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Original article

Oxidative stress in patients with essential hypertension: A comparison of dippers and non-dippers

Aymelek Gönenç [a,*], Aysun Hacışevki [a], Yusuf Tavil [b], Atiye Çengel [b], Meral Torun [a]

[a] Department of Biochemistry, Faculty of Pharmacy, Gazi University, Ankara, Turkey
[b] Department of Cardiology, Faculty of Medicine, Gazi University, Ankara, Turkey

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ABSTRACT

Background: Oxidative stress seems to play an important role in the pathophysiology of essential hypertension. We aimed to examine serum MDA, NO, 8-OHdG, ADMA, NT, CoQ10 and TAC as biomarkers of oxidative stress in dipper and non-dipper hypertensive patients.
Methods: Eighteen dipper hypertensives, 20 non-dipper hypertensives and 22 healthy control subjects were included in the study. Clinical assessment and ambulatory blood pressure monitoring were performed in patients. Serum MDA, TAC and NO levels were measured by using spectrophotometric methods. CoQ10 levels were measured by HPLC method. 8-OHdG, ADMA and NT were quantitated by ELISA methods.
Results: MDA levels were significantly higher in dipper and non-dipper groups compared to controls ($p<0.05$ and $p<0.01$, respectively). TAC levels were found at low level in patients dipper and non-dipper patients compared to control group ($p<0.01$). Higher ADMA and NT levels but lower CoQ10 levels were found in non-dipper group compared to healthy controls ($p<0.01$, $p<0.05$, and $p<0.05$, respectively). ADMA levels were found higher in non-dipper group than those of dipper group ($p<0.01$).
Discussion: Increased ADMA, NT levels and decreased CoQ10 levels in non-dipper hypertensive patients might indicate more severe oxidative stres compared with dipper hypertensive patients, which plays an important role in the development of cardiovascular diseases. Increased MDA and reduced TAC levels might be considered as prospective prognostic markers of the development of cardiovascular diseases in dipper and non-dipper hypertensive patients.

1. Introduction

Hypertension is an important worldwide public health challenge because it is one of the most common chronic conditions [1]. It is a major risk factor for cardiovascular disease [2]; it remains an important cause of coronary heart disease, cerebrovascular disease, peripheral artery disease and heart failure [3].

Blood pressure (BP) is characterized by alteration of rhythm along 24 h in hypertensive patients. Ambulatory blood pressure monitoring (ABPM) can supply information on day/night blood pressure variations and dipper status [4]. This variation presents a morning increase, small postprandial decline, manifest decrease in systolic and diastolic blood pressure during sleep compared to daytime. In some hypertensive patients BP values reveal 10–20% lower values in the night, compared to daytime measurements. This condition is called "dipper"change. But in other hypertensives, contrary to this normal change, nighttime BP lowering does not occur or shows a decrease less than 10% which is called "non-dipper"change [5]. Many studies have shown that non-dippers carry a high risk of cardiovascular disease such as atherosclerotic events and also high incidence of target organ damage compared to dippers [6,7].

The concept that structural and functional abnormalities in the vasculature, including endothelial dysfunction, increased oxidative stress and vascular remodeling, may antedate hypertension and contribute to its pathogenesis has gained support in recent years [8,9]. Oxidative stress is defined as the sustained increase in the levels of reactive oxygen species (ROS) such as hydrogen peroxide, superoxide anion radical, and other free radicals. An imbalance between ROS production and clearance leads to pathologically elevated levels of ROS, which cause cellular dysfunction by oxidizing various biochemical structures such as DNA, lipids, and protein [10]. The increased lipid peroxidation and reduced nitric oxide bioavailability have been demonstrated in both experimental and human hypertension [11–14]. In previous study, low levels of antioxidant enzymes and molecules were found in non-dipper hypertensives compared with dipper hypertensives [15].

Abbreviations: ABPM, Ambulatory blood pressure monitoring; ROS, Reactive oxygen species; MDA, Malondialdehyde; NO, Nitric oxide; 8-OHdG, 8-Hydroxy-2-deoxyguanosine; ADMA, Asymmetric dimethylarginine; NT, Nitrotyrosine; CoQ10, Coenzyme Q10; TAC, Total antioxidant capacity; SBP, Systolic blood pressure; DBP, Diastolic blood pressure.

* Corresponding author. Tel.: +90 312 2023152; fax: +90 312 2235018.
E-mail address: aymelek@gazi.edu.tr (A. Gönenç).

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In this study, we aimed to examine malondialdehyde (MDA), nitric oxide (NO), 8-hydroxy-2-deoxyguanosine (8-OHdG), asymmetric dimethylarginine (ADMA), nitrotyrosine (NT), coenzyme Q10 (CoQ10) and total antioxidant capacity (TAC) as biomarkers of oxidative stress in hypertensive dipper and non-dipper patients and compared with that of normotensives.

