The impact of protein oxidation on sustained and white coat hypertension

Erkan Yıldırım, Emrah İpek, Işıl Bavunoğlu, Nilgün Yıldırım[1], Mahir Cengiz*, Serap Yavuzer*, Hakan Yavuzer*, Hayriye Erman[2], Hafize Uzun***

Department of Cardiology, Region Training and Research Hospital; Erzurum-*Turkey*
Departments of *Medicine and **Biochemistry, Cerrahpaşa Faculty of Medicine, İstanbul University; İstanbul-*Turkey*
[1]Department of Medicine, Faculty of Medicine, Atatürk University; Erzurum-*Turkey*
[2]Department of Biochemistry, Göztepe Training and Research Hospital, Medeniyet University; İstanbul-*Turkey*

Abstract

Objective: The present study compared the unfavorable effects of protein oxidation and deoxyribonucleic acid damage on patients with white coat hypertension (WCH), sustained hypertension (HT), and normotensives.

Methods: Participants were allocated into 3 groups: 40 healthy controls, 36 patients with WCH, and 40 patients with sustained HT. Patients with risk factors for atherosclerosis, endocrine diseases, alcoholism, or masked hypertension were excluded. Plasma level of protein carbonyl (PCO), ischemia modified albumin (IMA), total thiol (T-SH), prooxidant-antioxidant balance (PAB), advanced protein oxidation products (AOPPs), and urinary level of 8-hydroxy-2'-deoxyguanosine (8-OHdG) were measured and relationship between these oxidative stress parameters and WCH and sustained HT was analyzed.

Results: Ambulatory 24-hour, daytime and night-time systolic and diastolic blood pressure readings of sustained HT group were significantly higher than those of WCH and control groups ($p<0.001$, all). AOPPs, PCO, IMA, 8-OHdG, and PAB levels were significantly higher in HT group than WCH and control groups ($p<0.001$, all). Additionally, T-SH level was significantly lower in HT group than WCH and control groups ($p<0.001$). A similar statistically significant relationship was detected between WCH and control groups.

Conclusion: Results indicate that increased level of AOPPs, PCO, IMA, 8-OHdG, PAB, and decreased level of T-SH are likely to be indicators of oxidative stress, which may play a key role both in WCH and sustained HT. *(Anatol J Cardiol 2017; 17: 210-6)*

Keywords: antioxidant levels, protein oxidation, white coat hypertension

Introduction

Oxidative stress is defined as an imbalance between antioxidant defense of the body and reactive oxygen and nitrogen species, in favor of the oxidants (1). Reactive oxygen species (ROS) and reactive nitrogen species (RNS) can damage biomolecules such as deoxyribonucleic acid (DNA), proteins, and lipids. Hypertension (HT) is among the most common risk factors for cardiovascular diseases. Experimental evidence indicates that HT is significantly associated with endothelial dysfunction due to production and release of ROS and RNS (1).

White coat hypertension (WCH) is defined as medical office blood pressure (BP) reading ≥140/90 mm Hg with normal 24-hour and daytime ambulatory BP monitoring (ABPM) or a normal home BP (2, 3) level. Endothelial dysfunction, atherosclerosis, and target organ damage can be found in WCH just as it can be detected in sustained HT (2). Compared to normotensives, patients with WCH have increased tendency to develop HT, metabolic syndrome, and diabetes (2, 4, 5). Risk of cardiovascular events in patients with WCH was found to be between risk of patients with HT and normotensives (2). Endothelial damage and angiogenesis are related to increased risk for worse prognosis in WCH. Endothelin-1 and vascular endothelial growth factor levels were found to be higher in patients with WCH (6). However, in some previous studies, data about levels of oxidative markers in patients with WCH were controversial (1, 2).

The present study examined the unfavorable effects of protein oxidation and DNA damage and compared the effects on patients with WCH, sustained HT and normotensives. We measured plasma levels of protein carbonyl (PCO), ischemia modi-

Address for correspondence: Dr. Erkan Yıldırım, Bölge Eğitim ve Araştırma Hastanesi Kardiyoloji Bölümü, Erzurum-*Türkiye*
Phone: +90 442 232 55 55 Fax: +90 442 232 50 25 E-mail: drerkan23@yahoo.com
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fied albumin (IMA), total thiol (T-SH), prooxidant-antioxidant balance (PAB), advanced protein oxidation products (AOPPs), and urinary levels of 8-hydroxy-2'-deoxyguanosine (8-OHdG), and analyzed the relationship between oxidative stress parameters and WCH.

