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Essential Hypertension, DNA Damage and Dyslipidemia in Two Ethnic Groups

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ABSTRACT To study assessed DNA damage and lipid profile of essential hypertensive patients and healthy control individuals (n=72) belonging to the *Baniya* and *Jat* Sikh ethnic groups. There were 44 patients (30 *Baniya* and 14 *Jat* Sikh) on single drug treatment (atenolol) for essential hypertension and 28 healthy normotensive individuals matched for age, sex, ethnicity and socioeconomic status. Following approval by the Institutional Ethics Committee and after informed consent, demographic information and physiometric and anthropometric measurements were taken from each participant. Leukocytic DNA damage was assessed using the Single Cell Gel Electrophoresis assay and serum lipid levels were determined using an automated analyzer. Significantly elevated (p=0.000) DNA damage [DNA migration length-36.71±0.97µm; damage frequency-97.89±0.64; Damage Index (DI)-266±4.04] as well as dyslipidemia were observed in the patients with non-significant gender and ethnic group differences. DNA damage (DNA migration length, DI) was also positively correlated with dyslipidemia (triglycerides-p=0.440, total cholesterol-p=0.621,low density lipoproteins-p=0.598) in both patient sub-groups. The disease-medicine-lifestyle-BMI interplay could be causing the observed increased genetic damage, which along with dyslipidemia, raises the risk for various cardiovascular diseases and malignancy in the studied patient group.

INTRODUCTION

The state hypertension increases the risk for stroke and coronary heart disease and is a main contributor to pre-mature death (Deepa et al. 2003; Yadav et al. 2008).In fact, dyslipidemia associated with hypertension plays an important role in the development of cardiovascular diseases (Zhang et al. 2007).Other numerous metabolic and physiological changes occur in hypertensive individuals and many of these processes stem from the associated increased oxidative stress (Vasdev 2006). A decrease in antioxidant enzymes and increase in oxidants in the hypertensive state have been reported to increase the production of reactive oxygen species. The reactive oxygen species/free radicals resulting from the oxidant-antioxidant imbalance tend to accumulate and are known to cause oxidative damage to the cellular macromolecules including the genetic material (Rao 2009).The genetic instability can include mutations, chromosomal aberrations and unscheduled DNA synthesis (Perara and Bapat 2007). Atenolol is a beta1-selective drug that binds to the beta-receptors and prevents the stimulation by catecholamine. This results in lowering of the heart rate and of the systemic blood pressure. The metabolism of atenolol is minimal because it is hydrophilic and therefore total absorbed drug is cleared via excretion in the urine in an unaltered manner (Wadworth et al. 1991).The genotoxic nature of atenolol as in its ability to induce sister chromatid exchanged, chromosome loss, chromosomal aberrations and induction of fragile sites (Telez et al. 2000, 2010) makes its use a cause for concern since genetic instability is an early event in carcinogenesis (Olaharski et al. 2006).

The present study was, therefore undertaken to investigate for any DNA damage and dyslipidemia in local *Baniya* and *Jat* Sikh hypertensive patients who were on regular atenolol drug-therapy. Normotensive healthy individuals belonging to the same ethnic group comprised the controls. To the best of our knowledge, this study is a first attempt to elucidate whether there are ethnic differences with respect to genomic instability in hypertensive patients and whether the anti-hypertensive medication induces any differential genetic damage responses.

METHODOLOGY

In this case-control study, (n=72) carried out between May 2007-December 2008.44 hyper-

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tensive (mean age 60.82±11.31y; mean hypertension:158.32/93.57mmHg in 27 males; 156.79/90.24mmHg in 17 females) belonging to two different population sub-groups, *Baniya* (n=30) and *Jat* Sikh (n=14) visiting the Guru Ram Das Hospital, Amritsar for treatment, participated voluntarily after informed consent. The study was cleared by the Institutional Ethics Committee. Records of hypertensive patients diagnosed by the attending physician for medical history, occupational, genetic and life style information were maintained on a pre-designed proforma. Healthy normotensive individuals (n=28; mean age 59.86 ± 11.29y; mean hypertension:127.56/85.00mmHg) matched for age, sex, ethnicity and socio-economic status form the control group. Anthropometric measurements (height, weight, hip and waist circumferences) were taken (Weiner and Lourie 1981) for each participant.

