters	Control	Essential hypertension		<i>p</i> -Value
		Newly diagnosed	During therapy	
SOD (U/g Hb)	936.4 ± 160.8	693.6 ± 82*	843.8 ± 104.3 ^{‡‡}	<i>p</i> < 0.001
Catalase (K/ml)	27.4 ± 2.9	16.8 ± 1.4*	19.7 ± 4 ^{‡‡}	<i>p</i> < 0.001
GPX (U/g Hb)	21.6 ± 3.8	15.1 ± 1.2*	20.8 ± 2.8 ^{‡‡}	<i>p</i> < 0.001
GST (mM/CDNB-GSH/min/mg protein)	4.3 ± 1	1.4 ± 0.2*	2.2 ± 0.5 ^{‡‡}	<i>p</i> < 0.001

Results are expressed as mean ± SD for all the parameters. One-way ANOVA and LSD post hoc were used to derive the *p*-value

SOD superoxide dismutase, GPX glutathione peroxidase, GST glutathione-s-transferase

* *p*-value significant compared with control (*p* < 0.05), [‡] *p*-value significant compared with control (*p* < 0.05), ^{‡‡} *p*-value significant compared with EHT newly diagnosed (*p* < 0.05)

Fig. 1 Photomicrographs showing varying intensities of the fluorescence in the comet tail (Class 0 undamaged, Class 1, 2 and 3 increasingly damaged and Class 4 maximally damaged)

Table 4 Comparison of MDA and Lymphocyte DNA damage between control and essential hypertensive subjects

Parameters	Control	Essential hypertension		<i>p</i> -Value
		Newly diagnosed	During therapy	
MDA ($\mu\text{M/g Hb}$)	0.17 ± 0.05	$0.64 \pm 0.16^*$	$0.49 \pm 0.06^{\ddagger\text{Y}}$	$p < 0.001$
Lymphocyte DNA damage (AU)	14.5 ± 4.2	$36.2 \pm 11.4^*$	$23.2 \pm 9.7^{\ddagger\text{Y}}$	$p < 0.001$

Results are expressed as mean $\pm$ SD for all the parameters. One-way ANOVA and LSD post hoc were used to derive the *p*-value

MDA malondialdehyde, AU arbitrary units

* *p*-value significant compared with control ($p < 0.05$), $\ddagger$ *p*-value significant compared with control ($p < 0.05$), Y *p*-value significant compared with EHT newly diagnosed ($p < 0.05$)

Fig. 2 Mean lymphocyte DNA damage was compared between controls and EHT Patients. Values are expressed in Mean $\pm$ S.D. * $p < 0.05$ statistically significant compared with control. $\ddagger$ $p < 0.05$ statistically significant compared with control. Y $p < 0.05$ statistically significant compared with EHT- newly diagnosed

Fig. 3 Mean TAS was compared between controls and essential hypertensive patients. Values are expressed in Mean $\pm$ S.D. * $p < 0.05$ statistically significant compared with control. $\ddagger$ $p < 0.05$ statistically significant compared with control. Y $p < 0.05$ statistically significant compared with EHT- newly diagnosed

hypertensive patients subsides after undergoing antihypertensive therapy for the period of 1 year.

An explanation for the observed reduction in antioxidant status among hypertensive patients could be due to the presence of increased free radicals and decreased antioxidants as it utilized for neutralizing free radicals. Free radical triggers the depletion of plasma antioxidants and increase lipid peroxidation. The reduction in antioxidant status and increased production of MDA in the hypertensive patients confirms the presence of oxidative stress. Possible mechanism of ROS production in hypertension is due to increased membrane peroxidation leads to vascular damage in hypertensive subjects which is a suggestive feature of oxidative stress in hypertension. Our results are also consistent with the previous study [28–32].

Oxidative stress due to over production of ROS outstripping antioxidant defense mechanism has been implicated in many pathophysiological conditions that affect the

cardiovascular system, such as hypercholesterolemia, diabetes and hypertension [33–37]. Decreased antioxidant capacity [38] and increased ROS release [39] were shown to be associated with essential hypertension [40, 41].

Our preliminary observations, provides evidence that oxidative DNA damage was associated with depletion of extra cellular antioxidants in essential hypertensive patients. Also TAS assay reflects a general antioxidant capacity of plasma to which protein thiols mainly contribute.

Increased oxidative status may initiate lipid peroxidation in cell membranes, damage membrane proteins or cause DNA fragmentation. These processes may result in a loss of heart contractile function and lead to severe myocardial cell damage [42]. Honda et al. [43] reported that reduced activities of the antioxidant enzymes were associated with increased levels of oxidative DNA damage. However in present study, the TAS levels, which provide a measure of

Fig. 4 The lymphocyte DNA damage was negatively correlated with total antioxidant status and the p value was highly significant ($p < 0.0001$) in all the three groups, respectively (Control, EHT-Newly Diagnosed and EHT-During Therapy)

total defenses against ROS were found at low level in hypertensive patients.