Methods

Informed consent

Protocol for sample collection was approved by the Local Ethics Committee and was carried out according to the requirements of the Second Declaration of Helsinki. All patients were fully informed about study procedures before providing written consent.

Study population

Study design

This study was designed as a cross-sectional observational study. Among 296 consecutive individuals with either previous WCH or sustained HT and normotensives, a total of 116 individuals were included in the study. Patients enrolled in this single center study were selected from general internal medicine outpatient clinic over 1-year period between March 2014 and March 2015. A total of 120 subjects with risk factors for atherosclerosis [smoking, low-density lipoprotein (LDL) >130 mg/dL, triglyceride (TG) >150 mg/dL, diabetes mellitus, metabolic syndrome, body mass index (BMI) >25 kg/m^2], endocrine diseases, or alcoholism were excluded. In addition, 36 patients using antioxidant substances or drugs for lipid metabolism (statins, fibrates) and 24 patients with masked HT were excluded. The individuals were allocated into 3 groups: healthy controls (23 females, 17 males; age: 42.4±4.6 years), WCH group (18 females, 18 males; age: 42.8±7.1 years), and sustained HT group (23 females, 17 males; age: 44.4±3.9 years). Individuals in sustained HT group consisted of grade 1 HT patients without history of antihypertensive drug therapy. BMI of each patient was calculated using the following formula: weight (kg)/height (m)^2.

Power analysis demonstrated that a total of at least 105 patients (35 patients in each group) were required in order to achieve 80% power in 2-sided test with 5% significance level to distinguish WCH patients from healthy individuals or patients with HT.

Measurement of blood pressure

Brachial arterial pressure was obtained by a single doctor using a mercury sphygmomanometer (Big Ben® round; Riester, Jungingen, Germany) standardized in accordance with American and British Hypertension Society and the World Health Organization recommendations (7).

WCH is defined as BP in the presence of a healthcare worker of ≥140/90 mm Hg on at least 3 occasions in a patient not taking any medication with normal 24-hour (<130/80 mm Hg) and daytime ABPM (<135/85 mm Hg) or normal home BP (<135/85 mm Hg) (2, 3). Patients with medical office BP of ≥140/90 (without any medication) were further evaluated by ABPM (Schiller BR-102 Plus; Schiller AG, Baar, Switzerland). Patients with normal 24-hour (<130/80 mm Hg) and day ABPM (<135/85 mm Hg) or normal home BP (<135/85 mm Hg) were enrolled in WCH group. Sustained HT group was selected from patients with increased 24-hour (≥130/80 mm Hg) and day ABPM (≥135/85 mm Hg) or home BP (≥135/85 mm Hg). Normotensive individuals in control group were also evaluated by ABPM in order to make comparison between patients with sustained HT and WCH in terms of mean day and night BP. Patients with normal 24-hour (<130/80 mm Hg) and day ABPM (<135/85 mm Hg) or a normal home BP (<135/85 mm Hg) were enrolled in control group.

Sample collection and measurements

Blood and urine samples were taken simultaneously in the morning (8–9 a.m.) after an overnight fast. Ethylenediaminetetraacetic acid-treated, anticoagulant-free tubes were used for blood samples. Plasma and serum were separated at least 30 minutes after centrifugation at 2500x g for 5 minutes (minimum). Each sample (serum, plasma, and urine) was divided into 4 aliquots and samples were stored at -80°C until biochemical analysis.

