ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

DNA Damage Assessment in Essential Hypertensive Patients Using the Single Cell Gel Electrophoresis (SCGE) Assay

Tarandeep Kaur and Gandhi G*

Department of Human Genetics, Guru Nanak Dev University, Amritsar, India

ABSTRACT

Leukocytic DNA damage in treated essential hypertension patients in different blood pressure ranges and in normotensive healthy controls was assessed using the alkaline Single Cell Gel Electrophoresis (SCGE/Comet) assay which is a sensitive method for DNA damage detection at cell level. A total of 100 individuals were assessed after informed consent. Atenolol-treated (mean 1.2±0.5y of treatment) hypertensive individuals (n=75; mean age 62.12±1.45y) visiting the local hospitals included 42 males and 33 females (mean systolic blood pressure (SBP) 151.2 mmHg; mean diastolic blood pressure (DBP) 90.0 mmHg). The control group comprised 25 age- and sex-matched controls (17 males, 8 females; mean age 64.50±1.95y; mean SBP 130.0 mmHg; mean DBP 76.0 mmHg). Using image analysis the comet parameters scored viz. Olive tail moment (OTM- 49.56±2.58), tail moment (TM-44.11±6.37) and percent tail DNA (21.8±0.90%) were significantly ($p \leq 0.001$) higher in patient group than the respective values in controls (5.11±1.01, 2.36±0.62 and 3.5±0.66%). Within the patient group, significant increase was observed for OTM and TM (which give migration and pattern of DNA damage) in mild hypertension and severe hypertension groups compared to high normal blood pressure group. Higher blood pressure levels and/or treatment with the drug (atenolol) could be causing the observed genetic damage in hypertensive patients.

Keywords: DNA damage, Gel electrophoresis, SCGE assay, hypertension

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Essential hypertension is sustained systolic blood pressure of >140mmHg and diastolic pressure of >90mmHg according to the Indian Hypertension Guidelines II [1]. Hypertension has become a major public health problem throughout the world and meta analysis has shown a rising trend in its prevalence also in different states of India over the last three decades [2]. Essential hypertension is known to be an independent risk factor for coronary artery disease, stroke and end stage renal diseases. Besides these susceptibilities, hypertension is also associated with decreased antioxidant status [3], and increase in oxidative stress [4] and reactive oxygen species [5]. Oxidative stress can cause genetic damage which can frequently promote age-related changes and malignancies thus adding to more complications for hypertensive individuals. High blood pressure requires treatment which in turn may also influence the level of oxidative stress. Beta-blockers act as antioxidants and are often prescribed to heart and hypertensive patients [6]. The imbalance of oxidant-antioxidant status manifests in genetic damage. Limited number of studies has assessed damage to genetic material in hypertensive patients on various treatment schedules [7,8] as well as in rat-models [9,10]. However, no studies have come to attention regarding atenolol-treated North-Indian patients. The present study hence investigated the relationship between leukocytic DNA damage in atenolol-treated hypertensive patients visiting the local hospitals. Of the number of methods used to assess genetic damage, the Single Cell Gel Electrophoresis (SCGE/comet) assay is a sensitive technique for the assessment of DNA damage [11]. Since damage to genetic material can cause cancer and other complications and as an earlier evaluation can assist in its management, it is important to assess genetic damage in hypertensive patients.

Review of work- DNA damage in atenolol-treated hypertensive patients and in matched controls was investigated. An association of DNA damage was further studied in patients in different blood pressure ranges. The relevant literature at global, national and regional levels is briefly reviewed.

The estimated total number of adults with hypertension in 2000 was 972 million worldwide- of these, 333 million were in economically developed countries and 639 million in economically developing countries [12]. The significance lies in the fact that these persons are susceptible to cardiovascular and renal problems. Increased blood pressure is also related to increased stress which can alter the genetic integrity.DNA damage was observed in blood, liver, brain and heart cells of animal models of renovascular hypertension; this was decreased by vitamin C implying that there is more oxidative stress in the hypertensive state [13]. Chromosomal telomere shortening has also been reported in hypertensive patients [14]. Treatment with atenolol increased frequency of micronuclei in treated essential hypertensive patients [15].

