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Oxidative DNA damage and oxidant status in hypertensive patients- A preliminary study

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ABSTRACT

Essential hypertensive patients on monotherapy (Atenolol, a beta-blocker), belonging to the Jat Sikh Punjabi population sub-group, and normotensive healthy, age-, sex- and socio-economic status-matched control individuals belonging to the same population sub-group were assessed for oxidative DNA damage and oxidative stress. Leukocytic oxidative DNA damage (measured using modified enzymatic comet assay/SCGE assay), oxidant status (total oxidative stress, total antioxidant capacity, malondialdehyde levels and oxidative stress index) and lipid profile was determined for each studied participant. The mean oxidative DNA damage and oxidative status of hypertensive patient group was found to be higher than in the control group ($p<0.001$). Statistically significant increases in total cholesterol, LDL, VLDL and triglycerides were also observed in the hypertensive patient group ($p<0.001$). Oxidative DNA damage showed a statistically significant positive correlation with blood pressure (SBP and DBP) and total oxidative stress levels and negative correlation with total antioxidant capacity. The findings suggest that oxidative stress could be a cause of observed DNA damage. Studies on a larger cohort are underway to determine confirmation of these findings.

Key Words: *Comet assay, Tail DNA percent, antioxidant capacity, malondialdehyde, oxidized purines, oxidized pyrimidines*

INTRODUCTION

Essential hypertension (≥140/90mmHg) requires lifelong treatment for which combinational therapy is the general prescriptive measure (Sever and Messerli, 2011). However, effects of these levels of treatments may themself prove to be deleterious, adding to the compromised state of health of the hypertensive patients in causing and/or increasing morbidity in terms of increased genetic damage which

increases susceptibility to malignancy. This may further be compounded by multidrug therapy. Recommended medications include calcium channel blockers, beta-blockers, ACE inhibitors, angiotensine II receptor blockers and diuretics (IHG, 2013) which may act as antioxidants (Wassmann *et al.,* 2004)and/or as receptor blockers/enzyme inhibitors (JNC7, 2004).

In fact, whether oxidative stress is a cause or a result of hypertension is not clear (Grossman, 2008) though the role of reactive oxygen species (ROS) in vascular function and in the development of hypertension is known (Touyz, 2004; Aggarwal *et al.,* 2013). In the present preliminary study, we report oxidative DNA damage in essential hypertensive patients on monotherapy viz. those on treatment with atenolol (beta-blocker). The significance of the study lies in the fact that monotherapy is an exceptional mode of therapy with few patients being prescribed a single drug. The patients had been prescribed Atenolol (beta-blocker) which acts by blocking beta-1 adrenergic receptors on heart muscle cells, preventing epinephrin and norepinephrin from stimulating the cardiovascular system as they increase heart rate, strength and activity leading to elevated blood pressure (Nezu *et al.,* 1985). This preliminary study further attempted to select a single population sub-group (Jat Sikh) to control for confounders based on variant genetic make-up in order to initiate a higher-order investigation.

MATERIALS AND METHODS:

Essential hypertensive patients on monotherapy (Atenolol) were identified in this hospital-based study to investigate for oxidative DNA damage in their peripheral blood leukocytes (PBL) and compare with genetic damage in normotensive healthy individuals. Oxidative DNA damage was assessed by the enzymatically modified comet assay (Collins *et al*., 2002) to score for levels of oxidized purines and oxidized pyrimidines. Demographic information and anthropometric measurements for obesity assessment were also taken. In order to find support for etiology of oxidative DNA damage, the oxidative stress in patients and controls was also assessed by estimating serum levels of lipid peroxidation as malondialdehyde, the total antioxidant capacity (TAC) and total oxidative stress (TOS). The lipid profile was also ascertained for all participants.

Blood pressure was taken after the study participants had been seated comfortably for ten minutes using a mercury sphygmomanometer as per AMA guidelines (ASH, 1992) and a record of the average of three readings were taken as the systolic blood pressure (SBP) and diastolic blood pressure (DBP) values.

