Assessment of DNA Damage in Peripheral Blood Leukocytes of Patients with Essential Hypertension by the Alkaline Comet Assay

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Received October 6, 2009; accepted May 6, 2010

Summary DNA damage was assessed in 50 subjects using the alkaline Single Cell Gel Electrophoresis assay and included 35 (18 males, 17 females) hypertensive individuals and 15 (7 males, 8 females) normotensives of the same age, sex and socio-economic status. Patients (32–62 years) had average systolic blood pressure >156 mmHg, diastolic pressure >98 mmHg, pulse and arterial pressure of >56 and >116 mmHg, respectively. Average BMI was 25.18 kg/m², 54% were overweight, 16.45% obese and waist hip ratio (WHR) >0.95. Chi-square test revealed that controls matched patients except for blood pressure values. Multiple regression analysis and analysis of variance (ANOVA) showed that DNA damage significantly associated with systolic ($p<0.05$), diastolic blood pressure ($p<0.05$) and mean arterial pressure ($p<0.05$). Mann–Whitney *U*-test revealed that 80.54% cells with tails and mean DNA migration length of $50.01\pm1.01\mu\text{m}$ were significantly higher ($p<0.001$) in patients compared to controls (27.21%; $20.33\pm0.74\mu\text{m}$). These differences between male and female patients/controls were, however, non-significant. The differences for drug-usage and WHR were non-significant while BMI and blood pressure values revealed significant DNA damage differences. The physiological state of hypertension probably has the potential to cause genetic damage as assessed in the present study by the SCGE assay since DNA migration length and percent cells with tails were highly significant ($p=0.001$) as compared to values in normo-tensive, healthy controls.

Key words Cells with tails, DNA migration length, Single cell gel electrophoresis, Hypertension.

Essential hypertension or the condition of elevated arterial blood pressure is a complex genetic trait caused by multiple susceptibility genes, the effects of which are modulated by gene-environment and gene–gene interactions (Shaughnessy 2000). It is a common chronic condition associated with reduced life expectancy with a potential to accumulate DNA damage (Pero *et al.* 1976) probably resulting from reactive oxygen species (ROS) produced by xanthine oxidase, NADPH/NADH oxidases and nitric oxide synthetase which become increased in hypertension (Huie and Padmaja 1993). Also, the angiotension II gene has been found to be associated with free radicals formation (Schupp *et al.* 2007). Free radicals react to form peroxynitrite which is a potent cytotoxic oxidant that leads to peroxidation of lipids, degradation of DNA and oligonucleosomal fragmentation (Hemnani and Parihar 1998). ROS also increase nitric oxide that causes tissue injury, protein oxidation and DNA damage (Xu and Touyz 2006). The imbalance or impaired status of antioxidants and free radicals in hypertensive patients further makes them vulnerable to oxidative stress (Kashyap *et al.* 2005). Peripheral polymorphonuclear leukocytes (which generate reactive superoxide anions) participate in oxidative stress and inflammation in patients with hypertension (Alexander 1995, Rajagopalan *et al.* 1996, Kristal *et al.* 1998). The pathogenic outcome of oxidative stress is oxidative damage, a major cause of vascular injury in hypertension (Touyz and Schiffrin 2007). Moreover, oxidative stress can have damaging effects on the DNA, which in turn

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are associated with age-related changes and causation of cancer. Since oxidative stress is induced in the hypertensive state, it is pertinent to investigate whether DNA damage is also induced in the hypertensive patients. Gur *et al.* (2007) observed a positive correlation between increased oxidative damage in hypertensive subjects and urinary 8-hydroxy-2-deoxyguanosine (8-OHdG), a DNA damage marker. There was reported an increase in oxidative stress and a reduction in the activity of antioxidant mechanisms though independent of the blood pressure value in whole blood and mono nuclear peripheral cells of hypertensive subjects (Redon *et al.* 2003). The single cell electrophoresis (SCGE), or 'comet', assay is a sensitive method for assessing DNA effects (Griffiths *et al.* 2002) such as DNA lesions, single-strand breaks, double-strand breaks and alkali-labile sites. The relative simplicity of the comet assay and the varied cell types that can be used has proved it to be a versatile and powerful tool for studying DNA damage. As not many studies have come to light regarding the assessment of DNA damage in hypertensive subjects and hardly any in the Indian populations, the present study investigated DNA damage in peripheral blood leukocytes of individuals with essential hypertension using the alkaline single cell gel electrophoresis assay.