Sample Size-Power Calculation

With a statistical power of 80% and alpha value of 0.05, the total sample size required is 42 on the basis of DNA migration length observed in the ethically matched control (referent) group, that is, 24.13±4.65μm(SD) and a postulated significant increase of p≤0.05 on Student's t-test analysis (after Albertini et al. 2003).

Blood Pressure Measurements

Blood pressure readings were obtained using a mercury sphygmomanometer. Averaged three readings each of systolic blood pressure (SBP) and diastolic pressure (DBP) were taken at 5 minutes interval from seated subjects. Hypertension was categorized as per IHG-II (2007).

Blood Sampling Protocol

Peripheral blood samples (5ml) were obtained by venipuncture and from this one ml of blood was immediately pipetted into heparinized tube for DNA damage assessment. The non-heparinized sample was centrifuged at 3000 rpm for 10 minutes to separate serum for lipid profiling (triglycerides-TG, total cholesterol-TC, Low Density lipoprotein cholesterol-LDL-C, High Density lipoprotein cholesterol-HDL-C).

Lipid Profiling

Serum levels of TG, TC, VLDL-C, HDL-C were measured on an automated analyzer (EEBA CHEM-7) using commercial kits (Angstorm, India) and LDL-C was calculated using the standard formula (Friedwald et al.1972).

The SCGE/Comet Assay

The Single Cell Gel Electrophoresis (SCGE) assay was carried out in alkaline conditions, basically as described by Singh et al. (1988) with slight modifications. On coated slides 1% Normal melting point agarose ,a second layer containing whole blood samples (30μl) mixed with 0.5% Low Melting Point Agarose (LMPA) was made, on which after its setting a third layer (0.5% LMPA) was put. The sample preparations were lysed (2.5 M NaCl, 100 mM EDTA-2Na; 10 mMTris–HCl, pH 10–10.5; 1% Triton X-100 and 10% DMSO were added just before use)) for 1-2 h to permit DNA unwinding and then placed in alkaline electrophoresis buffer (0.3 mol/lNaOH and 1mmol/l Na2ETDA, pH >13) for 20 min for DNA unwinding and for the expression of alkali labile-sites. Electrophoresis was carried out for 20 min (25 V/300 mA, 25 min) and the slides were rinsed gently thrice with neutralizing buffer (0.4M Tris,1000ml H_2O,pH 7.5) to remove excess of alkali and detergents. After treatment with fixing solution (15% trichloroacetic acid, 5% zinc sulfate, and 5% glycerol), the slide preparations were stained with silver nitrate (0.2% w/v ammonium nitrate, 0.2% w/v silver nitrate, 0.5% w/v tungstosilisic acid, 0.15% v/v formaldehyde, and 5% w/v sodium carbonate), coded and scored blind at 40X under a binocular microscope for DNA damage. Nucleoids (100/sample) were scored visually and tail lengths of comets under a transmission binocular microscope were measured using an occulo-micrometer. The cells were visually grouped into various categories (0-4) as per (Collins 2002).

Statistical Analysis

Statistical analysis was performed using SPSS version 16.0 software for windows. The Student's t-test was employed to compare indices of genetic damage patients with values in controls. The data were taken as Mean ± S.E.M.

