ORIGINAL ARTICLE

URINARY 8-OHdG: A MARKER OF OXIDATIVE STRESS TO DNA AND TOTAL ANTIOXIDANT STATUS IN ESSENTIAL HYPERTENSION WITH SOUTH INDIAN POPULATION

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ABSTRACT

Establishment of non-invasive urinary biomarker for the early prediction of essential hypertension (EH) is important. We evaluated whether estimation of urinary DNA, serves as a marker to predict the extent of cellular oxidative stress in essential hypertension. A total of 180 South Indian subjects aged 30-65 were recruited for the study. Of these hypertensive subjects investigated, 30 were newly diagnosed and were not on any antihypertensive drugs, but had systolic blood pressure 140-160 mmHg and diastolic blood pressure 95-100 mmHg and 75 hypertensive patients who already on drug therapy for one year and 75 were South Indian normotensive healthy controls with blood pressure $\leq 120/80$ mmHg. The 8-OHdG level in urine was significantly increased in hypertensive patients (both newly diagnosed and who already on drug therapy) compared with control group. The significant increase in 8-OHdG was observed in newly diagnosed hypertensive patients compared with hypertensive patients who already on drug therapy. There was a significant decrease in serum TAS value in essential hypertensive group when compared to control group. The urinary 8-OHdG was independently correlated with serum TAS. Decreased TAS levels, which reflect to increased oxidative stress, may be the reason of increased urinary 8-OHdG in South Indian hypertensive patients. Our preliminary data suggest that the competitive ELISA for 8-OHdG appears to be a simple method for quantifying the extent of oxidative stress.

KEY WORDS

Total Antioxidant Status, Urinary 8-OHdG, Essential Hypertension, Oxidative Stress.

INTRODUCTION

Hypertension is a major health problem in developed as well as developing countries with a common end result of elevated blood pressure (BP). Essential hypertension or pre-hypertension refers to the high blood pressure with no

identifiable causes. It can be diagnosed when the systolic blood pressure (SBP) of 140- 159 mmHg and /or diastolic blood pressure (DBP) of 80 to 90 mmHg at three random checks and need medical monitoring and lifestyle changes (1).

Essential hypertension is a common trait of multifactorial determination, imparting an increased risk of cardiovascular, cerebrovascular and renal diseases. 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 unknown.

Markers for DNA oxidation were few. Only in recent years 8-

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hydroxy-2-deoxy guanosine (8-OHdG) emerged as a marker of oxidative stress (2). Urinary 8-OHdG in particular, has been measured most frequently to indicate the extent of oxidative damage because it is noninvasive and technically less involved. It is well known that the study of oxidative DNA damage is clinically important (3).

Urinary 8-OHdG and its analogs, 8-hydroxyguanosine and 8-hydroxyguanine, are linked to many degenerative diseases (4). Reactive oxygen species (ROS) can interact with DNA to produce damage including single- and double-stranded DNA breaks, deletions and nucleoside modifications. Upon DNA repair, 8-OHdG is excreted in the urine. It is widely thought that continuous damage to DNA is a significant contributor to various diseases like cancer, hypertension etc. 8-OHdG, the oxidized form of the nucleoside 2'-deoxyguanosine present in DNA, is one of the most reliable and abundant markers of DNA damage because it reflects extremely low levels of oxidative damage (5). The association of ROS and the use of 8-hydroxydeoxyguanosine (8-OHdG) as a biomarker of oxidative stress have been investigated in many diseases, including bladder and prostate cancer (6), cystic fibrosis (7), atopic dermatitis (8) and rheumatoid arthritis (9).

Quantification of urinary 8-OHdG, a specific DNA repair product in the urine can be made with a simple ELISA. The present study was undertaken to investigate the relationship between urinary 8-OHdG and total antioxidant status in newly diagnosed hypertensive patients and in patients who already on drug therapy.