Materials and methods

Subjects

Individuals seeking medical attention for hypertension at the Health Centre of Guru Nanak Dev University, Amritsar, and healthy normo-tensive individuals of the same age, sex and socio-economic status comprised the study group. The individuals were given an explanation about the nature of the study and those who voluntarily agreed and gave written informed consent, were requested for their personal details and blood samples. The study was cleared by the Institutional Ethics Committee. The information of the patients and of the control group was noted on a specially designed questionnaire. A family pedigree of up to 3-generations was also taken to check for familial hypertension. The study was conducted during Jan.–Sept., 2007. Anthropometric measurements (as per Weiner and Lourie 1981) for calculating BMI (body mass index), as well as blood pressure, mean arterial pressure and pulse pressure readings (average of 3 using a mercury sphygmomanometer) were recorded for each individual.

Sample collection

Finger-prick blood samples (~300 μ l) were taken in heparin and phosphate-buffered saline and transported to the laboratory on ice. The Trypan-blue exclusion test revealed cell viability >85% and so the samples were processed for the alkaline SCGE assay (Singh *et al.* 1988) as modified by Ahuja and Saran (1999) using locally available chemicals (Fine Chem. Ltd., Qualigens, SRL, Glaxo Laboratory, Ranbaxy Lab., India). Blood mixed with 0.5% low melting point agarose (LMPA) was layered onto plain glass microscope slides pre-coated with 1.0% normal melting point agarose (NMPA), followed by another 0.5% LMPA layer. The slides were then subjected to lysis (pH=10; 2.5 M NaCl, 100 mM EDTA, 10 mM Tris aminomethane, 12.0 g NaOH pellets; the mixture was freshly added to 1% Triton X-100 and 10% DMSO) to liberate DNA, followed by alkaline treatment in electrophoresis buffer (25 min in 300 mM NaOH, 1 mM EDTA; pH>13), an electrophoretic run (pH>13.0; 300 mA, 25 V; 20–25 min; 0.8 V/cm), neutralization (0.4 M Tris, pH=7.5) and silver (20% AgNO₃) staining (Delincee 1995). The slides were manually scored (100 cells/sample *viz.* 50/slide) under a binocular microscope (40 $\times$) for cells with tails; an ocular micrometer was used to measure the DNA migration/tail length which was calculated by subtracting the measurement of radius of the core/cell from the total length of the comet.

Statistical analysis

The data were non-parametric and the Mann–Whitney “U” test was applied. A Chi-square test

Table 1. Demographic information of the hypertensive patients and control individuals

Characteristics	Males			Females		
	Hypertensives		Controls	Hypertensives		Controls
	Range	Mean $\pm$ S.E.M.	Range	Range	Mean $\pm$ S.E.M.	Range
Age (years)	32–69	52.44 $\pm$ 2.0	37–56	35–60	50.35 $\pm$ 2.05	35–52
Weight (kg)	58–110	80.16 $\pm$ 3.31	61–75	40–85	63.47 $\pm$ 2.62	43.00–76.50
Height (cm)	162–186	177.89 $\pm$ 1.7	174.00–183.00	144–164	154.65 $\pm$ 1.54	148–168
BMI (kg/m ²)	17.32–31.80	25.29 $\pm$ 0.86	18.21–23.93	14.87–39.34	26.72 $\pm$ 1.29	17.36–29.15
WHR	0.85–1.16	1.47 $\pm$ 0.49	0.80–1.10	0.73–1.13	0.94 $\pm$ 0.22	0.76–0.98
Blood pressure systolic (mmHg)	140.00–180.00	153.06 $\pm$ 2.71	120–130	140–170	160.00 $\pm$ 2.23	110–130
Diastolic (mmHg)	90–110	97.78 $\pm$ 0.90	80–90	90–110	99.41 $\pm$ 1.35	70–90
Mean arterial pressure (mmHg)	105–130.0	115.5 $\pm$ 1.87	93.20–103.30	93.20–130.30	117.81 $\pm$ 2.08	83.2–103.3
Pulse pressure (mmHg)	40–80	54.4 $\pm$ 2.8	30–45	40.00	59.11 $\pm$ 2.28	40.00–45.00