Measurement of serum PAB concentrations

The method of Alamdari et al. (8) was used for PAB assay. Standard solutions were prepared by mixing varying proportions (0–100%) of 1 mM hydrogen peroxide (H_2O_2) with 3 mM uric acid (in 10 mM sodium hydroxide). We dissolved 60 mgs of 3,3′,5,5′-tetramethylbenzidine (TMB) powder in 10 mL absolute dimethyl sulfoxide (DMSO). We prepared TMB cation by adding 400 µL of TMB/DMSO to 20 mL of acetate buffer (0.05 M buffer, pH 4.5).Next, 70 µL of fresh chloramine-T (100 mM) solution was added, mixed well, and solution was incubated for 2 hours in the dark at room temperature. After incubation, we added 25 U of peroxidase enzyme solution to the 20 mL TMB cation and it was dispensed in 1 mL and kept at -20°C. We prepared TMB solution by adding 200 µL of TMB/DMSO to 10 mL of acetate buffer (0.05 M buffer, pH 5.8). Working solution was prepared by mixing 1 mL of TMB cation with 10 mL of TMB solution. Working solutions were used immediately. A 10-µL aliquot of each sample, standard or blank (distilled water), was mixed with 200 µL of working solution in each well of a 96-well plate, which was then incubated at 37°C for 12 minutes in the dark. Next, 100 µL of 2 N hydrogen chloride was added to each well, and absorbance was measured using an enzyme-linked immunosorbent assay (ELISA) plate reader (ELX 800 UV; BioTek Instruments, Winooski, VT, USA) at 450 nm, with a reference wavelength of 620 or 570 nm. Standard curve was determined using values obtained from standard samples. Values of the unknown samples were then calculated according to values obtained from standard curve. PAB values were expressed in arbitrary units, which correspond to percentage of H_2O_2 in the standard solution. Coefficients of intra- and inter-assay variation were 5.2% (n=20) and 6.0% (n=20), respectively.

Measurement of total T-SH concentration

To determine plasma T-SH concentration, 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB) was used, as introduced by Hu (9). The intra- and inter-assay variation coefficients were 1.9% (n=20) and 4.4% (n=20), respectively.

Measurements of concentration of plasma AOPPs

A modification of the Gelişgen et al. (10) method was used for spectrophotometric determination of concentration of AOPPs: 200 µL of plasma was diluted 1:5 in phosphate-buffered solution (PBS) and 10 µL of 1.16 M potassium iodide (KI) was added to each tube followed by addition of 20 µL of absolute acetic acid 2 minutes later. Absorbance of the reaction mixture was immediately measured at 340 nm against a blank containing 2000 µL of PBS, 200 µL of acetic acid and 100 µL of KI. Linear range of chloramine-T absorbance at 340 nm occurs between 0 and 100 µmol/L. Concentration of AOPPs was expressed in µmol/L of chloramine-T equivalents. Coefficients of intra- and inter-assay variation were 2.9% (n=15) and 3.4% (n=15), respectively.

Measurement of serum IMA concentration

A commercially available ELISA kit (Cat. No: E90825Hu; USCN Life Science, Inc., Houston, TX, USA) was used to measure serum ischemia-modified albumin (IMA) levels. Coefficients of intra- and inter-assay variations were 4.1% (n=10) and 5.0% (n=10), respectively.

Measurement of serum PCO concentration

Serum protein carbonyl (PCO) level was measured with ELISA kit (OxiSelect™ Protein Carbonyl ELISA Kit, Cat. No: STA-310; Cell Biolabs Inc., San Diego, CA, USA). Coefficients of intra- and inter-assay variations were 4.0% (n=10) and 5.3% (n=10), respectively.

Measurement of urine 8-OHdG concentration

An ELISA kit (OxiSelect™ Oxidative DNA Damage ELISA Kit, Cat. No: STA-320; Cell Biolabs Inc., San Diego, CA, USA) was used to detect urine 8-Oxo-2'-deoxyguanosine (8-OHdG) concentration, which was stated as ng/mg creatinine. Coefficients of intra- and inter-assay variations were 3.0% (n=20), and 3.9% (n=20), respectively.

Other biochemical parameters were measured using routine methods with commercial kits. Urine creatinine (spot) level was measured using Jaffe method with Architect c8000 analyzer (Abbott Diagnostics, Lake Forest, IL, USA). Serum creatinine clearance was calculated using Cockroft-Gault formula. Albumin excretion in 24-hour urine samples was measured using Roche Hitachi P800 analyzer (Roche Diagnostics, Rotkreuz, Switzerland) with ALBT2 microalbumin kit in 24-hour urine samples and mean value was calculated as daily albumin excretion (11). Normoalbuminuria was defined as albumin excretion of ≤30 mg/24h. Microalbuminuria (MAU) and clinical albuminuria were defined as albumin excretion of 30–299 mg/24h and ≥300/24h, respectively (11).