Table 1: General information on hypertensive patients and on healthy controls

	Range	*Patients (n=44) (%)*	*Controls (n=28) (%)*	χ^2
Age (y)	21-50	7 (15.91)	6 (21.42)	0.352
	51-80	37 (84.09)	22 (78.57)	
Sex	Male	27 (61.36)	14 (50.00)	0.90
	Female	17 (38.64)	14 (50.00)	
Occupation	Farming	4 (9.09)	2 (7.14)	
	Business	15 (34.09)	10 (35.71)	
	Service	9 (20.45)	4 (14.28)	
	House wives	16 (36.36)	12 (42.86)	
Height (cm)	140-165	22 (50.00)	21 (75.00)	**4.44**
	166-180	22 (50.00)	7 (25.00)	
Weight (kg)	60-85	27 (61.36)	28 (100)	**14.162**
	86-120	17 (38.64)	0	
Diet	Vegetarian	36 (81.82)	26 (92.86)	1.74
	Non vegetarian	9 (20.45)	2 (7.14)	
Alcohol Consumption	Alcoholics	11 (25.00)	1 (3.57)	**5.66**
	Non alcoholics	33 (75.00)	27 (96.43)	
Smoking habit	Smokers	6 (13.64)	0	**4.165**
	Non Smokers	38 (86.36)	28 (100)	
Mobile usage	Users	31 (70.45)	20 (71.43)	0.079
	Non Users	13 (29.55)	8 (28.57)	

Values in bold are significant (χ^2-test, $p \leq 0.05$)

Pearson's correlation coefficient was calculated to find out relationship between dependent and independent parameters. Multiple regression analysis and multivariate analysis of variance (ANOVA) were also performed to determine the independent association between DNA damage and various parameters (demographic, anthropometric and lipid profile). The p value of <0.05 was considered as significant.

RESULTS

Table 1 depicts the demographic characteristics of the hypertensive patients (n=44, mean age 60.82±11.31y) and of the age- , sex-, diet- and mobile usage- matched healthy normotensive individuals (n=28, mean age-59.86± 11.29y). Chi-square analysis revealed that both groups matched for age, sex, diet, mobile usage but showed significant differences for height, weight, alcohol consumption and smoking.

On performing ANOVA, a significant effect of SBP, TC, LDL-C (p=0.000), DBP (p=0.008), TG (p=0.002) on damage index (DI) and of SBP, TC, LDL-C (p=0.000), DBP (p=0.007), TG (p=0.003) on mean DNA migration length in hypertensive patients whereas BMI contributed to increased DF (p=0.002). In controls, association of DI with WHR (p=0.013) and LDL-C (p=0.045) was observed. These factors also showed positive correlation with DNA damage (Table 2).

The student's t-test revealed highly significant differences (p=0.000) on comparing the hypertensive patients and controls for TC, HDL-C, TG, LDL-C, SBP and DBP (Table 3). No gender-and population group-differences for various DNA damage indices in patients and controls were observed (Table 4).However, Chi-square analysis revealed difference in different damage categories. Significant difference (p=0.0128) in DNA damage in Baniya and Jat Sikh patients was observed. In controls also, the significant difference in category II (p=0.0003) and III(p=0.0114) was found between *Baniya* and *Jat* Sikh individuals (Table 5).

DISCUSSION

The findings of the present case-control study in hypertensive patients belonging to two subpopulations, *Baniya* and *Jat* Sikh, assessed for DNA damage are in accordance with those reported in literature. Increased genetic damage was reported in hypertensive patients belonging to South Indian (Subash et al. 2010) and Turkish (Demirbag et al. 2006, 2005) populations. The observed genomic instability may stem from the condition of hypertension itself, the use of medication, increased BMI/WHR values, lipid profile, lifestyle, food habits, etc. Hypertension is associated with dyslipidemia as indicated by the higher levels of total cholesterol, triglycerides, LDL, VLDL and lower val-

Table 2: Multivariate ANOVA, Pearson correlation and multiple linear regression analysis of on DNA damage parameters in hypertensive patients and normotensive controls