BMI, body mass index; WHR, waist hip ratio.

was performed to check whether the sample group matched the control. Multiple regression analysis and analysis of variance (ANOVA) was carried out to determine if confounding variables induced any DNA damage. The Mann–Whitney “*U*” was also applied to find the significance within the factors associated with hypertension and DNA damage *viz.* BMI, blood pressure, *etc.* Results were taken as significant at $p < 0.05$ and $p < 0.001$.

Results and discussion

The patient group ($n=35$) comprised individuals with systolic blood pressure >140 mmHg and diastolic blood pressure >90 mmHg (WHO/ISH 2003). The control group ($n=15$) comprised healthy individuals with no history of hypertension. The demographic information of the subjects ($n=50$) is given in Table 1. The hypertensive males ($n=18$) were between 32–69 years (mean 52.44 ± 0.22 years) with average of 153.06 ± 2.71 mmHg systolic and 97.78 ± 0.90 mmHg diastolic blood pressure measurements. The 17 hypertensive females were between 35–59 years (mean 50.35 ± 2.05) with systolic blood pressure of 140–170 mmHg (mean 160 ± 2.23 mmHg), and diastolic blood pressure 90–110 mmHg (mean 99.41 ± 1.35 mmHg) values. The control group comprised normotensive, healthy individuals ($n=15$; 7 males, 8 females; mean 46.33 ± 1.71 years; mean systolic blood pressure 122.00 ± 1.68 mmHg; diastolic blood pressure 82.33 ± 1.75 mm). The personal attributes of the patients and controls are depicted in Table 2. The 2×2 Chi-square (χ^2) contingency test revealed that patient and control groups matched except for their age, blood pressure readings and BMI status. Multiple regression analysis showed a relationship between DNA damage and diastolic blood pressure, ($p=0.039$) and mean arterial pressure, ($p=0.041$) in male patients and with BMI in controls. In the analysis of variance, significance was only observed for systolic blood pressure ($p=0.047$) in combined patient group.

DNA damage

Genetic damage was scored as per cent cells with tails and DNA migration length. An initial analysis of results separately for males (in patient

Table 2. Personal attributes and Chi square (χ^2) analysis of hypertensive patients and normal control individuals

Attributes	Range	Patient group	Control group	χ^2_{cal}	χ^2_{tab}	
Age range (years)	32–50	14	11	4.670	3.84	S
	51–69	21	4			
Dietary pattern	Veg	19	9	0.139	3.84	NS
	Non veg	16	6			
Mobile phone users	User	15	7	0.062	3.84	NS
	Non user	20	8			
Alcohol consumption	Drinker	10	3	0.400	3.84	NS
	Non drinker	25	12			
BMI (kg/m ²)	<18.5–24.5	10	13	14.26	3.84	S
	25.5–31.5	25	2			
Systolic blood pressure (mmHg)	>120–139	0	15	50.00	9.49	S
	140–159	16	0			
	160–179	19	0			
Diastolic blood pressure (mmHg)	70–89	0	10	29.69	9.49	S
	90–100	29	5			
	101–111	6	0			
Mean arterial pressure (mmHg)	93.2–113.2	12	15	50.70	3.84	S
	114.2–134.2	23	0			
Pulse pressure (mmHg)	40–55	17	15	12.05	3.84	S
	56–71	18	0			
Waist hip ratio	0.8–0.96	17	11	2.61	3.84	NS
	0.97–1.13	18	4			

NS, not significant; S, significant; BMI, body mass index.