Statistical analysis

Statistical analyses were performed using SPSS software version 20.0 (SPSS Inc., Chicago, IL, USA). Normal distribution of data was tested with 1-sample Kolmogorov-Smirnov test. Difference by gender was analyzed with chi-square test. All statistical analyses were performed using analysis of variance to compare multiple-group means. Posthoc evaluation was made using Bonferroni method. All data were expressed as mean±SD. Pearson's correlation was used for numerical data. Spearman's correlation was used for nominal data. To assess diagnostic accuracy, we performed receiver operating characteristic (ROC) curve analysis. The area under the curve (AUC) of ROC curve was then estimated. $P<0.05$ values were considered statistically significant.

Results

General characteristics and laboratory findings of studied groups are shown in Table 1. Age, gender distribution, and BMI were not statistically significantly different between groups. Disease duration in HT group was 4.5±2.5 years, and in WCH group was 2.3±2.3 years. C-reactive protein level was significantly higher in HT group compared with control group ($p<0.001$). Office systolic BP (SBP) and diastolic BP (DBP) measurements of normotensive group were significantly lower than those of HT group ($p<0.001$, $p<0.001$ respectively) and WCH group ($p<0.001$, $p<0.001$ respectively). Office SBP and DBP were significantly higher in WCH group than HT group and normotensives ($p<0.001$). Ambulatory 24-hour (average), daytime and nighttime SBP and DBP readings of HT group were significantly higher than those of WCH and control groups ($p<0.001$, all). There was no significant difference in glucose, high-density lipoprotein, low-density lipoprotein, triglyceride, uric acid, creatinine, and creatinine clearance levels between all groups.

Oxidative stress parameters of all groups are provided in Table 2. In HT group, AOPPs, PCO, IMA, 8-OHdG, and PAB levels were significantly higher than in control group ($p<0.001$, all). Additionally, T-SH levels were significantly lower in HT group than in control group ($p<0.001$).

Results of correlation analysis among tested parameters are presented in Table 3. T-SH was significantly negatively correlated with PCO, IMA, 8-OHdG, PAB, office SBP and DBP, and all ABPM. AOPPs were positively correlated with PCO, IMA, 8-OHdG, office SBP and DBP, and all ABPM. PCO was positively correlated with IMA, 8-OHdG, PAB, office SBP and DBP, and all ABPM. IMA was positively correlated with 8-OHdG, PAB, office SBP and DBP, and all ABPM. 8-OHdG was positively correlated with PAB, office SBP and DBP, and all ABPM. PAB was positively correlated with office SBP and DBP, and all ABPM. Disease duration was positively correlated with 8-OHdG.

Comparison of ROC curves with sensitivity, specificity, AUC, cut-off, and asymptotic significance of T-SH, AOPPs, IMA, PCO, 8-OHdG, and PAB levels in all groups is shown in Table 4.

Table 1. Demographic characteristics and laboratory findings of study groups

	Control (n=40)	WCH (n=36)	HT (n=40)	*P*
Gender, F/M	23/17	18/18	23/17	0.75
Age, years	42.4±4.6	42.8±7.1	44.4±3.9	0.21
Disease duration, years	–	2.3±2.3	4.5±2.5	**<0.001**
BMI, kg/m^2	23.6±1.1	23.8±1.3	23.7±1.3	0.82
Glucose, mg/dL	85.6±5.3	88.7±8.8	88.2±5.9	0.11
Creatinine, mg/dL	0.7±0.2	0.8±0.2	0.8±0.3	0.13
Uric acid, mg/dL	4.3±0.9	4.6±0.9	4.6±0.9	0.23
LDL, mg/dL	108.4±19.9	112.1±9.9	109.7±17.8	0.63
TG, mg/dL	93.7±27.6	104.1±28.1	97.1±34.4	0.31
HDL, mg/dL	58.9±13.3	53.4±10.5	53.9±11.1	0.07
CRP, mg/L	1.5±1.3	2.2±1.1	2.6±1.1	**<0.001**[a]
CrCl, mL/min	110.8±30.4	121.5±29.6	117.7±27.6	0.27
Office SBP, mm Hg	122.5±6.7	148.9±3.9	136.5±4.9	**<0.001**[b]
Office DBP, mm Hg	75.9±5.3	95.1±3.7	86.5±3.9	**<0.001**[c]
Average SBP, mm Hg*	118.9±8.5	118.6±8.4	135.1±4.1	**<0.001**[d]
Average DBP, mm Hg*	74.5±5.8	74.6±5.3	85.4±4.3	**<0.001**[e]
Daytime SBP, mm Hg*	122.4±9.1	122.3±8.9	139.5±4.2	**<0.001**[f]
Daytime DBP, mm Hg*	67.4±4.1	66.8±3.8	88.5±6.5	**<0.001**[g]
Nighttime SBP, mm Hg*	110.8±8.3	110.7±7.8	121.4±6.4	**<0.001**[h]
Nighttime DBP, mm Hg*	67.3±4.1	66.8±3.8	75.5±6.5	**<0.001**[i]