Anthropometric measurements of height, weight, waist circumference (WC) and hip circumference (HC) were taken as per Weiner and Lourie (1981). The abdominal adiposity as a function of WC, waist hip ratio (WHR) and general obesity as a function of body mass index (BMI) were also determined according to recommended cut-offs for Indians (Misra *et al.,* 2009).

Intra-venous blood samples (~10ml from each participant) were used for genetic damage assessment and aliquots for serum separation were analyzed for lipid levels and oxidant status. The levels of the total cholesterol(TC), high density lipoproteins (HDL) and triglycerides(TG) were determined on an automated lipid analyzer (Erba, India) using kits (Angstrom Biotech, India), as per the manufacturer's instructions and the very low density lipoproteins (VLDL=triglycerides*1/5) and low density lipoproteins (LDL=total cholesterol-HDL-VLDL) were then calculated (Friedewald *et al.,* 1972).

Oxidative stress parameters of total antioxidant capacity (TAC), total oxidative stress (TOS) and malondialdehyde (MDA) levels were assessed in blood serum samples by standard methodology (Erel, 2004, 2005; Beuge and Aust, 1978). Oxidative stress index (OSI) was also calculated as ratio of TOS levels to TAC levels.

Genetic damage in PBL was determined using the modified single cell gel electrophoresis (SCGE/comet) assay with foramidopyrimidine DNA-glycolase (FPG) and endonuclease III (Endo III) enzymes (Collins *et al., 2002*) after checking for cell viability(Coligan *et al*., 2003). DNA damage was scored using the comet assay Software Program and a record of percent DNA in tail, olive tail moment (OTM), tail moment (TM) and tail length (TL) was made. The values are mean±S.E.M. Test of significance for categorical (χ^2) and continuous (t-test) variables were applied. Correlation and regression analyses were performed and significance levels were set at $p \leq 0.001$.Statistical analysis was carried out on SPSS version 16 for windows.

RESULTS

Demographic and clinical information of the study participants (Table 1) were matched in terms of age, gender, height, weight, hip circumference (HC), waist circumference (WC), body mass index (BMI) and waist hip ratio (WHR) but differed in relation to systolic blood pressure (SBP), pulse pressure (PP) and mean arterial pressure (MAP). The participants had no smoking habits and did not use mobile phone.

Serum lipid levels (Table 2) for all individuals were significantly elevated ($p \leq 0.01$) in hypertensive patients. Oxidative stress levels of malondialdehyde (MDA), TOS, oxidative stress index (OSI) were significantly ($p \leq 0.01$) increased in hypertensive patients while total antioxidant capacity (TAC) was significantly ($p \leq 0.01$) reduced in hypertensive patients.

Oxidative damage to purines and pyrimidines was detectable following incubation with enzymes (Table 3) and showed significant elevation ($p \leq 0.01$) for the assessed DNA damage parameters in patients from values observed in normotensive controls.

On correlation and regression analyses, DNA damage showed significant association with WHR (r=0.88, p=0.04), SBP and DBP in hypertensive patients while as hypothesized DNA damage was negatively

correlated with TAC and positive with TOS (Table 4) suggesting that increased oxidative stress could be leading cause to DNA damage.

DISCUSSION

The Atenolol- treated hypertensive patients were hyperlipidemic with manifestation of oxidative stress and oxidative DNA damage compared with normotensive individuals. TOS levels were two times raised in hypertensive patients compared to values in healthy individuals, while TAC levels were two times decreased in patient group. Increased oxidative stress manifests as lipid peroxidation, further producing additional ROS that can enhance oxidative damage to proteins and DNA and could lead to severe myocardial cell damage (Chen *et al*., 1995). The role of ROS in cardiovascular dysfunction along with hyperlipidemia, diabetes, hypertension and chronic heart failure has been known (Taniayama *et al.,* 2003).