Table 3. DNA damage in hypertensive and control individuals

Groups		Cells with tails/cells scored (%)	Pooled cells with tails/cells scored (%)	Mean DNA migration length (μm) $\pm$ S.E.M.	Pooled mean DNA migration length (μm) $\pm$ S.E.M.
Hypertensive individuals	Males ($n=18$)	1401/1800 (79.33 ^{***a,b})	2791/3500 (80.54 ^{**})	50.74 $\pm$ 1.18 ^{***a,b}	50.01 $\pm$ 1.01 ^{**}
	Females ($n=17$)	1390/1700 (81.76 ^{***a,b})		49.28 $\pm$ 0.85 ^{***a,b}	
Control individuals	Males ($n=7$)	181/700 (25.80 ^b)	410/1500 (27.21)	19.95 $\pm$ 0.76 ^b	20.33 $\pm$ 0.74
	Females ($n=8$)	229/800 (28.62 ^b)		20.71 $\pm$ 0.73 ^b	

^{**} Highly significant when compared with total control group ($p \leq 0.001$, Mann–Whitney *U*-test).

^a Highly significant when compared with parallel control group ($p \leq 0.001$, Mann–Whitney *U*-test).

^b Non-significant compared to each other.

Table 4. Effect of BMI on DNA damage in hypertensive and control individuals

BMI range ^a (kg/m ²)	No.	Mean BMI (kg/m ²)	Age range (year)	Mean age (year)	Systolic blood pressure (mmHg)	Mean pressure (mmHg)	Diastolic blood pressure range (mmHg)	Mean pressure (mmHg)	Mobile users	Alcohol users	No. of cell scored	Cells with tails (%)	Mean migration length (μm) ±S.E.M. ^b
Normal	6	20.40	38–69	53.60	150–170	159.9	90–110	99.8	2	3	600	500 (83.30)**	49.65±1.29**
At risk	4	23.80	38–60	50.00	140–170	150.0	90–100	95.0	1	2	400	266 (66.20)**	42.79±0.50**
Obese I	21	26.70	32–62	50.70	140–170	155.75	90–110	98.09	9	3	2100	1683 (80.05)**	51.3±1.04**
Obese II	4	31.10	48–58	54.50	150–180	160.00	100–110	106.6	2	2	400	342 (84.50)**	47.98±0.87**
Total	35										3500	2791 (78.50)**	49.99±1.01**
Normal	10	20.20	37–56	49.45	—	—	—	—	5	3	1000	192 (28.95)	18.95±0.63
At risk	3	23.70	35–55	40.20	—	—	—	—	2	2	300	135 (21.10)	19.46±1.27
Obese I	2	28.50	43–47	45.00	—	—	—	—	—	—	200	83 (41.50)	26.95±0.36
Total	15										1500	410 (27.21)	20.33±0.74

^a As per WHO/IASO/IOTF 2000.^b Calculated as an average of individual DNA migration length in that group.** Highly significant compared to parallel control group ($p<0.01$, Mann–Whitney U -test).**Table 5.** Effect of blood pressure on DNA damage in hypertensive and control individuals

Blood pressure categories ^a mmHg	No.	Age range (year)	Mean pressure range (mmHg)	Mean pressure range (mmHg)	Pulse pressure range (mmHg)	Mean pressure (mmHg)	Mobile user	Alcohol user	Number of cell scored	Cells with tails (%)	Mean migration length (μm) ±S.E.M. ^b
Grade I (mild)	16	38–62	53.60	105.0–123.3	111.89	40–60	50.12	5	1600	1227 (77.60)**	48.33±1.100**
Grade 2 (moderate)	18	32–69	51.38	93.2–130.0	120.80	40–80	62.50	3	1800	1492 (83.10)**	50.685±0.895**
Grade 3 (severe)	1	54	54.00	126.0	126.00	80	80.00	1	100	72 (72.00)**	60.60±1.250**
Total	35								3500	2791 (80.54)**	50.01±1.010**
Normal controls	15	35–56	46.30	83.8–103.3	95.70	30–45	40.30	6	1500	410 (27.21)	20.33±0.740

^a As per WHO 2003.^b Calculated as an average of individual DNA migration lengths in that group.** Highly significant when compared with total control group and within the group ($p<0.01$, Mann–Whitney U -test).