It is well recognized that hypertension is also a major health problem in India [16]. The prevalence of hypertension has been increasing in India, both in rural and urban regions. In 1960-1980's the prevalence of hypertension in urban area was between 2.6-5.2% and it increased to 20-33% in the last decade [17, 18, 19]. In studies from Punjab, Ahlawat *et al*. 2002

[20] reported a prevalence of 45.80% in women from Chandigarh while Sidhu *et al.* 2001 [21] reported a prevalence of only 15.10% in the women of Punjab and in a later study, the prevalence of hypertension was 20.15% [22]. In 2008, Yadav *et al*. [23] reported a prevalence of 32.2% in a north Indian upper socio-economic status population. Dhawan and Jain (2005) [24] documented increased levels of 8-Hydroxy-2'-deoxyguanosine (8-OHdG) in hypertensive patients from North West region of India Garlic supplementation reduced this oxidative DNA damage and increased the total antioxidants status as observed in their blood and urine samples. In our laboratory, hypertensive patients from local hospitals on different drugs were observed to have increased genetic damage compared to healthy controls [25]. The review shows that atenolol-treated hypertensive patients from Punjab have not been assessed for genetic damage and so this study was carried out.

METHODOLOGY

A case-control study was carried out which included 75 patients on atenolol- therapy and 25 healthy controls, matched for socio-economic status, age and sex. The patients were diagnosed with essential hypertension by the doctors and were on atenolol (a beta-blocker agonist) treatment. The informed voluntary written consent was obtained from all the participants of the study and the study was cleared by the Institutional Ethics Committee. The demographic information of the subjects was recorded on a questionnaire. For each individual an average of three readings, for systolic blood pressure (SBP) and diastolic blood pressure (DBP), were taken using a mercury sphygmomanometer and about one ml of intravenous blood sample was drawn. Cell viability of each sample was checked before performing the alkaline SCGE assay by the method of Singh *et al.* (1988) [26] with slight modifications. For the assay, agarose pre-coated slides were layered with blood samples mixed in low melting point agarose (0.75%, LMPA) and sandwiched with a third agarose layer. Preparations were lysed, electrophoresed at pH ≥ 13 and stained using silver nitrate. Coded slides were scored for DNA damage (100cells/sample) using image analysis software (Comet assay software program (CASP)-http://www.casp.of.pl). The comet parameters taken were Olive tail moment (OTM), tail moment (TM) and percent DNA tail [27, 28]. Values were taken as means ± S.E.M. Group differences for the extent of primary DNA damage were tested by the Student's t-test using SPSS version 16.0 for windows 7. The p-values, $p \leq 0.05$ and $p \leq 0.01$, were taken as significant.

Presentation and Analysis of Data

The study included 75 essential hypertensive patients and 25 age- and sex- matched healthy individuals (control group) with no family history of hypertension. The patient group comprised 42 males and 33 females who were on 50mg daily dose of atenolol for an average of 1.2±0.5 years. There were 17 males and 8 females in the control group. In Table1, the base line characteristics of patients and control group are presented. The patients were in the age range of 40-83y with mean of 62.12±1.45 years and the control group was in the range of 48-83y (mean 64.50± 1.95y). The mean SBP/DBP for patients and control group was 151.2/90 mmHg and 130/76 mmHg, respectively. Most of the patients (n=51) and controls (n=21) were taking vegetarian diet. All the individuals in the study were non smokers. Alcohol drinking was also not

so common both, in patient and control groups. The individuals in the patient group were divided into different hypertension stages on the basis of blood pressure levels (IHG-II) and included the high normal (n=23;130-139/85-89mmHg), mild stage I hypertension (n=26;140-159/90-99mmHg), moderate stage II hypertension (n=12;160-179/100-109mmHg) and severe stage III hypertension (n=14;≥180/≥110mmHg). The DNA damage parameters as also studied by Dhawan *et al.* (2009) [29] included Olive tail moment (product of tail length and the fraction of total DNA in the tail), tail moment (combines information on tail length and tail intensity) and percent DNA tail (the intensity of the tail compared with the intensity of the whole comet) are given in Table 2. The comet assay results demonstrated that average values of OTM (49.56±2.58), TM (44.11±6.37) and percent DNA tail (21.8±0.9%) in essential hypertensive patients were significantly increased from the respective values in the control group (5.11±1.01; t_{cal}=16.06, df = 98, p < 0.001;2.36±0.62; t_{cal}=6.52, df = 98, p < 0.001;3.5±0.66 %; t_{cal}=16.19, df = 98, p < 0.001). Within the patient group, though there was a non-significant increase in percent DNA tail in mild hypertension (23.34±1.8%) and severe hypertension (22.41±1.9%) when compared with that in the high-normal blood pressure group (19.58±1.2%), yet a significant increase (p<0.05) was found for OTM when values in mild hypertension group (55.38±5.24) and severe hypertension group (54.06±4.87) were compared to the high normal blood pressure group (40.23±3.36). Similarly, a significant increase was also observed for TM for values in severe hypertension (61.70±21.1) and mild hypertension (42.39±5.71 groups compared to those in high normal blood pressure (64.19±27.7) group.