MATERIALS AND METHODS

Subjects: The study design included three groups of human subjects as participants (controls, newly diagnosed EH patients and EH patients who already on drug therapy). These subjects were enrolled from Dr. K. M. Cherian Heart Foundation,

Chennai. All participants underwent a detailed clinical examination. Different parameters such as Age, BP, Hb and ECG were recorded. The study was approved by both research and ethics committees of our institute. An informed consent was obtained from these subjects prior to participation in the study.

All volunteers enrolled in this study were aged between 30 – 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. EHT patients who were on antihypertensive drugs for one year were included in group 3.

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: 3ml of peripheral venous blood was collected in heparinized tubes. 5ml urine was collected into a sterile container to measure 8-OHdG. Heparinized blood was centrifuged at 3000 rpm for 10 min for plasma separation. Plasma sample was used for the analysis of total antioxidant status (TAS), lipid profile and glucose.

Measurement of Biochemical Markers: Plasma triglyceride, total cholesterol, LDL, HDL and fasting glucose concentrations were measured with an auto analyzer (Randox Daytona, clinical biochemistry analyzer) by using commercial kits.

Measurement of Total Antioxidant Status (TAS): Total antioxidant status of plasma was determined by ferric reducing antioxidant power assay (10-12), whereby at low pH, reduction of a ferric tripyridyl triazine (Fe^{3+} -TPTZ) complex (Sigma Aldrich, St. Louis, MO, USA) to a ferrous form, which has an intense blue color, that can be monitored by measuring the absorbance at 593 nm using spectrophotometer. It is 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.

Evaluation of urinary 8-OHdG: Urine specimens were centrifuged at 3000g for 10 minutes or filtered through 0.45 mm filter, prior to use in the assay. The supernatant was used for the measurement of the 8-OHdG levels using a competitive

in vitro enzyme-linked immunosorbent assay (ELISA) kit (Cell Biolabs, Inc., San Diego, CA 92126, USA). The detection range of the ELISA assay was 0 to 20 ng/mL. All of the 8-OHdG measurements were performed within 6 months of post-collection.

Statistical analysis: Results are presented as mean $\pm$ SD or frequency expressed as percentage, categorical variables were compared by using chi-square 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. Association of urinary 8-OHdG with demographical, clinical and antioxidative parameters was assessed by Pearson correlation coefficient. Values of probability less than 0.05 was considered statistically significant. The statistical analysis was performed with SPSS 10.0 for windows. The statistical power estimated was 94% to detect main effects and α set at 0.05.

RESULTS AND DISCUSSION

The mean urinary 8-OHdG concentration and total antioxidant status (TAS) of all the three groups are illustrated in Fig 1 and 2 respectively, showing that newly diagnosed essential hypertensive groups had significantly higher urinary 8-OHdG levels (15.9 ± 5.9) and significantly lower TAS levels

Fig 1: Mean Urinary 8 - OHdG was compared between controls and essential hypertensive patients

Mean urinary 8-OHdG level of the patient group was significantly higher ($P < 0.05$) than control (group 1). Mean urinary 8-OHdG level of newly diagnosed EH patients (group 2) was significantly higher ($P < 0.05$) when compared with EH patients during therapy (group 3).

(678.8 ± 104.7) than control group ($P < 0.05$). After one year of treatment with antihypertensive drugs, there was a significant reduction of urinary 8-OHdG levels (11.7 ± 2.9) and significantly increased TAS levels (789.5 ± 153.9) in group 3 when compared