Hypertension is often associated with obesity (Wolk *et al.,* 2003) and body mass index (BMI) is an indicator/risk factor for hypertension (Feldstein *et al.,* 2005). With increase in age, BMI values and DNA damage also increases (Krajcovicova *et al.,* 2007). Increase in waist hip ratio (WHR) also significantly increases pulse pressure (Feldstein *et al.,* 2005). There is often an associated increase in various lipid components in hypertensive patients (Maharajan *et al.,* 2008). In accordance with the results of the present study, hypertensive patients were deficient for antioxidant enzymes but with increased lipid peroxidation (MDA) and DNA oxidation byproducts (Redon *et al.,* 2003). Quantitatively increased oxidative DNA damage was represented in hypertensive patients, while treatment decreased urinary 8-OHdG levels and TAS levels in treated hypertensive patients (Yildiz *et al.,* 2008; Subash *et al.,* 2010). Lee *et al.,* (2005) also showed that DNA damage caused by ROS was more common in hypertensive patients than in normotensive while reduction of antioxidant enzymes was associated with increased level of oxidative DNA damage (Dittmar *et al., 2008*) as many antihypertensive agents are genotoxic and carcinogenic (Brambille *et al.,* 2006). The monotherapy with atenolol in patients of the present study could also be genotoxic. Chromosomal breaks were two times higher in hypertensive patients on beta-blocker therapy as compared to values in control individuals in a study by (Telez *et al.,* (2010). Thukral *et al*., (2012) have also reported a significant increase in DNA damage and dyslipidemia in Punjabi hypertensive patients. The increased DNA damage observed in hypertensive patients pose them to be at a risk for developing neoplasia and target organ damage.

CONCLUSION

These preliminary results emphasize the increased oxidative DNA damage and oxidative stress in essential hypertensive patients on atenolol treatment raising the possibility that hypertensive state is

associated with increased free radical damage to DNA. Further studies on a large scale on oxidative DNA damage in hypertensive patients can clarify the role of oxidative stress in the pathogenic mechanism of hypertension.

REFERENCES

Aggarwal SS, Gross CM, Sharma S, Fineman JR, Black SM. 2013. Reactive Oxygen Species in Pulmonary Vascular remodeling. *Compr Physiol* **3(3)**:1011-1034.

American Society of Hypertension, "Recommendations for routine blood pressure measurement by indirect cuff sphygmomanometry," *AM J of Hypertens* **5**: 207–209, 1992.

Brambilla G, Mattioli F, Martelli A. 2010. Genotoxic and carcinogenic effects of gastrointestinal drugs. *Mutagenesis* **25(4)**:315-326.

Buege JA, Aust SD. 1978. Microsomal lipid peroxidation. *Methods Enzymol* **52**: 302-310.

Chen LY, Nichols WW, Hendricks J, Mehta JL. 1995. Myocardial neutrophil infiltration, lipid peroxidation, and antioxidant activity after coronary artery thrombosis and thrombolysis. *Am Heart J* **129**: 211–218.

Coligan JE, Kruisbeck AM, Margulies DH, Shevach EM, Strober W. 1995. Current protocols in immunology. Wiley-Liss: New York.

Collins AR. 2002. The Comet Assay, principles, applications and limitations, in methods in molecular biology. In: V V Didenko (Ed.): In-situ Detection of DNA Damage: Methods and Protocols. Vol.203. Totowa, NJ: Humana Press Inc., pp.163–177.

Dittmar M, Knuth M, Beineke M, Epe B. 2008. Role of Oxidative DNA Damage and Antioxidative Enzymatic Defence Systems in Human Aging. *Open Anthropol J* **1**: 38-45.

Erel, Ozcan 2004. A novel automated method to measure total antioxidant response against potent free radical reactions. *Clin Biochem,* **37**:112-119.

Erel, Ozcan 2005. A new automated colorimetric method for measuring total oxidant status. *Clin Biochem*, **38**:1103-1111.