Table1 Characteristics of Essential Hypertensive Patients and Control Individuals

Group	Sex		Age range in years (mean)	SBP (mmHg) (mean±S.E.M)	DBP (mmHg) (mean±S.E.M)	Diet		Smokers		Alcohol Drinkers	
	Male (%)	Female (%)				Veg.	Non-veg	Yes	No	Yes	No
Patients (n=75)	42 (56)	33 (44)	40-83 (62.10)	151.5±2.79	90.0±1.18	51	24	-	75	19	56
Controls (n=25)	17 (68)	8 (32)	48-83 (64.50)	130.0±1.53	76.0±1.24	21	4	-	25	3	22

Table 2 DNA Damage in Patients in different Blood Pressure Ranges

Group	Blood pressure group* (SBP/DBP) mmHg	No. of individuals (M/F)	Olive tail moment mean±S.E.M (range)	Tail moment mean±S.E.M (range)	Percent DNA tail mean±S.E.M (range)
Patients	High normal (131-139/85-89)	23 (10/13)	40.23±3.36**[a] (9.80-71.93)	24.88±2.74**[a] (3.40-53.48)	19.58±1.20**[a] (4.10-32.13)
	Mild (140-159/90-99)	26 (15/11)	55.38±5.24**[a,b] (13.90-108.60)	42.39±5.71**[a,b] (1.83-100)	23.34±1.80**[a] (7.80-46.48)
	Moderate (160-179/100-109)	12 (10/2)	49.57±6.59**[a] (21.85-98.90)	64.19±27.70**[a] (5.64-359.70)	22.29±2.50**[a] (11.19-40.90)
	Severe (≥180/≥110)	14 (7/7)	54.06±4.80**[a,b] (30.55-35.40)	61.70±21.10**[a,b] (15.07-326.79)	22.41±1.90**[a] (13.05-38.77)
	Total	75 (42/33)	49.56±2.58**[a] (9.80-108.60)	44.11±6.37**[a] (1.83-359.70)	21.8±0.90**[a] (4.10-46.48)
Controls	Normal (130-76)	25 (17/8)	5.11±1.01 (0.00-15.75)	2.36±0.62 (0.04-13.17)	3.5±0.66 (0.00-9.88)

*According to Indian Hypertension Guidelines-II, 2007. **[a] Statistically significant when compared to total control group (p≤0.001, Student's t-test). **[b] Statistically significant when compared to high normal blood pressure group (p≤0.05, Student's t-test). SBP- Systolic Blood Pressure; DBP- Diastolic Blood Pressure.

ISSN: 0975-8585

DISCUSSION

The results revealed that DNA damage was significantly higher in the atenolol- treated essential hypertensive patients as compared to normotensive individuals. Patients in different blood pressure categories were studied also to find any association between leukocyte DNA damage and different blood pressure ranges. In the patient group, TM was highest in moderate hypertension group and percent DNA tail and OTM in mild hypertension group. .These differences may be resulting from the varying life style activities of the patients within these ranges. The overall results reveal that OTM was ~10 times, TM ~22 times and percent DNA tail ~7 times higher in patient group than in control group and these values are highly significant ($p < 0.001$). OTM, TM and percent DNA tail reflect overall DNA damage in the cells. OTM represents the migration and pattern of DNA damage [30], TM represents the amount of DNA and distance it migrates into the comet tail and percent DNA tail gives a relative indication of the number of DNA strand breaks within the cell [31]. Tail Moments (OTM and extent tail moment) and percent DNA tail are most sensitive parameters and are most frequently used. Among these TM is preferred parameter for both high and low damaged cells as it provides most stable estimates for DNA damage [32]; in the present study, these comet parameters were studied and their values observed to be highly significant from controls ($p < 0.001$) indicating DNA damage in the hypertensive patients. A loss of balance between status of oxidative stress and level of antioxidants in hypertension occurs [33] generating reactive oxygen species which caninteract with nitrogenous bases of DNA strands causing oxidative damage to DNA resulting in base or sugar modifications, covalent cross links and single- and double strand-breaks [34]. The treatment modalities of hypertension can also influence the level of oxidative stress. Though the prescribed beta-blockers also possess antioxidant properties [35] however their genotoxicity has also been reported [7] indicating that treatment with these can also cause DNA damage.