2. Subjects and methods

2.1. Subjects

Newly diagnosed thirty-eight essential hypertensive patients (19 men and 19 women, mean age 52.92 ± 1.76 years) were recruited from the department of cardiology, then compared to twenty-two non-hypertensive healthy volunteers (10 men and 12 women, mean age 50.09 ± 2.17 years). Exclusion criteria for entry in the study were presence of hypercholesterolemia, hypertriglyceridemia, diabetes mellitus, chronic renal failure, cancer, ischemic heart disease, cerebrovascular disease, and congestive heart failure. No person in the study group was administrated any vitamin supplementation or any drugs. Hypertension was considered to be present if the systolic pressure was >140 mmHg and/or diastolic pressure was >90 mmHg. Blood pressure was measured using a mechanical sphygmomanometer in hospital setting. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were taken as the first and fifth phases of Korotkoff sounds. ABPM device (Tracker NIBP2, Delmar Reynolds, Hertford, UK) was applied to patients for a single 24-hour period. Blood pressure measurements were taken at 30-min period in nighttime (between 2400 and 0600 h) and at 15-min period in daytime (between 0600 and 2400 h). Hypertensive patients were divided into two groups: 18 dipper patients and 20 non-dipper patients. Patients with blood pressure decrease of 10% or more during nighttime were accepted as dipper status, whereas patients with blood pressure decreases less than 10% were accepted as non-dipper hypertensive cases. Healthy control subjects with blood pressure <140/90 mmHg in multiple measurements and with same age range with hypertensive patients, were also enrolled in the study. The Institution's Ethics Committee for studies on human subjects approved the study and each patient and healthy subject consented to participate in the research.

2.2. Methods

2.2.1. Determination of MDA levels

Serum MDA levels were determined with the spectrophotometric method [16]. Serum (100 μL) was mixed with 1000 μL 0.67% TBA and 500 μL of 20% tricloroacetic acid. The mixture was incubated at 100 °C for 20 min. After cooling, the mixture was centrifuged at 12 000 *g* for 5 min and the absorbance was measured at 530 nm using a Beckman spectrophotometer.

2.2.2. Determination of NO levels

Plasma NO was measured with a nitrate/nitrite assay kit (Cayman, Ann Arbor, MI, USA). This assay determines nitric oxide concentrations based on the enzymatic conversion of nitrate to nitrite by nitrate reductase. The inter-assay CV is 5.3%.

2.2.3. Determination of 8-OHdG levels

Serum 8-OHdG was measured with a competitive ELISA kit (DNA Damage ELISA kit; Assay Designs, Ann Arbor, MI, USA). Absorbance was measured by Versamax microplate reader (Molecular Devices, Sunnyvale, CA, USA) at 450 nm. The concentration of 8-OHdG in each sample was determined by generating standard curves from standardized samples contained in each ELISA kit. Results are expressed in nanograms per milliliter. The inter-assay coefficient of variation of the DNA damage is 3.4.

2.2.4. Determination of serum TAC levels

Serum TAC levels were determined according to the ABTS radical cation ($ABTS^{\bullet+}$) decolorization assay described by Re et al. [17]. In this method, when the aliquot of serum is added to the $ABTS^{\bullet+}$ solution, decolorization as a result of the presence of serum antioxidants which reverse the formation of $ABTS^{\bullet+}$ is observed. After addition of 1.0 mL of diluted $ABTS^{\bullet+}$ solution to 10 μL serum or Trolox standard in PBS, the absorbance reading was taken at 30 °C exactly 6 min after initial mixing. Percentage inhibition values of samples and standards were calculated.

2.2.5. Determination of plasma CoQ10 levels

Plasma levels of CoQ10 was measured using a high performance liquid chromatography (HPLC) system (Hewlett-Packard 1050) equipped with a Lichrosorb RP18 column (10 μm, 150×4.6 mm; Phenomenex, Torrance, CA, USA) and with a guard column [18]. The mobile phase was methanol/ethanol (30:70 v/v). Flow rate was 1 mL/min and the UV detector was set up at 275 nm. The interassay error coefficient was 7.3%.

2.2.6. Determination of serum ADMA levels

The serum ADMA concentration was assessed by the ADMA ELISA kit (Immundiagnostik AG, Bensheim, Germany). This assay is based on the method of competitive enzyme linked immunoassays. The color changes from blue to yellow and the absorbance is measured in the photometer at 450 nm. The interassay coefficient of variation is 6.1%.

2.2.7. Determination of plasma NT levels

The plasma samples were assayed for NT content using Nitrotyrosine ELISA Kit (Oxis International Inc., Portland, OR) according to manufacturer's instructions. The samples were incubated in microtiter wells coated with antibody to nitrotyrosine. A biotynylated secondary antibody was used and the product of the reaction of streptavidinperoxidase with tetramethyl benzidine as a substrate was measured by absorbancy at 450 nm. The inter-assay coefficient of variation of NT is 5.5%.

2.2.8. Determination of other blood parameters

Serum total cholesterol, HDL-cholesterol and triglycerides were measured spectrophotometrically on a Hitachi P-800 autoanalyzer (Roche, Almere, The Netherlands) by using a commercial kit (Roche, Mannheim, Germany). Serum hs-CRP was determined by automated particle-enhanced immunoturbidimetric assay performed on a Hitachi P-800 autoanalyzer. Serum level of hs-CRP was measured by the nephelometric method on the basis of particle-bound goat antihuman CRP (Beckman Instruments, Inc, Fullerton, CA).

2.3. Statistical analysis

A minimum sample of $n = 20$ was calculated to detect a difference of approximately 40% between controls and hypertensive patients with power of 0.8 and alpha at 0.05. All results are expressed as means ± SE. Statistical analyses were performed by using SPSS packed program (version 10 software, SPSS Inc. Chicago, Illinois, USA). Because some of the data set were not normally distributed according to Kolmogorov–Smirnov test, statistical comparisons between group were performed using non-parametric tests, Mann–Whitney U and Kruskal–Wallis test. p-value <0.05 was considered statistically significant.

3. Results

Clinical characteristics and laboratory data from patient and control groups were shown in Table 1. Office SBP and DBP were not different between dipper and non-dipper patients. Office SBP and DBP in two patient groups were higher than the control group ($p<0.01$). The levels of HDL-cholesterol in non-dipper patient group were statistically lower