Table 6. Effect of medication on DNA damage in hypertensive patients

Medication	No. of patients	Age range (year)	Mean arterial pressure range (mmHg)	Mean pulse pressure range (mmHg)	Mobile user	Alcohol user	Number of cell scored	Cells with tails (%)	Mean migration length (μm) +S.E.M. ^a		
Atenolol (50mg)	6	32-69	56.16	140-170	158.3	90-110	103.30	3	4	499 (83.10)	49.60±0.85
Amlodipine (5 mg)	9	35-60	49.10	150-180	158.8	90-100	101.10	2	4	696 (77.30)	48.81±0.75
Atenolol + Amlodipine (55 mg)	11	35-59	49.80	140-170	156.8	90-100	96.36	2	3	904 (82.18)	51.92±1.52
Atenolol + Bisulfate+Nimodipine (88.85 mg)	1	57	57.00	170	170.0	100	100.00	—	—	85 (85.00)	56.12±0.20
Amlodipine + Glycovidin (75 mg)	2	57-58	57.50	140-150	145.0	90-100	95.00	1	—	128 (64.00)	50.83±1.38
Atenolol +Sleeping Pills (50 mg each)	1	58	58.00	150	150.0	90	90.00	—	1	84 (84.00)	45.39±0.94
Total-treated patients	30	—	—	—	—	—	—	—	—	2396 (79.26)**	50.44±0.94**
Un-treated patients	5	43-55	48.6	140-165	—	90-100	—	1	2	395 (79.00)**	48.00±0.62**
Controls	15									1500 (410 (27.21)	20.33±0.74

^a Calculated as an average of individual DNA migration lengths in that group.** Highly significant when compared to control group ($p < 0.01$, Mann-Whitney U -test).

and control groups) and for females (in patient and control groups) revealed statistical significance for both DNA migration and per cent cells with tails though no significant increase was observed for different age ranges (Gandhi and Jyoti 2010). However, the differences between male and female patients were not significant for either per cent cells with tails ($Z_{\text{cal}}=1.174$, $Z_{\text{tab}}=2.576$; $p=0.241$) or DNA migration length ($Z_{\text{cal}}=1.023$, $Z_{\text{tab}}=2.576$, $p=0.306$); neither were these respective values significant in the control males and females ($Z_{\text{cal}}=0.406$, $Z_{\text{tab}}=2.576$, $p=0.685$; $Z_{\text{cal}}=0.465$, $Z_{\text{tab}}=2.576$, $p=0.645$). Hence these values for females and males (Table 3) were pooled in both (control and patient) groups. These combined values on analysis revealed statistical significance with each other ($Z_{\text{cal}}=5.561$, $Z_{\text{tab}}=3.291$, $p < 0.001$; $Z_{\text{cal}}=5.262$, $Z_{\text{tab}}=3.291$, $p < 0.001$) indicating significant genetic damage in hypertensive patients compared to healthy controls.