Genetic endpoint	*Independent variables*	*Patients*					*Controls p value*
		Multivariate ANOVA		*Pearson correlation*	*Multiple linear regression*	*p value*	
		Mean Squares	*F*	*'r' value*	*Beta (95%CI)*		
DamageFrequency	Age(y)	132.308 15.384	8.600	0.412	0.412 (0.048- 0.262)	**0.005**	0.620
	Body mass index(kg/m$^{2)}$	37.073 17.651	2.100	0.218	0.218 (-0.063- 0.38)	0.155	**0.002**
DamageIndex	Waist hip ratio	2.778 736.375	0.004	-0.009	-0.009 (-140.462- 132.16)	0.951	**0.013**
	Systolic blood pressure (mm/Hg)	17984.046 308.250	58.342	0.765	0.765 (0.790- 1.367)	**0.000**	0.760
	Diastolic blood pressure (mm/Hg)	4768.819 622.898	7.656	0.393	0.393 (0.359- 2.294)	**0.008**	0.243
	Cholesterol (mg/dl)	11448.005 463.870	24.679	0.608	0.608 (0.214- 0.506)	**0.000**	0.140
	Triglycerides(mg/dl)	6161.011 589.751	10.447	0.446	0.446 (0.106- 0.458)	**0.002**	0.961
	Low density lipoproteins(mg/dl)	9955.348 499.409	19.934	0.567	0.567 (0.193- 0.512)	**0.000**	**0.045**
Mean DNA Migration (μm)	Systolic blood pressure (mm/Hg)	1055.221 17.454	60.465	0.768	0.768 (0.193- 0.327)	**0.000**	0.532
	Diastolic blood pressure (mm/Hg)	285.945 35.772	7.994	0.400	0.400 (0.093- 0.557)	**0.007**	0.157
	Cholesterol(mg/dl)	689.654 26.160	26.363	0.621	0.621 (0.054- 0.123)	**0.000**	**0.009**
	Triglycerides(mg/dl)	346.388 34.333	10.089	0.440	0.440 (0.024- 0.109)	**0.003**	0.983
	Low density lipoproteins(mg/dl)	640.166 27.338	23.417	0.598	0.598 (0.052- 0.127)	**0.000**	0.353

The significant $p \leq 0.05$ values of effective variables on DNA damage parameters are given in bold
CI-Confidence Intervals

Table 3: Lipid profile and blood pressure measurements in hypertensive patients and normotensive controls

Parameter	*Hypertensive patients (n=44)*	*Normotensive controls (n=28)*	*p-value*
Cholesterol(mg/dl)	233.75 ± 6.83242	160.05 ± 7.414	0.000***
High density lipoproteins(mg/dl)	29.23 ± 1.1007	39.39 ± 1.845	0.000***
Triglycerides (mg/dl)	182.27 ± 6.397	165.97 ± 6,575	0.080
Low density lipoproteins (mg/dl)	168.07 ± 6.504	85.79 ± 2.634	0.000***
Systolic blood pressure(mm/Hg)	155.57 ± 2.872	131.07 ± 1.243	0.000***
Diastolic blood pressure(mm/Hg)	93.07 ± 1.19	80.89 ± 0.77	0.000***

Values are given as mean ± SEM
Hypertensive versus normotensive subjects: $^{****}p<0.001$ (highly significant). (Student's t-test).

Table 4: DNA damage in hypertensive patients and normotensive controls

Group	*No. of individuals*	*Damage frequency (DF)† ± S.E.M.*	*Damage index (DI)† ± S.E.M.*	*Mean DNA migration † ± S.E.M. (μm)*
Sample Group (n=44)	Males (n=27)	97.74*** ± 0.95	267.00*** ± 4.99	37.42*** ± 1.18
	Females (n=17)	98.12*** ± 0.74	264.65*** ± 7.01	35.58*** ± 1.69
	Total	97.89*** ± 0.64	266.00*** ± 4.04	36.71*** ± 0.97
Control Group (n=28)	Males (n=14)	57.92 ± 2.75	149.00 ± 6.22	23.74 ± 0.98
	Females (n=14)	60.93 ± 4.26	158.00 ± 9.57	24.75 ± 1.48
	Total	62.61 ± 2.64	154.00 ± 5.74	24.13 ± 0.88