All data were expressed as the mean±SD. Normal distribution of data was tested with 1-sample Kolmogorov-Smirnov test. Difference in gender was analyzed using chi-square test. All statistical comparisons were performed using analysis of variance to compare multiple-group means. BMI - body mass index; CRP - C-reactive protein; CrCl - creatinine clearance; DBP - diastolic blood pressure; ESR - erythrocyte sedimentation rate; F - female; HDL - high density lipoprotein; HT - hypertension; LDL - low density lipoprotein; M - male; SBP - systolic blood pressure; TG - triglyceride; WCH - white coat hypertension. * Ambulatory blood pressure monitoring.

[a]Control and WCH, *P*<0.001; control and HT, *P*<0.001; WCH and HT, *P*<0.001; [b]control and WCH, *P*<0.001; control and HT, *P*<0.001; WCH and HT, *P*<0.001; [c]control and WCH, *P*<0.001; control and HT, *P*<0.001; WCH and HT, *P*<0.001; [d]control and WCH, *P*<0.001; control and HT, *P*<0.001; WCH and HT, *P*<0.001; [e]control and HT, *P*<0.001; control and WCH, *P*<0.001; WCH and HT, *P*<0.001; [f]control and WCH, *P*<0.001; control and HT, *P*<0.001; WCH and HT, *P*<0.001; [g]control and WCH, *P*<0.001; control and HT, *P*<0.001; WCH and HT, *P*<0.001; [h]control and WCH, *P*<0.001; control and HT, *P*<0.001; WCH and HT, *P*<0.001; [i]control and WCH, *P*<0.001; control and HT, *P*<0.001; WCH and HT, *P*<0.001

Table 2. Oxidative stress parameters between the study groups

	Control (n=40)	WCH (n=36)	HT (n=40)	*P*
T-SH, µM	0.89±0.21	0.64±0.14	0.54±0.13	**<0.001**[a]
AOPPs, µM	66.2±28.9	76.4±55.4	112.7±64.8	**<0.001**[b]
PCO, nmoL/mg.pr.	1.03±0.3	1.42±0.73	1.59±0.8	**0.001**[c]
IMA, ng/mL	30.8±17.5	82.1±66.4	153.2±140.5	**<0.001**[d]
8-OHdG, ng/mg creatinine	3.8±1.9	4.6±2.9	10.7±4.9	**<0.001**[e]
PAB, AU	0.61±0.12	0.72±0.17	1.03±0.26	**<0.001**[f]

All data were expressed as the mean±SD. Normal distribution of data was tested with 1-sample Kolmogorov-Smirnov test. Difference by gender was analyzed using chi-square test. All statistical comparisons were performed using analysis of variance to compare multiple-group means. AOPPs - advanced oxidation protein products; AU - arbitrary units corresponding to percentage of H2O2 in standard solution; HT - hypertension; IMA - ischemia modified albumin; PAB - prooxidant-antioxidant balance; PCO - protein carbonyl; T-SH - total thiol; WCH - white coat hypertension; 8-OHdG-8-hydroxy-2'-deoxyguanosine.