Feldstein, C. A., Akopian, M., Olivieri, A. O., Kramer, A. P., Nasi, M., Garrido, D. 2005. A comparison of body mass index and waist-to-hip ratio as indicators of hypertension risk in an urban Argentine population: A hospital-based study. *Nutr Metab Cardiovasc Dis* **15**: 310-315.

Friedewald WT, Levy RI, Fredrickson DS. 1972. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* **18**: 499–502.

Grossman, E. 2008. Does increased oxidative stress cause hypertension? *Diabetes care* **31(2)**: 185-189.

Indian hypertension guidelines III, "Management of Hypertension" the journal of association of physicians of India, **61**:18-23, 2003.

Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC). 7th *Report of the JNC*. Washington, DC: National Institutes of Health; 2004.

Krajcovicova-Kudlackova M, Valachovicova M, Paukova V, Dusinska M. 2008. Effects of diet and age on oxidative damage products in healthy subjects. *Physiol Res* **57(4)**: 647-651.

Lee J, Lee M, Kim JU, Song KI, Choi YS, Cheong SS. 2005. Carvedilol reduces plasma 8-hydroxy-2'-deoxyguanosine in mild to moderate hypertension: a pilot study. *Hypertension* **45**: 986-990.

Maharjan BR, Jha JC, Vishwanath P, Alukar VM, Singh PP. 2008. Oxidant-antioxidant status and lipid profile in the hypertensive patients. *J Nepal Health Res Counc* **6(13)**: 63-68.

Misra A, Chowbey P, Makkar BM, Vikram NK, Wasir JS, Chadha D, Joshi SR, Sadikot S, Gupta R, Gulati, S, Munjal YO. 2009. Consensus statement for diagnosis of obesity, abdominal obesity and the metabolic syndrome for Asian Indians and recommendations for physical activity, medical and surgical management. *JAPI* **57**: 163-170.

Nezu M, Miura Y, Adachi M, Adachi M, Kimura S, Toriyabe S, Ishizuka Y, Ohashi H, Sugawara T, Takahashi M. 1985. The effects of epinephrine on norepinephrine release in essential hypertension. *Hypertension* **7**:187-195.

Redón J, Oliva MR, Tormos C, Giner V, Chaves J, Iradi A, Sáez GT. 2003. Antioxidant Activities and Oxidative Stress Byproducts in Human Hypertension. *Hypertension* **4**: 1096-1101.

Sever PS, Messerli FH. 2011. Hypertension management 2011: optimal combination therapy. *Eur Heart J* **32(20)**: 2499-2506.

Subash P, Gurumurthy P, Sarasabharathi A, Cherian KM. 2010. 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* **25(2)**: 127-132.

Subash P, Premagurumurthy K, Sarasabharathi A, Cherian KM. 2010. Total antioxidant status and oxidative DNA damage in a South Indian population of essential hypertensives. *J Hum Hypertens* **24**: 475-482.

Taniyama Y, Griendling KK. 2003. Reactive Oxygen Species in the Vasculature Molecular and Cellular Mechanisms. *Hypertension* **42**:1075-1081.

Téleza M, Ortiz-Lastrab E, Gonzalezc AJ, Floresd PF, Huertaa I, Ramíreza JM, Barasoaina M, Criadoe B, Arrietaa I. 2010. Assessment of the genotoxicity of atenolol in human peripheral blood lymphocytes: Correlation between chromosomal fragility and content of micronuclei. *Mutat Res* **695**: 46–54.

Thukral K, Sharma R, Chandey M, Gandhi G. 2012. Essential Hypertension, DNA Damage and Dyslipidemia in Two Ethnic Groups. *Int J Hum Genet* **12(2)**: 105-111.

Touyz RM. 2004. Reactive oxygen species and angiotensin II signaling in vascular cells -- implications in cardiovascular disease. *Braz J Med Biol Res* **37(8)**:1263-1273.