***very highly significant ($p<0.001$) compare to values in control group (Student's t-test)
†calculated for each individual and then averaged to obtain the values for each group

Table 5: Grades of DNA damage in peripheral blood leukocytes patients and controls by ethinicity

Ethnic group	*No. of Individuals*	*DNA damage cell categories†*			
		0††	*I*	*II*	*III*
Patients					
Baniya	Male (n=18)	0	53	343	1404
	Female (n=12)	5	7	462	726
Jat Sikh	Male (n=9)	4	78	123	695
	Female (n=5)	0	20	189	291
χ^2			0.011 (p=0.9176)	0.812 (p=0.3675)	6.200 **(p=0.0128)**
Controls					
Baniya	Male (n=9)	0	214	187	501
	Female (n=6)	7	140	142	311
Jat Sikh	Male (n=5)	0	145	137	218
	Female (n=8)	10	154	241	395
χ^2			1.438 (p=0.2305)	12.874 **(p=0.0003)**	6.3999 **(p=0.0114)**

Values in bold are significant (χ^2-test, $p<0.05$)
††cells without tails (not subjected to analysis)
†as per Collins (2002)

ues of cardio-protective HDL (Jeeyar 2005; Mohanty et al. 2006). This has also been observed in the present study. Increased amounts of lipids can provide more substrate for lipid peroxidation by ROS. An increase in oxidised lipoproteins are important causation factors for coronary artery disease (Ghosh et al. 2006) while in the hypertensive state, reduced degrees of LDL oxidation may probably have decreased cardiovascular risk (Guxens et al. 2009). Other important factors with direct and strong contribution in inducing oxidative macromolecular damage including damage to DNA in the hypertensive state are the increased generation of reactive oxygen species and depleted anti-oxidants leading to oxidative stress. Imbalance in oxidant and anti-oxidant levels in hypertensive patients resulted in accumulation of DNA damage with time (Khanna et al. 2008). The endogenous and exogenous levels of anti-oxidants of the host had a preventive role and limited oxidative damage as observed with high blood pressure (Kashyap et al. 2005). In an experimental model of lead-induced hypertension, vitamin C supplementation enhanced the antioxidant activity (Attri et al. 2003).These observations emphazise the presence of oxidative stress and genetic damage which is directly lowered, on antioxidant supplementation. Antihypertensive drugs can also exert other health and genotoxic effects. Carlberg et al. (2005) had reported that atenolol-treatment caused higher cardiovascular mortality rates were documented in those receiving /not receiving atenolol therapies (Grossman et al. 2001) Atenolol also induced sister-chromatid-exchanges and increased the micronuclei frequency in cultured peripheral lymphocytes in treated patients (Telez et al. 2000). Recently, hypertensive individuals on chronic long-term atenolol therapy were reported to have an increased expression of fragile sites and chromosomal aberrations (Telez et al. 2010). As damage to DNA can lead to cellular dysfunction, cancer and other diseases (Bohr 2002), hypertensive patients with genetic damage may also be prone to such problems. However, earlier detection of DNA damage can assist in its management and provide for interventions such as antioxidant supplements so that health of hypertensive patients is not further compromised.

The increased DNA damage and observed abnormal lipid profile in both *Baniya* and *Jat* Sikh hypertensive patients may reflect differences in their dietary, life-style and occupational practices besides personal dietary preferences and habits. *Baniya* is an occupational caste originating from Western and Central India whereas *Jat* people are historically an Indo-Aryan ethnic group. Both groups differ greatly according to their personal lifestyle. Hence, it can be hypothesized that these differences may also be playing a contributory role in inducing DNA damage in combination with the state of hypertension and the effects of anti-hypertensive treatment as the matching healthy normotensive persons had comparatively much lesser damage. On the other hand, the non-significant differences in DNA damage and lipid profile parameter between the two patient sub-groups requires clarification in future studies carried out on a larger sample size.

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