[a]Control and WCH, *P*<0.001; control and HT, *P*<0.001; WCH and HT, *P*=0.026; [b]control and HT, *P*<0.001; WCH and HT, *P*=0.009; [c]control and WCH, *P*=0.03; control and HT, *P*=0.001; [d]control and WCH, *P*=0.048, control and HT, *P*<0.001; WCH and HT, *P*=0.003; [e]control and HT, *P*<0.001; WCH and HT, *P*<0.001; [f]control and WCH, *P*=0.024; control and HT, *P*<0.001; WCH and HT, *P*<0.001

Discussion

Oxidative stress plays a vital role in the development and progression of HT (1). As seen in previous reports (2,12), we detected increased levels of circulating protein oxidation markers such as AOPPs, PCO, IMA, urine 8-OHdG, and decreased antioxidant defenses such as reduced plasma concentration of T-SH in both patients with HT and those with WCH, relative to those in normotensive controls. The present study results demonstrate significant differences in levels of both oxidants (AOPPs, PCO, IMA, urine 8-OHdG) and antioxidants (T-SH) between normotensives, patients with WHC, and patients with sustained HT. Current results may indicate some important role of increased oxidant and decreased antioxidant levels in pathogenesis of WCH and HT.

Imbalance between oxidants and antioxidants can impair vascular function by changing proteins and DNA as a result of protein dysfunction and even cell death in the pathophysiological process of both WCH and HT (2, 12). HT is itself considered a state of oxidative stress that can contribute to the development of HT-induced end-organ damage and atherosclerosis (13, 14). However, there is limited data regarding the relationship between oxidative stress markers and WCH (2, 15). Although some authors did not find any difference in oxidative stress markers between WCH cases and normotensives (15), in study by Caner et al. (12), it was demonstrated that oxidative stress was increased in patients with WCH and HT. Additionally, decreased antioxidant levels may indicate increased oxidative stress associated with WCH (16).

In hypertension, AOPPs are newer markers of oxidative stress. AOPPs may play some role in oxidative stress and inflammation with activation of neutrophils, monocytes, and T-lymphocytes (17). In vitro, AOPPs can inhibit inducible nitric oxide (NO) production with macrophages and induce ROS production in endothelial cells via nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activation (17). NADPH oxidase activation leading to stimulation of angiotensin II type 1 (AT1) receptors may result in increase in production of ROS and consequent decrease in NO in arterial HT (18). Present study showed that AOPPs significantly increased in both HT and WCH groups compared to normotensive group. This result may indicate role of AOPPs in pathophysiology of WCH by inhibiting NO production and inducing ROS production. However, increase in AOPPs may be result of WCH, and this issue remains to be further explored in additional studies.

Table 3. Correlation of study parameters with each other and blood pressure

	T-SH R	AOPPs R	PCO R	IMA R	8-OHdG R	PAB R
Disease duration	-0.192	0.177	0.064	0.194	0.396**	0.118
T-SH		-0.149	-0.297*	-0.379**	-0.410**	-0.432**
AOPPs	-0.149		0.194*	0.314*	0.411**	0.141
PCO	-0.297*	0.194*		0.276*	0.243*	0.262*
IMA	-0.379**	0.314*	0.276*		0.396**	0.338**
8-OHdG	-0.410**	0.411**	0.243*	0.396**		0.372**
PAB	-0.432**	0.141	0.262*	0.338**	0.372**	
Office SBP	-0.630**	0.297*	0.293*	0.430**	0.422**	0.509**
Office DBP	-0.619**	0.257*	0.331*	0.417**	0.366**	0.467**
Average SBP[a]	-0.478**	0.384**	0.269*	0.418**	0.510**	0.610**
Average DBP[a]	-0.442**	0.319**	0.237*	0.427**	0.500**	0.591**
Daytime SBP[a]	-0.511**	0.406**	0.245*	0.425**	0.544**	0.609**
Daytime DBP[a]	-0.473**	0.331**	0.236*	0.412**	0.481**	0.565**
Nighttime SBP[a]	-0.431**	0.388**	0.197*	0.429**	0.413**	0.527**
Nighttime DBP[a]	-0.369**	0.327**	0.209*	0.494**	0.490**	0.538**

