Lymphocyte DNA damage and total antioxidant status in patients with white-coat hypertension and sustained hypertension

Beyaz önlük hipertansiyonunda ve devamlı hipertansiyonda lenfosit DNA hasarı ve total antioksidan durumunun değerlendirilmesi

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Objectives: We assessed lymphocyte DNA damage and total antioxidant status (TAS) in patients with white-coat hypertension (WCH) and sustained hypertension (SHT).

Study design: The study included 23 patients (14 females, 9 males; mean age 46±6 years) with WCH, 21 patients (13 females, 8 males; mean age 45±7 years) with newly diagnosed SHT, and 19 age- and sex-matched healthy volunteers as controls. All subjects underwent echocardiographic examination, office blood pressure measurements, and 24-hour ambulatory blood pressure monitoring. DNA damage was assessed by the alkaline comet assay in peripheral lymphocytes, and plasma TAS levels were determined using an automated measurement method.

Results: The two hypertensive groups had similar echocardiographic measurements and office systolic and diastolic blood pressures. The mean daytime and nighttime pressures were significantly higher in the SHT group ($p<0.05$). Patients with WCH had similar daytime and nighttime pressures compared to the controls ($p>0.05$). Patients with SHT had significantly increased lymphocyte DNA damage ($p<0.001$, for both WCH and control groups) and decreased TAS level ($p=0.012$ *vs* WCH group; $p<0.001$ *vs* controls). Patients with WCH did not differ significantly from the control group with regard to lymphocyte DNA damage ($p=0.052$), but had significantly lower TAS levels ($p<0.001$). In the SHT group, lymphocyte DNA damage was correlated with TAS ($r= -0.818$, $p<0.001$), age ($r=0.453$, $p=0.039$), total cholesterol ($r=0.550$, $p=0.010$), and LDL-cholesterol ($r=0.539$, $p=0.012$). In multiple linear regression analysis, lymphocyte DNA damage was independently correlated with serum TAS level ($ß= -0.717$, $p<0.001$). In the WCH group, lymphocyte DNA damage was only correlated with serum TAS level ($r= -0.458$, $p=0.028$).

Conclusion: Decreased TAS showing increased oxidative stress and increased lymphocyte DNA damage may contribute to target organ damage in patients with WCH.

Key words: Antioxidants/metabolism; blood pressure; blood pressure monitoring, ambulatory; DNA Damage; hypertension; lymphocytes; oxidative stress.

Amaç: Beyaz önlük hipertansiyonu (BÖH) ve devamlı hipertansiyon olan hastalarda lenfosit DNA hasarı ve total antioksidan durumu (TAD) araştırıldı.

Çalışma planı: Çalışmaya BÖH'lü 23 hasta (14 kadın, 9 erkek; ort. yaş 46±6), tanısı yeni konmuş devamlı hipertansiyonlu 21 hasta (13 kadın, 8 erkek; ort. yaş 45±7) ve kontrol grubu olarak, yaş ve cinsiyet açısından hipertansiyon gruplarıyla uyumlu 19 sağlıklı gönüllü alındı. Tüm olgularda ekokardiyografik ölçümler, kan basıncı ölçümleri ve 24 saat ambulatuvar kan basıncı izlemesi yapıldı. DNA hasarı periferik lenfositlerde alkalin tek hücre elektroforez (comet) yöntemi ile ölçüldü; serum TAD düzeyleri otomatik ölçüm yöntemiyle belirlendi.

Bulgular: Hipertansiyon grupları arasında ekokardiyografik ölçümler ve anlık ölçülen sistolik ve diyastolik kan basınçları açısından anlamlı fark yoktu. Gündüz ve gece ölçülen kan basınçları devamlı hipertansiyonlu grupta anlamlı derecede yüksek bulunurken ($p<0.05$), BÖH grubundaki basınçlar kontrol grubuyla benzerlik gösterdi ($p>0.05$). Devamlı hipertansiyonlu grupta lenfosit DNA hasarı anlamlı derecede yüksek (BÖH ve kontrol grubu için, $p<0.001$), TAD düzeyi anlamlı derecede düşük (BÖH grubu için, $p=0.012$; kontrol grubu için, $p<0.001$) bulundu. Kontrol grubu ile karşılaştırıldığında, BÖH grubunda lenfosit DNA hasarı açısından anlamlı farklılık görülmezken ($p=0.052$), TAD düzeyi anlamlı derecede düşük idi ($p<0.001$). Hipertansif grupta lenfosit DNA hasarı, TAD düzeyi ($r= -0.818$, $p<0.001$), yaş ($r=0.453$, $p=0.039$), total kolesterol ($r=0.550$, $p=0.010$) ve LDL-kolesterol ($r=0.539$, $p=0.012$) ile anlamlı ilişki gösterdi. Çoklu lineer regresyon analizinde lenfosit DNA hasarı serum TAD düzeyiyle bağımsız ilişkili bulundu ($ß= -0.717$, $p<0.001$). Lenfosit DNA hasarı BÖH grubunda sadece serum TAD düzeyiyle ilişkiliydi ($r= -0.458$, $p=0.028$).