Our study clearly shows that, increased oxidative DNA damage and decreased antioxidant levels were frequently observed in the newly diagnosed essential hypertensive patients, suggesting that oxidative stress is important in the pathogenesis of essential hypertension. Consequently, prevention of DNA damage may be inadequate in hypertensive patients with particularly newly diagnosed EH patients. Furthermore, in this group, presence of low level of TAS and high level of lymphocyte DNA damage may be plausible.

Essential hypertension may leads to severe troublesome problem in humans. Therefore, we should focus on the prevention of development of essential hypertension as well as on the reduction of the complication of hypertension. Oxidative damage is closely related to the development and complication of essential hypertension.

Furthermore, Oxidative DNA damage also should be importantly considered because it may change genes and result in problems for offspring of those with DNA damage. Doctors do not know in their daily practice who might have more serious oxidative damage, especially DNA

damage, among hypertensive patients. Therefore, we need accurate and sensitive method like COMET assay for oxidative DNA damage so that we can use the appropriate drug for antihypertensive medications, for hypertensive patients who have more DNA damage.

In conclusion, the finding of increased lymphocyte DNA damage of EHT patients indicates the potential genetic hazards posed by oxidative stress and emphasizes that ROS occurs more in newly diagnosed essential hypertensive patients than in persons with normal blood pressure. Thus the study shows that essential hypertension is associated with increased oxidative stress and reduced antioxidant status. Adequate control of blood pressure with antihypertensive therapy decreases oxidative DNA damage and improves the antioxidant status in essential hypertensive patients.

Compliance with Ethical Standards

Conflict of interest The author declares no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee. The study was approved by both research and ethics committee of our institute.

Informed consent A fully informed consent was obtained from all the subjects prior to participation in the study.