The findings of increased DNA damage in essential hypertensive patients in the present study are in accordance with those reported in literature. Dincer *et al*. (2008) [36] observed increased 8-OHdG levels in hypertensive patients with chronic kidney disease with low antioxidant status Similar results were also observed by Yilidz *et al*. (2008) [37]. They found that DNA damage was significantly increased in white-coat hypertensive patients and the total antioxidant status was decreased. Treatment modalities could also influence the level of genetic damage in hypertension [10]. Among main cardiovascular risk factors, hypertension was observed as the strongest determinant of oxidative stress and DNA damage [38]. However, a significant decrease in DNA damage and blood pressure and increase in antioxidant status was observed with grape juice supplementation [39]. Recently, Subash *et al.* (2010) [40] also reported increased DNA damage and decreased antioxidant status in treated compared to untreated hypertensive patients. Both genetic and lifestyle factors can contribute to the development of hypertension and DNA damage. Lifestyle factors such as smoking, age, gender, alcohol consumption [41], mobile phone usage [42], dietary supplements [43] can modify the level of DNA damage in humans. Genotoxicity and carcinogenicity of antihypertensive agents have also been reported for many drug classes (Brambilla *et al.,* 2006). Atenolol among other beta-blockers was observed to cause chromosomal damage in those treated with it [7].

Therefore, the observed increase in DNA damage in patient group in the present study may be due to disease or treatment as patient and control groups were matched for other parameters. The results imply that these patients are at a risk of developing neoplasia and target organ damage as compared to healthy controls.

Anthropological Significance

Human beings shuttle between home, work, community and family, forming a conduit through which life is constantly protected and supported. This scenario can increase the oxidative stress which can result in hypertension. Drug specifications and dietary supplementation have been reported to reduce blood pressure and counteract oxidative stress, and thereby offer cardio-protection in essential hypertensives on one hand, while having a capacity to induce genetic damage on the other. As the hypertensive condition can be managed after appropriate intervention, the results of the study can be utilized to promote strategies to prevent and/or delay hypertension and associated genetic damage and to improve the care of the patient with associated disorders.

CONCLUSION

Significantly increased DNA damage was detected in hypertensive patients on treatment with atenolol compared to normal individuals. DNA damage can produce gross chromosomal alterations in addition to point mutations and thus can be involved in the inactivation or loss of proto-oncogenes and tumor suppressor genes which can lead to cancer. Therefore, treated essential hypertension seems to be an important clinical situation requiring a close follow-up.

REFERENCES

[1] Indian Hypertension Guidelines-II. http://www.apiindia.org/hypertension guidelines/ hyperhome.htm. Assessed July3,2010.
[2] Gupta R. India Journal of Human Hypertension 2004; 18: 73–78.
[3] Subash P, Premagurumurthy K, Sarasabharathi A and Cherian KM. Journal of Human Hypertension2010; 24: 475–482.
[4] Grossman E. Diabetes care 2010; 31(2): 185-189.
[5] Harrison DG and Gongora MC. Med. Clin. N. Am. 2009; 93: 621–635.
[6] Ericson G. Journal of American Medical Association 2009. Online at http://mednews.wustl.edu/news/page/normal/5837.html.
[7] Télez M, Eduardo OL, Gonzalezc AJ, Floresd P, Huertaa I, Ramíreza JM, Barasoaina M, Criadoe B, and Arrietaa I. Mutation Research 2010; 695: 46–54.
[8] Brambilla G, Mattioli F, Martelli A. Mutagenesis 2010; 25(4): 315-326.
[9] Robianno L, Martelli A, Allavena A, Mazxei M, Gazzaniga GM, Brambilla G. Cancer Res. 1991; 51:2273-2279.
[10] Almeida MR, Lema E.de.O, da-Silva VJ, Campos MG , Antunes LM , Salman AK, Dias FL. Mutat Res 2008; 657(2): 155-15.