Sonuç: Artmış oksidatif stresin göstergesi olan azalmış TAD düzeyi ve artmış lenfosit DNA hasarı, BÖH'li olgularda hedef organ hasarı oluşumuna katkıda bulunabilir.

Anahtar sözcükler: Antioksidan/metabolizma; kan basıncı; kan basıncı izlemesi, ambulatuvar; DNA hasarı; hipertansiyon; lenfosit; oksidatif stres.

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The term "white-coat hypertension" (WCH) refers to a transient increase in blood pressure (BP), resulting from an alerting reaction and pressor response, observed in certain individuals when attending a clinic or doctors' office.[1] About 25% of patients with high BP in the clinic have a normal BP on ambulatory BP monitoring (ABPM).[2] It was reported that patients with WCH had a better prognosis than patients with sustained hypertension,[3] whereas other data suggest that it precedes the onset of sustained or persistent hypertension and increases the risk for cardiovascular disease.[4,5] Target organ damage, particularly hypertensive retinopathy and left ventricular hypertrophy, have been reported in patients with WCH.[6,7]

Peripheral lymphocyte DNA damage is caused by multiple factors including mainly oxidative stress[8,9] and other causes such as coronary artery disease[10] and inflammation.[11] It has been demonstrated that DNA damage contributes significantly to the development and progression of atherosclerosis.[12] It has also been shown that essential hypertension (HT) is associated with decreased antioxidant capacity[13] and increased release of reactive oxygen species (ROS).[14] DNA damage caused by ROS occurs more commonly in hypertensive patients than in normotensives.[15]

The relationship between WCH and oxidative stress was investigated in two studies.[16,17] However, lymphocyte DNA damage and total antioxidant status (TAS) have not been investigated in patients with WCH. Plasma TAS is an accurate index of oxidative stress and provides a measure of total plasma defenses against ROS.[18] An inverse relationship between TAS and lymphocyte DNA damage was observed in previous studies.[19,20] In this study, we aimed to investigate serum TAS levels and lymphocyte DNA damage in cases with WCH.

PATIENTS AND METHODS

Subjects. The study consisted of 44 consecutive patients. There were 23 patients with WCH (14 females, 9 males; mean age 46±6 years) and 21 patients (13 females, 8 males; mean age 45±7 years) with newly diagnosed sustained HT (SHT) of mild to moderate degree, without a previous history of antihypertensive medication. Nineteen age- and sex-matched healthy volunteers (10 females, 9 males; mean age 47±4 years) were also included as controls. They were chosen from nonmedical staff of our hospital or their relatives, did not have coronary risk factors or cardiac symptoms, and were found to have normal electrocardiographic and echocardiographic examinations.

Inclusion criteria were age 18 to 55 years and, for women, being on a regular menstrual cycle. All subjects were from the same geographical region and had a similar dietary pattern (Mediterranean type of diet), as assessed by a well-trained dietitian who collected diet histories. Written informed consent for participation was obtained from all individuals and the institutional ethics review board approved the study.

Exclusion criteria were the presence of following conditions: neoplastic diseases, inflammatory diseases such as infections and autoimmune disorders, liver and kidney diseases, organic coronary artery disease, vasospastic angina, heart failure, cardiomyopathies, secondary and malignant HT, history of diabetes, recent major surgical procedure; previous antihypertensive therapy, use of antioxidant drugs such as statins and vitamins such as E and C, diuretic use, alcohol use, smoking; and electrocardiographic findings including ST-segment or T-wave changes specific for myocardial ischemia, Q waves, and incidental left bundle branch block. Patients with a body mass index ≥30 kg/m^2 and left ventricular hypertrophy (left ventricle mass index >125 g/m^2 in men, >110 g/m^2 in women)[21] were also excluded.