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

Parameters	Control	Essential Hypertension (EH) (Newly Diagnosed-ND)	Essential Hypertension (EH) (During Therapy-DT)	P-Value
Number	75	30	75	-
Gender (Male/Female) ^a	62/13	22/08	61/14	NS
Age (Years)	40.0 $\pm$ 8.8	46.0 $\pm$ 10	43.0 $\pm$ 8.5	-
SBP (mmHg)	121.2 $\pm$ 5.2	158.3 $\pm$ 10.3 [‡]	147.4 $\pm$ 7.6 ^{‡*}	$P < 0.0001$
DBP (mmHg)	80.0 $\pm$ 1.9	99.5 $\pm$ 3.7 [‡]	93.0 $\pm$ 7.4 ^{‡*}	$P < 0.0001$
Glucose (mg %)	95.2 $\pm$ 7.7	93 $\pm$ 6.8	97.6 $\pm$ 10 [*]	$P < 0.0424$
Total Cholesterol (mg %)	155 $\pm$ 14.8	172.4 $\pm$ 23 [‡]	165.2 $\pm$ 20.6 [‡]	$P < 0.0001$
TAG (mg %)	97.8 $\pm$ 14.1	139.2 $\pm$ 20.7 [‡]	112.3 $\pm$ 30.6 ^{‡*}	$P < 0.0001$
HDL Cholesterol (mg %)	41.49 $\pm$ 5.5	34.0 $\pm$ 6 [‡]	37.0 $\pm$ 5.2 ^{‡*}	$P < 0.0001$
LDL Cholesterol (mg %)	94.0 $\pm$ 13	110.5 $\pm$ 21.3 [‡]	105.0 $\pm$ 18.9 [‡]	$P < 0.01$
Urinary 8-OHdG (ng/mg of creatinine)	5.7 $\pm$ 2.4	15.9 $\pm$ 5.9 [‡]	11.7 $\pm$ 2.9 ^{‡*}	$P < 0.0001$
TAS (mM/L)	900.8 $\pm$ 182	678.8 $\pm$ 104.7 [‡]	789.5 $\pm$ 153.9 ^{‡*}	$P < 0.0001$

One-Way ANOVA and LSD post-hoc was used to derive the P value; *P value Significant Compared with HT-Newly diagnosed Vs HT-During therapy ($P < 0.05$); [‡]P value Significant Compared with Control Vs HT-During therapy ($p < 0.05$); [‡]P value Significant Compared with Control Vs HT-Newly diagnosed ($P < 0.05$); ^aChi-square test. SBP, systolic blood pressure; DBP, diastolic blood pressure; TAG, triacylglycerol; TAS, total antioxidant status; EH, essential hypertension; ND, newly diagnosed; DT, during therapy.

Fig 2: Mean TAS was compared between controls and essential hypertensive patients

Mean TAS level of the patient group was significantly lower ($P<0.05$) than control (group 1). Mean TAS level of newly diagnosed EH patients (group 2) was significantly lower ($P<0.05$) when compared with EH patients during therapy (group 3).

with controls ($P<0.05$) and newly diagnosed essential hypertensive patients ($P<0.05$).

The inter-comparison of baseline characteristics of SBP, DBP, glucose, lipid profile, urinary 8-OHdG and TAS were different in between the essential hypertensive patients and the control groups are summarized in Table 1. The urinary 8-OHdG was correlated with other variables like age, glucose, lipid profile and TAS in EH-during therapy group was demonstrated in Table 2. Only TAS was highly significant when compared with urinary 8-OHdG. The correlation of urinary 8-OHdG with TAS of all the three groups was illustrated in Fig 3.

Table 2: Correlation between urinary 8-OHdG with other variables of EH patients who already on drug therapy

Variables	Correlation coefficients	P Value
Age (Years)	-0.0124	NS
SBP (mmHg)	0.0273	NS
DBP(mmHg)	0.2464	NS
Glucose (mg %)	0.1700	NS
Total Cholesterol (mg %)	0.0443	NS
TAG (mg %)	0.0176	NS
HDL Cholesterol (mg %)	-0.1020	NS
LDL Cholesterol (mg %)	0.0673	$P<0.0331$
TAS (mM/L)	-0.9332	$P<0.0001$

The urinary 8-OHdG was correlated with other variables in group 3 (EH-During Therapy). The urinary 8-OHdG was negatively correlated with TAS and the P value was highly significant ($P<0.0001$).

Fig 3: Correlation of urinary 8-OHdG with TAS

The urinary 8-OHdG was independently correlated with plasma TAS in all the groups (Control, EH-Newly Diagnosed and EH-During Therapy). The negative correlation was highly significant ($P<0.0001$) in all the 3 groups.