Effect of body mass index (BMI)

BMI is an indicator of a risk factor for hypertension (Feldstein *et al.* 2005). Endothelial dysfunction and hypertension may be related to chronic oxidative stress induced by obesity and therefore BMI and WHR can be related with significant DNA damage. Krajcovicova *et al.* (2007) observed that with increase in age, BMI values increased as did DNA damage. A longitudinal association of BMI and oxidative DNA damage (8-OHdG levels) in 174 individuals has also been reported (Mizoue *et al.* 2007). Kasai *et al.* (2001) had investigated an association of BMI with other life style patterns like smoking and meat intake with DNA damage and their results revealed significant reduction in urinary 8-OHdG level with increase in BMI. In the present study, patients and controls were categorized for BMI as per WHO criteria (2000). There were significant differences (Table 4) between 2 ranges (23.00–24.90 kg/m² and 25.0–29.9 kg/m²) for both, increased percentage of cells with tails ($t_{\text{cal}}=2.529$, $t_{\text{tab}}=2.450$, $df=6$; $p=0.045$) and increased mean DNA migration length ($t_{\text{cal}}=2.968$, $t_{\text{tab}}=2.070$, $df=23$; $p=0.007$) and between the categories <23.00 kg/m² and 23.00–24.90 kg/m² for increased mean DNA migration length only ($t_{\text{cal}}=2.506$, $t_{\text{tab}}=2.310$, $df=8$, $p=0.030$).

Effect of blood pressure

Increased blood pressure has vulnerability to oxidative stress (Kashyap *et al.* 2005) and a relationship between 8-OHdG level and hypertension has been reported (Negishi *et al.* 2001). Hypertension is also associated with increased ROS formation (Harrison *et al.* 2007) and these ROS cause oxidative stress which reduces the level of vasodilators and nitric oxide, and thereby cause tissue injury and DNA damage (Xu and Touyz 2006). In the present study, the per cent cells with tails and mean DNA migration length showed significant differences between different ranges of blood pressure (Table 5). The damage continuously increased with increase in blood pressure values (ranges according to WHO 2003). For cells with tails, the ranges between 140–159 mmHg/90–99 mmHg and 160–179 mmHg/100–109 mmHg ($t_{\text{cal}}=10.862$, $t_{\text{tab}}=3.290$, $df=32$, $p=0.001$), between 140–159 mmHg/90–99 mmHg and ≥ 180 mmHg/ ≥ 110 mmHg ($t_{\text{cal}}=7.034$, $t_{\text{tab}}=4.070$, $df=15$, $p=0.001$) and between 160–179 mmHg/100–109 mmHg and ≥ 180 mmHg/ ≥ 110 mmHg ($t_{\text{cal}}=21.21$, $t_{\text{tab}}=3.29$, $df=31$, $p=0.001$). For mean DNA migration length, a significant difference was observed for blood pressure ranges 160–170 mmHg/100–109 mmHg and ≥ 180 mmHg/ ≥ 110 mmHg ($t_{\text{cal}}=2.162$, $t_{\text{tab}}=1.96$, $df=31$, $p=0.001$) and for ranges between 150–159/90–99 mmHg and $\geq 180/\geq 110$ mmHg ($t_{\text{cal}}\leq 2.80$, $t_{\text{tab}}=2.13$, $df=15$; $p=0.06$).