Blood pressure measurements. Blood pressure was measured using a mechanical sphygmomanometer in the medical office setting. In each subject, the average of three BP measurements was calculated, which were obtained on separate days and after 15 minutes of comfortably sitting. Then each subject wore an ambulatory BP device (Tracker NIBP2, Del Mar Reynolds Medical, Hertford, UK) with an appropriate cuff size for 24 hours. Blood pressure was recorded every 15 min during daytime (from 07.00 AM to 23.00 PM) and every 30 min during nighttime (from 23.00 PM to 07.00 AM), which provided approximately 80 records. The recordings were considered satisfactory when valid readings exceeded at least 80% of the total.

Diagnosis of WCH and SHT. White-coat hypertension was determined when the mean office readings of systolic BP was ≥140 mmHg, diastolic BP was ≥90 mmHg, while the mean daytime systolic BP was <135 mmHg, diastolic BP was <85 mmHg, and the mean nighttime systolic BP was <125 mmHg, diastolic BP was <75 mmHg.[22]

Patients whose office systolic BP was ≥140 mmHg and/or diastolic BP was ≥90 mmHg were diagnosed as

having SHT, if their mean daytime systolic BP was ≥135 mmHg and/or diastolic BP was ≥85 mmHg and their mean nighttime systolic BP was ≥125 mmHg and/or diastolic BP was ≥75 mmHg.[22] Criteria for control subjects were the same as the WCH group for daytime and nighttime ABPM monitoring, with the exception that their office systolic BP was <140 mmHg and/or diastolic BP was <90 mmHg.

Echocardiography. Echocardiographic examination was performed using a commercially available system (Aloka Prosound SSD-5000, Aloka Inc., Tokyo, Japan) with a 3-MHz transducer. Measurements were made during normal breathing at end-expiration. M-mode echocardiographic measurements were made according to the recommendations of the American Society of Echocardiography.[23] Left ventricular end-systolic (LVESD) and end-diastolic diameters (LVEDD), end-diastolic interventricular septal thickness (IVSth), and end-diastolic posterior wall thickness (PWth) were measured. Left ventricular ejection fraction (EF) was determined using the Teichholz formula.[24] Left ventricular mass (LVM) was calculated using the Devereux formula: LVM = (1.04 [(LVDd + IVSth + PWth)^3 − (LVDd)^3] − 13.6).[25] Then, LV mass index (LVMI) was obtained by the following formula: LVM/body surface area.

Blood sampling protocol. Peripheral venous blood samples were drawn from all the subjects in the fasting state and placed into heparinized tubes. One milliliter of blood was immediately pipetted into another tube to measure DNA damage. The remaining blood was centrifuged at 3,000 rpm for 10 min for plasma separation. Plasma samples were stored at -80 °C until analyses for TAS, and levels of triglyceride, total cholesterol, low density lipoprotein (LDL) cholesterol, high density lipoprotein (HDL) cholesterol, and fasting glucose. Plasma levels of triglyceride, total cholesterol, LDL-cholesterol, HDL-cholesterol, and fasting glucose were measured on an automated analyzer (Abbott Aeroset, Abbott Diagnostics, Abbott Park, IL, USA) using commercial kits (Abbott).

Determination of DNA damage by the alkaline comet assay. Lymphocyte isolation for the comet assay was performed by density gradient separation (Histopaque 1077, Sigma-Aldrich, Inc., St. Louis,