Wassmann S, Wassmann K, Nickenig G. 2004. Modulation of Oxidant and Antioxidant Enzyme Expression and Function in Vascular Cells. *Hypertension* **44**: 381-386.

Weiner JS, Lourie JA. 1981. Human biology: A guide to field methods. Practical human biology. Academic press Inc. New York.

Wolk R, Shamsuzzaman ASM, Somers VK. 2003. Obesity, Sleep Apnea, and Hypertension. *Hypertension* **42**:1067-1074.

Yıldız A, Gür M, Yılmaz R, Demirbağ R, Çelik H, Aslan M, Koçyiğit A. 2008. Lymphocyte DNA damage and total antioxidant status in patients with white-coat hypertension and sustained hypertension. *Türk Kardiyol Dern Arş - Arch Turk Soc Cardiol* **36(4)**: 231-238.

Table1.Demographic, anthropometric and physiometric measurements of hypertensive patients and normotensive controls

Parameters	**Hypertensive Patients (n=5)**		**Normotensive Controls (n=3)**	
	Range	Mean±S.E.M.	Range	Mean±S.E.M.
Age	60-78	66.2±3.32	58-70	67.00±5.57
Height (cm)	152.4-186	166.96±5.53	152.4-186	169.47±9.70
Weight (kg)	54-90	78.20±6.55	40-90	66.67±14.53
Hip Circumference (cm)	90-166	123.80±17.13	102-117	108.67±4.41
Waist Circumference (cm)	94-172	127.40±17.85	102-112	105.33±3.33
Body Mass Index (kg/m^2)	23.25-33.79	28.04±2.16	17.22-26.01	22.49±2.68
Waist Hip Ratio	0.55-1.05	0.77±0.11	0.55-0.67	0.62±0.04
Systolic Blood pressure (mmHg)	140-180	156.00*±8.12	110-130	120.00±5.77
Diastolic Blood Pressure (mmHg)	80-120	100.00±7.07	75-85	80.00±2.89
Pulse Pressure (mmHg)	50-60	56.00*±2.45	30-45	40.00±5.00
Mean Arterial Pressure (mmHg)	99.80-139.80	118.48*±7.34	89.85-99.85	93.20±3.32

*Statistically significant differences between hypertensive patients and normal control individuals (Students' t-test; p≤0.05)

Table2. Lipid Levels and Oxidative Stress in essential hypertensive patients and normotensive controls

Variables	**Hypertensive Patients (Mean±S.E.M.)**	**Normotensive Controls (Mean±S.E.M.)**	**t-value**	**p-value**
Cholesterol(TC)	168.62±10.27	104.97±15.55	3.415*	0.030
High Density lipoproteins (HDL)	47.04±3.68	22.03±5.91	3.825**	0.009
Low density Lipoproteins (LDL)	79.68±10.08	55.25±9.78	1.740	0.159
Triglycerides (TG)	209.48±13.61	38.47±11.94	3.526**	0.012
Very low density lipoproteins (VLDL)	41.89±2.72	27.69±2.38	3.526**	0.012
Malondialdehyde (MDA) (nmol/mL)	10.44±0.61	5.44±1.47	3.721**	0.010
Total Antioxidant capacity (TAC) (mmol Trolox Eqv./L)	0.37±0.04	0.77±0.07	5.621***	0.001
Total oxidative stress (TOS) (µmol H_2O_2 Eq./L)	0.70±0.06	0.35±0.04	4.113**	0.006
Oxidative stress Index (OSI)	0.20±0.04	0.05±0.01	3.090*	0.021

**Statistically highly significant ($p \leq 0.01$) when compared to normal control individuals
* Statistically significant ($p \leq 0.05$) when compared to respective values in normal control individuals

Table 3. DNA damage parameters in Hypertensive patients and normotensive controls