Effect of medication

During the interval when this study was conducted, most of the patients ($n=30$) were on anti-hypertensive medications (Table 6). As per WHO recommendation (2005), 6 main classes of drugs prescribed worldwide are diuretics, β -blockers, angiotensin converting enzyme inhibitors, calcium antagonists, α -blockers and angiotensin II antagonists. In the University Health Care Centre, Amritsar mainly β -blockers (Atenolol- $\text{C}_{14}\text{H}_{22}\text{N}_2\text{O}_3$) and calcium channel blockers (Amlodipine- $\text{C}_{20}\text{H}_{25}\text{ClN}_2\text{O}_5$ and Nimodipine- $\text{C}_{14}\text{H}_{25}\text{N}_7\text{O}_7$) are prescribed. Literature perusal has revealed that amlodipine, induced neither mutagenicity in bacteria nor chromosomal aberrations in mice (Ahr *et al.* 1997). It significantly inhibited lipid peroxidation which is the main cause of oxidative stress and reactive oxygen species formation (Harrison *et al.* 2007) and Nimodipine also appeared to inhibit lipid peroxidation but not statistically. Mason *et al.* (1999) also showed that Amlodipine inhibited nuclear changes associated with apoptosis, including chromatin condensation and inter-nucleosomal DNA cleavage at low or nanomolar levels, and induced neuroprotective activity and decreased reactive oxygen species formation. Andreassi *et al.* (1999) also reported that calcium antagonists did not induce cytogenetic damage in peripheral blood lymphocyte cultures. Atenolol, however mainly induced chromosomal loss, especially of chromosome 17 (Telez *et al.* 2000, 2003), and Nimodipine also induced micronuclei production (Telez *et al.* 2001). Saez *et al.* (2004) observed that there was increased oxidative stress and decreased antioxidant mechanism in patients taking antihypertensive drugs. However in the present study, the results did not reveal significant DNA damage between treated (79.26% cells with tails; mean migration length of $50.44 \pm 0.94 \mu\text{m}$) and the untreated (79.00% cells with tails; mean migration length $48.00 \pm 0.62 \mu\text{m}$) patient groups, probably because of the small number of untreated patients ($n=5$) as compared to treated patients ($n=30$) and also perhaps because of their different lifestyles. The damage observed has probably resulted from the increased blood pressure values themselves since damage was much lower in normo-tensive (control) group. Antihypertensive agents (β -adrenergic blockers, ACE inhibitors, AT_1 receptor antagonists, and Ca^{2+} -channel blockers may be mediated in part by decreasing vascular oxidative stress (Godfraind 2005, Cifuentes and Pagano 2006, Flammer *et al.* 2007) and Grossmann (2008) has concluded that lowering blood pressure with antihypertensive medications is associated with reduced oxidative stress and so oxygen stress is probably not the cause, but rather a consequence of, hypertension.

Individuals with essential hypertension are vulnerable to oxidative stress (because of an imbalanced status of antioxidants and free radicals) and to oxidative and DNA damage (Kashyap *et*

et al. 2005, Xu and Touyz 2006). In the present study, DNA damage was highly significant ($p \leq 0.001$) in patient samples. The damage may be resulting from the state of hypertensiveness *via* reactive oxygen species since these are both a consequence and cause of hypertension (Huie and Padmaja 1993). Angiotension II has also been found to be associated with free radical formation by activation of the NADPH oxidase enzyme that can cause DNA damage *via* Ang II type I receptor binding and the subsequent formation of oxidative stress (Schupp *et al.* 2007). An increase in the level of 8-hydroxy guanine (8-OHdG) is also an indicator of the relationship between oxidative DNA damage, hypertension and cardiovascular disease risk (Negishi *et al.* 2000). A vegetarian diet/supplementation can provide some protection against oxidative stress and so decrease oxidative DNA damage (Kazimirova *et al.* 2004, Dhawan and Jain 2005, Krajcovicova *et al.* 2007). Confounding factors like alcohol consumption and mobile use can also cause DNA damage (Zhu *et al.* 2002, Gandhi and Anita 2005). Furthermore, pulse pressure significantly increased with increase in WHR and caused oxidative stress (Feldstein *et al.* 2005). Our sample group also had 24 patients with a WHR >0.92 – 1.12 in the higher BMI range. Increases in age and BMI also increased DNA damage (Krajcovicova *et al.* 2007) and a longitudinal association of BMI and oxidative DNA damage has been also documented (Mizoue *et al.* 2007). Hypertension is a leading risk factor for cerebrovascular, cardiovascular and renal problems (Kashyap *et al.* 2005) and the results of the present study highlight that damage to genetic material is also elevated in patients with essential hypertension. As generation of DNA damage is considered to be an important initial event in carcinogenesis, the observed DNA damage hence may have other far-reaching consequences as it can further contribute to the risk of development of degenerative and mitochondrial diseases as well as neoplasia. Interventions aimed at increasing the antioxidant defense system and reducing oxidative stress may prove useful for mitigating these outcomes in hypertensive patients.

Acknowledgements

Part of the work was carried out with a grant from PSCST awarded to JJ.

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