Pearson's correlation was used for numerical data. Spearman's correlation was used for nominal data. AOPPs - advanced oxidation protein products; BP - blood pressure; DBP - diastolic blood pressure; IMA - ischemia modified albumin; PAB - prooxidant-antioxidant balance; PCO - protein carbonyl; SBP - systolic blood pressure; T-SH - total thiol; WCH - white coat hypertension; 8-OHdG -8-hydroxy-2'-deoxyguanosine. [a]Ambulatory blood pressure monitoring. *P<0.05; **P<0.001

Table 4. Sensitivity, specificity, area under curve, cut-off, and asymptotic significance of receiver operating curve analysis of oxidative stress parameters and blood pressure

		Sensitivity (%)	Specificity (%)	AUC	Cut-off	Asymptotic Sig.
Control vs WCH	IMA	86.1	65	0.803	38.9	**<0.001**
	T-SH	80.6	77.5	0.853	0.75	**<0.001**
	PAB	72.2	67.5	0.714	0.64	**0.001**
	PCO	63.9	67.5	0.670	1.07	**0.011**
Control vs HT	IMA	90	85	0.948	47.8	**<0.001**
	T-SH	87.5	85	0.926	0.69	**<0.001**
	AOPP	70	70	0.765	78.1	**<0.001**
	PAB	85	85	0.934	0.73	**<0.001**
	PCO	70	70	0.733	1.11	**<0.001**
	8-OHdG	90	80	0.925	4.71	**<0.001**
WCH vs HT	IMA	87.5	69.4	0.739	56.1	**<0.001**
	T-SH	70	61.1	0.691	0.61	**0.004**
	AOPP	70	69.4	0.708	79.2	**0.002**
	PAB	77.5	69.4	0.837	0.79	**<0.001**
	8-OHdG	87.5	75	0.877	5.64	**<0.001**

To assess diagnostic accuracy, receiver operating characteristic curve analysis was performed. Area under the curve was then estimated. AOPPs - advanced oxidation protein products; AUC - area under curve; HT - hypertension; IMA - ischemia modified albumin; PAB - prooxidant-antioxidant balance; PCO - protein carbonyl; ROC - receiver operating curve; T-SH - total thiol; WCH - white coat hypertension; 8-OHdG- 8-hydroxy-2'-deoxyguanosine. Curves were created for all parameters and their cooperative power to discriminate 2 sets of patients, control and WCH groups, controls and HT, and WCH and HT

Exposure of proteins to ROS can alter the physical and chemical structure of the target, causing consequent oxidation of side-chain groups, protein scission, backbone fragmentation, cross-linking, unfolding, and formation of new reactive groups (19). Oxidation of hydrophobic amino acyl residues to hydroxy and hydroperoxy (P-OOH) derivatives, PCO, oxidation of T-SH groups, and dityrosine formation can lead to decrease in or loss of protein's biological function (19). PCO is among the most important products of protein oxidation and accumulation of oxidized proteins leads to cellular and tissue damage (20). In the present study, we had similar findings: PCO levels increased in both sustained HT and WCH groups compared to normotensives.

Measurement of T-SH is a good marker of excess free radical generation, as the conformation of albumin is changed, and T-SH groups are oxidized (21, 22). Our study demonstrated significantly higher levels of T-SH in normotensive group compared to both WCH and HT groups indicating the importance of oxidative stress in WCH and HT. Changes in protein function as result of oxidative damage can trigger the physiological processes leading to WCH.

IMA is another biomarker of cardiovascular damage. Transition metals can bind tightly to exposed N-terminus of albumin under physiological conditions. Some structural changes occur in N-terminal part of the protein, reducing its binding capacity in case of myocardial ischemia, possibly due to exposure to ROS. Although it is not specific to cardiac ischemia, the test has recently been licensed by the US Food and Drug Administration for diagnostic use in suspected myocardial ischemia (23). We used IMA as a marker of oxidative stress in our study group. The significantly higher levels of IMA found in both WCH and HT groups may indicate role of increased ROS and cardiovascular damage in WCH and HT groups compared to normotensives. Production of IMA as a result of oxidative stress may be cause or result of WCH. This indicates the complex relationship between oxidative stress, endothelial dysfunction, and pathophysiology of WCH.