[11] Collins AR, Oscoz AA, Brunborg G, Gaiva˜o I, Giovannelli L, Kruszewski M, Smith CC, Ste˘tina R. Mutagenesis 2008; 1–9.
[12] Kearney PM, Whelton M, Reynolds K, Munter P, Whelton PK, He J. Lancet 2005; 365(9455): 217-223.
[13] Nishi EE, Campos RR, Bergamaschi CT, Almeida VR, Ribeiro DA. Human & Experimental Toxicology. 2010; 29:593.
[14] Yang Z, Huang X, Jiang H, Zhang Y, Liu H, Qin C, Eisner GM, Jose P, Rudolph L, Ju Z. Hypertension 2009; 53: 639-645.
[15] Télez M, Martinez B, Criado B, Lostoc C, Ortega B, Flores P, Ortiz-Lastra E, Alonso RM, Jimenez RM, Arrieta I. Mutagenesis 2000; 15(3): 195-202.
[16] Mehta S. Chest 2003; 123: 989-992.
[17] Gupta R, Gupta S, Gupta VP and Prakash H. J. Hyperten 1995; 13: 1193-1200.
[18] Shanthirani CS, Pradeepa R, Deepa R, Premalatha G, Saroja R, Mohan V. Jour. Assoc. Physicians India 2003; 51: 20-27.
[19] Prabhakaran D, Shah P, Chaturvedi V, Ramakrishnan L, Manhapra A and Reddy KS. Natl Med J India 2005; 18: 59-65.
[20] Ahlawat SK, Singh MMC, kumar R, Kumari S, Sharma BK. J Ind Med Assoc 2002; 10: 547-555.
[21] Sidhu,S, Kamal N, Kumari K In: The Science of Man in Service of Maned. by MK Bhasin and SL Malik 2001; 157-160. University of Delhi, Delhi
[22] Sidhu S, Kumari K, Prabjot. J Hum Ecol 2005; 17(3): 211-215.
[23] Yadav S, Boddula R, Genitta G, Bhatia V, Bansal B, Kongara S, Julka S, Kumar A, Singh HK, Ramesh V, Bhatia E. Indian J Med Res 2008; 128: 712-720.
[24] Dhawan V, Jain S. Molecular and Cellular Biochemistry 2005; 1275: 85-94.
[25] Jyoti J. M.Sc. dissertation submitted to Department Human Genetics, Guru Nanak Dev University, Amritsar (Punjab) India. 2007
[26] Singh NP, McCoy MT, Tice RR, Schneider EL. Experimental Cell Research 1998; 175: 184-191.
[27] Kumaravel TS, Jha AN. Mutat. Res. 2006; 605: 7-16.
[28] Collins AR, Oscoz AA, Brunborg G, Gaiva˜o I, Giovannelli L, Kruszewski M, Smith CC and Ste˘tina R. Mutagenesis 2008; 1–9.
[29] Dhawan A, Bajpayee M, Parmar D. Cell Biol Toxicol 2009; 25: 5-32.
[30] Patton WP, Chakravarthy U, Davies RJH and Archer DB. Invest Ophthalmol. Vis Sci 1999; 40: 3268–3275.
[31] Heaton PR, Ransley R, Charlton CJ, Mann SJ, Stevenson J, Smith BHE, Rawlings and EJ Harper. J Nutr 2002; 132: 1598S–1603S.
[32] Lee E, Oh E, Lee J, Sul D, Lee J. Toxicological Sciences 2004; 81: 121–132.
[33] Mclntyre M, Bohr DF, Dominiczak F. Hypertension 1999; 34: 539-545.
[34] Loft S, Poulsen HE. Journal of Molecular Medicine 1996; 74: 297-312.
[35] Gomes A, Costa D, Lima JLF, Fernandes E. Bioorganic Medicinal Chemistry 2006; 14**:** 4568–4577.
[36] Dincer Y, Sekercioglu N, Pekpak M, Gunes KN, Akcay T. Ren. Fail. 2008; 30(10): 1006-1011.

[37] Yıldız A, Gür M, Yılmaz R, Demirbağ R, Çelik MH, Aslan M, Koçyiğit A. Türk Kardiyol Dern Arş - Arch Turk Soc Cardiol 2008; 36(4): 231-238.
[38] Fandos M, Corella D, Guillen M, Portoles O, Carrasco P, Iradi A, Martinez-Gongalez MA, Estruch R, Covas MI, Lamuela-Raventos RM, Michavilla MT, Cerda C, Torregrosa R, Redon J, Chaves FF, Tormos MC, Ocete D, Saez GT. Free Radical Research 2009; 43(12): 1179-1186.
[39] Park YK, Lee SH, Park E, Kim JS, Kang MH. Ann. N. Y. Acad Sci 2009; 1171: 385-390.
[40] Subash P, Gurumurthy P, Sarasabharathi A, Cherian KM. Indian Journal of Clinical Biochemistry 2010; 25(2): 127-132.
[41] Manikantan P, Balachandar V, Sasikala K, Mohanadevi S, Lakshmankumar B. Internet Journal of Toxicology 2010; 7: 2.
[42] Gandhi G, Anita. Indian Journal of Human Genetics 2005; 11(2): 99-104.
[43] Watters JL, Satia JA, Kupper LL, Swenberg JA, Schroeder JC, Switzer BR. Cancer Epidemiology, Biomarkers & Prevention 2007; 16: 1428-1436.