Our data clearly demonstrate that oxidative DNA damage occurs in essential hypertensive patients more than in normal blood pressure subjects and that the oxidative DNA damage is significantly diminished by the administration of antihypertensive drugs for the period of one year. To the best of our knowledge, this is the first report in South Indian population, to evaluate the association between urinary 8-OHdG, oxidative DNA damage with total antioxidant status in both newly diagnosed and those already on drug therapy for one year.

The imbalanced Reactive oxygen species (ROS) formation results in oxidative modifications of macro molecules and subsequently, genomic instability (13). Formation of 8-OHdG is regarded as a useful indicator of oxygen radical induced DNA damage (14). Intracellular accumulation of this compound has been demonstrated in animal models of oesophageal squamous cell carcinoma (OSCC) (15) and adenocarcinoma (16). Several lines of evidence show that oxidative damage may be an important mechanism underlying several pathophysiological states, for example, atherosclerosis caused by oxidative modification of low-density lipoprotein (17); diabetic complications caused by oxidative damage of lipids, proteins (18) and DNA (19); aging caused by oxidative damage of proteins and myocardial damage/loss through oxidative injury.

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 (20). Antioxidant systems (such as TAS) prevent the damage of DNA (21). Honda et al reported that reduced activities of the antioxidant enzymes increased levels of oxidative DNA damage. Recently the involvement of oxidative stress in hypertension has been demonstrated in experimental hypertension and in humans (22-24). Oxidative damage of DNA occurring during atherosclerosis was also demonstrated by feeding rabbits with cholesterol. Oxidative damage as manifested by expression of 8-OHdG, was found in plaques in rabbits (25). 8-OHdG has been suggested to serve as a new sensitive biomarker of the in vivo oxidative DNA damage in diabetes (26).

Isoprostanes, such as the lipid (arachidonic acid) peroxidation metabolite, have been emerged as one of the best indices of oxidative injury. However, we used urinary 8-OHdG as the marker of oxidative DNA damage in this study, because we focused on the oxidative DNA damage, but not lipid oxidation or general oxidative damage. The good correlation between 8-OHdG and isoprostanes was well documented in one report (27).

All this data and other reports, jointly support that oxidative stress could be used as significant risk predictor in essential hypertensive patients. The reason that urinary 8-OHdG was selected as a marker of oxidative DNA damage in this study was because of its convenient use in clinical practice and also they allow the assay to be non-invasive. However, no data on urinary 8-OHdG and its comparison with TAS in patients with essential hypertension are available.

Corroborating our preliminary observations, the present study provided 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.

However, in current study, plasma TAS levels, which provide a measure of total defenses against ROS were found at low level in hypertensive patients. In our study, the major decrease in mean TAS levels were observed in newly diagnosed EH group. In addition, the major increases in urinary 8-OHdG values were observed in newly diagnosed EH patients and an independent negative correlation between urinary 8-OHdG and TAS levels were detected.

In our study, increased oxidative DNA damage and decreased

serum TAS levels were frequently observed in the newly diagnosed, early stages of HT, suggesting that oxidative stress is important in the pathogenesis of essential hypertension.

The above results demonstrated that ROS might be critical in the early stages of human HT, which may represent a novel pathophysiological mechanism for increased risk of cardiovascular diseases and target organ damage in patients with essential hypertension.

In conclusion, it is likely that DNA damage caused by ROS occurs more in newly diagnosed essential hypertensive patients than in persons with normal blood pressure and that antihypertensive drug significantly reduces DNA damage in essential hypertension in vivo. Accordingly, it remains within the future scope to see the effect of antioxidants in combination with antihypertensive drug interaction with damaged DNA in essential hypertensive patients and a large clinical trial might be needed.

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

Furthermore, hypertension is one of the most important risk factors of atherosclerosis. 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 that might have more serious oxidative damage, especially DNA damage, among hypertensive patients. Therefore, we need simple, accurate and specific test kits like urinary 8-OHdG for oxidative DNA damage so that we can use the appropriate drugs for antihypertensive medications, for hypertensive patients who have more DNA damage.

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