Method of Comet assay	**DNA Damage parameters**	**Hypertensive patients**	**Normotensive controls**	**t-value**	**p-value**
Alkaline standard comet	Percent Tail DNA	22.08±1.96	5.25±1.79	5.754**	0.001
	Tail length (TL)	218.40±27.15	56.67±5.49	4.440**	0.004
	Tail Moment (TM)	49.07±8.91	2.90±1.07	3.877**	0.008
	Olive Tail Moment (OTM)	63.64±9.14	7.17±3.14	4.533**	0.004
Comet assay with enzyme Endonuclease III (Endo III)	Percent Tail DNA	8.96±1.92	1.78±0.50	3.618*	0.018
	Tail length (TL)	51.8±9.73	19.67±0.88	3.290*	0.030
	Tail Moment (TM)	17.45±2.19	1.14±0.53	7.223***	0.001
	Olive Tail Moment (OTM)	20.03±3.24	2.04±0.70	5.420**	0.004
Comet assay with enzyme formamidopyrimidine DNA glycosylase (FPG)	Percent Tail DNA	11.39±2.52	2.15±0.19	3.663*	0.021
	Tail length (TL)	62.40±7.69	19.67±1.20	5.489**	0.005
	Tail Moment (TM)	19.20±2.07	1.36±0.77	8.060***	0.000
	Olive Tail Moment (OTM)	20.95±3.16	2.17±0.83	5.754**	0.003

***Very highly statistically significant ($p \leq 0.001$),**Statistically highly significant ($p \leq 0.01$), *Statistically significant ($p \leq 0.05$) when compared to normal control individuals

Table 4. Pearson's correlation coefficients of blood pressure (SBP and DBP) and Oxidative Stress Parameters with DNA damage related parameters in hypertensive patients

Variables		**SBP (mmHg)**		**DBP (mmHg)**		**Total Antioxidant capacity (TAC) (mmol Trolox Eqv./L)**		**Total oxidative stress (TOS) (µmol H_2O_2 Eq./L)**		**Oxidative stress index (OSI)**	
		r- value	p-value	r- value	p-value	r- value	p-value	r- value	p-value	r- value	p-value
Alkaline comet	Percent Tail DNA	0.773	0.125	0.656	0.230	-0.751	0.143	0.726	0.165	0.836	0.078
	Tail length (TL)	0.673	0.213	0.839	0.076	-0.889*	0.044	0.914*	0.03	0.822	0.088
	Tail moment (TM)	0.866	0.058	0.909*	0.032	-0.987**	0.002	0.991***	0.001	0.993***	0.001
	Olive tail moment (OTM)	0.884*	0.046	0.885*	0.046	-0.965**	0.008	0.961**	0.009	0.993***	0.001
Comet assay with enzyme Endonuclease III (Endo III)	Percent Tail DNA	0.950**	0.013	0.846	0.071	-0.797	0.107	0.788	0.113	0.884*	0.046
	Tail length (TL)	0.820	0.089	0.865	0.058	-0.879*	0.049	0.861	0.061	0.820	0.089
	Tail moment (TM)	0.872*	0.054	0.728	0.163	-0.733	0.158	0.709	0.180	0.828	0.083
	Olive tail moment (OTM)	0.970**	0.006	0.902*	0.036	-0.734	0.158	0.751	0.143	0.826	0.085
Comet assay with enzyme formamido pyrimidine DNA glycosylase (FPG)	Percent Tail DNA	0.832	0.832	0.667	0.219	-0.637	0.248	0.616	0.268	0.753	0.142
	Tail length (TL)	0.911*	0.032	0.910*	0.032	-0.825	0.086	0.816	0.092	0.810	0.096
	Tail moment (TM)	0.843	0.073	0.662	0.223	-0.532	0.356	0.499	0.392	0.615	0.269
	Olive tail moment (OTM)	0.962**	0.009	0.869*	0.056	-0.704	0.185	0.713	0.176	0.800	0.104

**statistically highly significant correlation ($p \leq 0.01$)
* statistically significant correlation ($p \leq 0.05$)