References

- Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL Jr, Jones DW, Materson BJ, Oparil S, Wright JT Jr, Roccella EJ. National Heart, Lung, and Blood Institute Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure; National High Blood Pressure Education Program Coordinating Committee. The Seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. The JNC 7 report. *JAMA*. 2003;289:2560–72.
- De Bruyn VH, Nuns DW, Cappelli-Biggazzi M, Dole WR, Lamping KG. Effect of acute hypertension in the coronary vessels. *J Hypertens*. 1994;12:163–72.
- Briones AM, Touyz RM. Oxidative stress and hypertension: current concepts. *Curr Hypertens Rep*. 2010;12:135–42.
- Makazono K, Watanabe N, Matsuno K, Sasaki J, Sato T, Inow M. Does superoxide underlie the pathogenesis of hypertension? *Proc Natl Acad Sci USA*. 1991;88:10045–8.
- Hartmann A, Herkommer K, Gluck M, Speit G. DNA damaging effect of cyclophosphamide on human blood cells in vivo and in vitro studied with the single cell gel test (comet-assay). *Environ Mol Mutagen*. 1995;25:180–7.
- Awara WM, El-Nabi SH, El-Gohary M. Assessment of vinyl chloride-induced DNA damage in lymphocytes of plastic industry workers using a single cell gel electrophoresis technique. *Toxicology*. 1998;128:9–16.
- Halliwell B, Aruoma O. DNA damage by oxygen-derived species: its mechanism and measurement in mammalian systems. *FEBS Lett*. 1991;281:9–19.
- Ames BN. Endogenous DNA damage as related to cancer and ageing. *Free Radical Res Commun*. 1989;7:121–8.
- Botto N, Rizza A, Colombo MG, Mazzone AM, Manfredi S, Masetti S, et al. Evidence for DNA damage in patients with coronary artery disease. *Mutat Res*. 2001;493(1–2):23–30.
- Schottenfeld D, Beebe-Dimmer J. Chronic inflammation: a common and important factor in the pathogenesis of neoplasia. *CA Cancer J Clin*. 2006;56:69–83.
- Marvar PJ, Vinh A, Thabet S, et al. T-Lymphocytes and vascular inflammation contribute to stress-dependent hypertension. *Biol Psychiatry*. 2012;71(9):774–82.
- Dinh QN, Drummond GR, Sobey CG, Chrissobolis S. Roles of inflammation, oxidative stress, and vascular dysfunction in hypertension. *BioMed Res Int*. 2014;2014:1–11.
- Botto N, Masetti S, Petrosi L, Vassalle C, Manfredi S, Biagini A, Andreassi MG. Elevated levels of oxidative DNA damage in patients with coronary artery disease. *Coron Artery Dis*. 2002;13(5):269–74.
- Beutler E, Duron O, Kelly BM. Improved method for the determination of blood glutathione. *J Lab Clin Med*. 1963;61:882–8.
- Omaye ST, Turnbull JD, Sauberlich HE. Selected methods for determination of ascorbic acid in cells, tissues and fluids. *Methods Enzymol*. 1979;62:3–11.
- Desai ID. Vitamin E analysis method for animal tissues. *Method Enzymol*. 1984;105:138–42.
- Sedlak J, Lindsay RH. Estimation of total protein bound and non protein sulphhydryl groups in tissue with Ellman's reagent. *Anal Biochem*. 1968;25:192–205.
- Benzie IFF. Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Methods Enzymol*. 1999;299:15–27.
- Winterbourn CC, Hawkins RE, Brian M, Carrell RW. The estimation of red cell superoxide dismutase activity. *J Lab Clin Med*. 1975;85(2):337–41.
- Aebi H. Catalase in vitro. *Method enzyme*. 1984;105:121–6.
- Flohe L, Gunzler WA. Assay of glutathione peroxidase. In: Packer L, editor. *Methods in enzymology*. New York: Academic Press; 1984.
- Coombs B, Stakelum GS. A liver enzyme that conjugates sulfo-bromophthalein with glutathione. *J Clin Invest*. 1961;40:981–8.
- Ohkawa H, Ohishi N, Yagi K. Assay of lipid peroxide in animal tissue by thiobarbituric acid reaction. *Anal Biochem*. 1979;95(2):351–8.
- Singh PN, McCoy MT, Tice RR, Schneider EL. A simple technique for quantitation of low levels of DNA damage in individual cells. *Exp Cell Res*. 1988;175:184–91.
- Burlinson B, Tice RR, Speit G, Agurell E, Brendler-Schwaab SY, Collins AR, et al. Fourth international workshop on genotoxicity testing: resulting of the in vivo comet assay workgroup. *Mutat Res*. 2007;627(1):31–5.
- Erdogan C. The evaluation of oxidative stress in patients with CRF. *CCA*. 2002;322:157–67.
- Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power. The FRAP assay. *Anal Biochem*. 1996;239:70–6.
- Subash P, Gurumurthy P, Sarasabharathi A, Cherian KM. Urinary 8-OHdG: a marker of oxidative stress to DNA and total antioxidant status in essential hypertension with South Indian population. *Indian J Clin Biochem*. 2010;25(2):127–32.
- de la Sierra A, Larrousse M. Endothelial dysfunction is associated with increased levels of biomarkers in essential hypertension. *J Hum Hypertens*. 2010;24:373–9.
- Juraschek SP, Guallar E, Appel LJ, Miller ER. Effects of vitamin C supplementation on blood pressure: a meta-analysis of randomized controlled trials. *Am J Clin Nutr*. 2012;95:1079–88.
- Savica V, Bellingeri G, Kopple JD. The effect of nutrition on blood pressure. *Annu Rev Nutr*. 2010;30:365–401.
- Beg M, Gupta A, Khanna VN. Oxidative stress in essential hypertension and role of antioxidants. *JIACM*. 2010;11(4):287–93.
- Steinberg D, Witztum JL. Is the oxidative modification hypothesis relevant to human atherosclerosis? Do the antioxidant trials conducted to date refute the hypothesis? *Circulation*. 2002;105:2107–11.
- Chisolm GM, Steinberg D. The oxidative modification hypothesis of atherogenesis: an overview. *Free Radic Biol Med*. 2000;28:1815–26.
- Kachhawa K, Varma M, Sahu A, Kachhawa P, Kumar R. Oxidative stress and antioxidant enzyme levels in hypertensive chronic kidney disease patients. *IJBAR*. 2004;5(10):488–90.
- Gonzalez J, Valls N, Brito R, Rodrigo R. Essential hypertension and oxidative stress: new insights. *World J Cardiol*. 2014;6(6):353–66.
- Rodrigo R, Gil D, Miranda-Merchak A, Kalantidis G. Antihypertensive role of polyphenols. *Adv Clin Chem*. 2012;58:225–54.
- Russo C, Olivieri O, Girelli D, Faccini G, Zenari ML, Lombardi S, et al. Anti-oxidant status and lipid peroxidation in patients with essential hypertension. *J Hypertens*. 1998;16:1267–71.
- Prabha PS, Das UN, Koratkar R, Sagar PS, Ramesh G. Free radical generation, lipid peroxidation and essential fatty acids in uncontrolled essential hypertension. *Prostaglandins Leukot Essent Fatty Acids*. 1990;41:27–33.
- Rodrigo R, Libuy M, Feliu F, Hasson D. Oxidative stress-related biomarkers in essential hypertension and ischemia-reperfusion myocardial damage. *Dis Markers*. 2013;35(6):773–90.

41. Biswas UK, Kumar A. A study on lipid profile, oxidative stress and carbonic anhydrase activity in patients with essential hypertension. *J Clin Diagn Res.* 2010;4:3414–20.
42. Chen LY, Nichols WW, Hendricks J, Mehta JL. Myocardial neutrophil infiltration, lipid peroxidation, and antioxidant activity after coronary artery thrombosis and thrombolysis. *Am Heart J.* 1995;129:211–8.
43. Honda M, Yamada Y, Tomonaga M, Ichinose H, Kamihira S. Correlation of urinary 8-hydroxy-2-de-oxyguanosine (8-OHdG), a biomarker of oxidative DNA damage, and clinical features of hematological disorders: a pilot study. *Leuk Res.* 2000;24:461–8.