One of the predominant forms of free radical-induced lesions of DNA is oxidatively modified product 8-OHdG. Although 8-OHdG can be detected in human tissue, body fluid, or blood samples, urinary 8-OHdG measurement is typically preferred to indicate extent of oxidative damage since it is a non-invasive and simple procedure (24). HT was reported to be related to increased oxidative stress and 8-OHdG formation. In previous studies, urinary 8-OHdG was said to be a useful biomarker in assessment of ROS-induced DNA damage in epidemiological and clinical settings (25). Similarly, we detected higher urinary levels of 8-OHdG in WCH and HT groups as well as in patients with longer disease duration, highlighting the role of this molecule in oxidative damage in patients with HT and WCH. In clinical practice, the relationship of 8-OHdG to disease duration is a valuable laboratory tool for the clinician.

PAB values can be measured rapidly, easily, and cost effectively, and indicate oxidant–antioxidant ratio as a marker of oxidative stress. Oxidative stress can be defined as an imbalance between production of ROS and the capacity to remove ROS. ROS production is a normal result of cellular processes that is tightly controlled by antioxidants under physiological conditions (26, 27). Molecular damage, neuronal adaptation and critical failure of biological function occur when ROS levels exceed antioxidant capacity of cells due to age or increased metabolic demand (26). Present study evaluated PAB using a simple, rapid, and inexpensive assay. Significant increase in PAB values was detected in HT and WCH groups. Possible explanation for elevation in PAB values may be impairment of oxidant-antioxidant balance in HT and WCH patients.

Results of correlation analysis are also in parallel with other findings in present study. Significant negative correlation of T-SH with other oxidative parameters, clinical SBP, DBP, and ABPM indicates impaired oxidant-antioxidant status. Additionally, significant positive correlation of each oxidative marker with other oxidative parameters, clinical SBP, DBP, and ABPM may show strong relationship and cooperation of these oxidative stress parameters in pathophysiology of both WCH and HT.

Study limitations

A relatively small sample size is the major limitation of this study. Age can influence extent of protein oxidation; however, since distribution of age groups was almost identical, it was not considered to have a significant impact on our results. Additionally, effect of subclinical atherosclerosis on protein oxidation cannot be excluded.

Conclusion

Our results show that increased AOPPs, PCO, IMA, 8-OHdG, PAB, and decreased T-SH levels may likely be the result of oxidative stress due to imbalance between ROS production and deactivation. In pathophysiology of WCH and sustained HT, oxidative stress parameters may play some role and interact with each other during this process. Level of 8-OHdG in particular may be more specific, sensitive, and valuable, because it can be measured rapidly and cost-effectively in laboratories with high-positive predictivity. Further clinical studies are needed to support current findings and conclusions.

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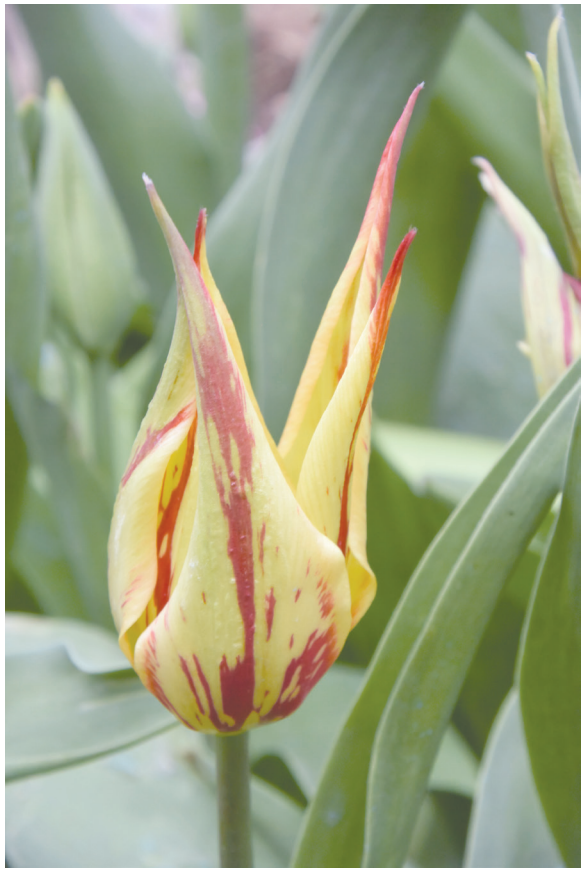

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