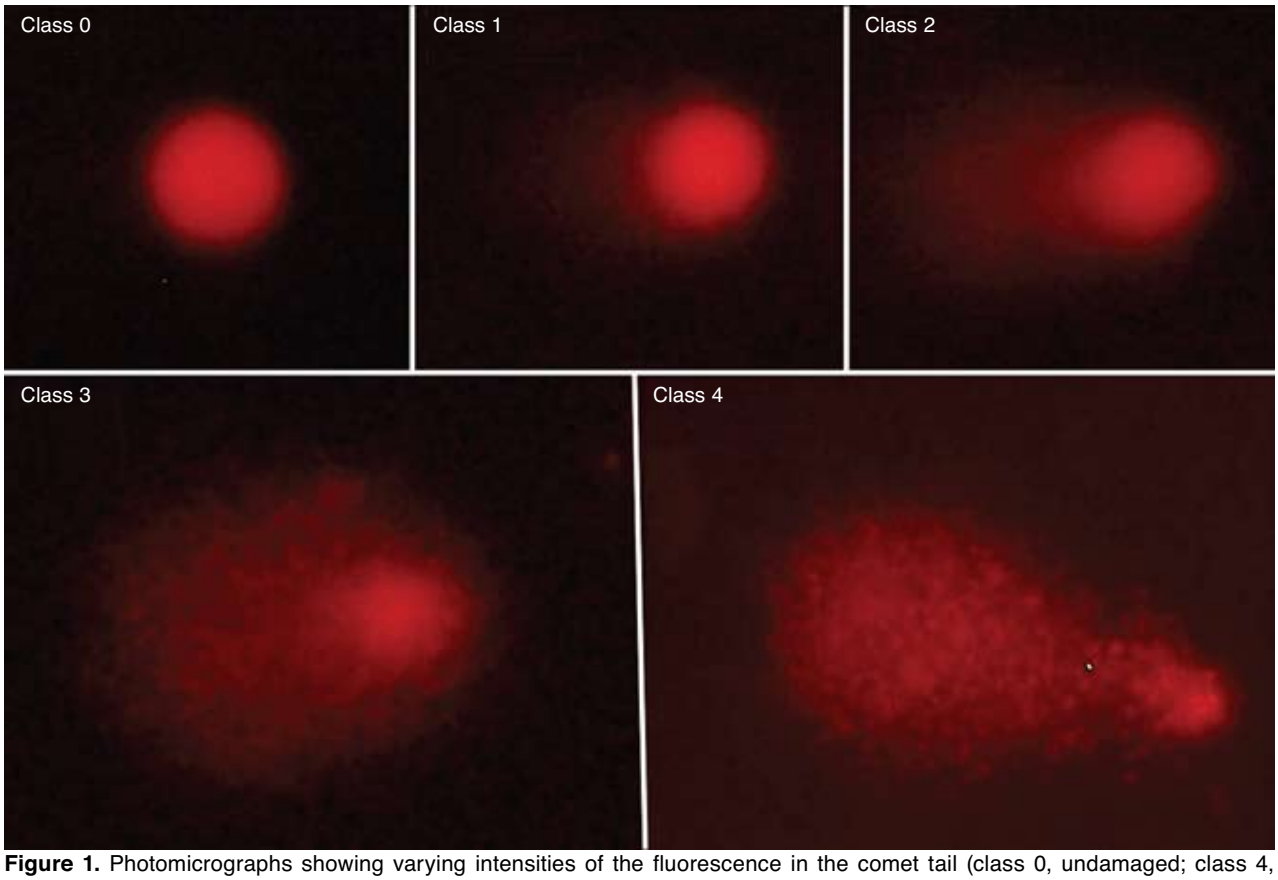

Figure 1. Photomicrographs showing varying intensities of the fluorescence in the comet tail (class 0, undamaged; class 4, maximally damaged).

MO, USA). Heparinized blood of 1 ml was carefully layered over 1 ml Histopaque and centrifuged for 35 min at 500 × g and 25 °C. The interface band containing lymphocytes was washed with phosphate-buffered saline (PBS) and then collected by 15 min centrifugation at 400 × g. The resulting pellets were resuspended in PBS to obtain 20,000 cells in 10 μl. Membrane integrity was assessed by trypan blue exclusion.

Endogenous DNA damage in lymphocytes was analyzed by the alkaline comet assay as described by Singh et al.[26] with minor modifications. Fresh lymphocyte cell suspension of 10 μl (about 20,000 cells) was mixed with 80 µl of 0.7% low-melting point agarose (LMPA) (Sigma) in PBS at 37 °C. Subsequently, 80 µl of this mixture was layered onto slides that had previously been coated with 1.0% hot (60 °C) normal-melting point agarose (NMPA) and the slides were covered with a coverslip at 4 °C for at least 5 min to allow the agarose to solidify. After removing the coverslips, the slides were submersed in freshly prepared cold (4 °C) lysing solution (2.5 M NaCl, 100 mM EDTA-2Na; 10 mM Tris–HCl, pH 10–10.5; 1% Triton X-100 and 10% DMSO were added just before use) for at least one hour. The slides were then immersed in freshly prepared alkaline electrophoresis buffer (0.3 mol/l NaOH and 1 mmol/l Na_2ETDA, pH >13) at 4 °C for unwinding (40 min) and then electrophoresed (25 V/300 mA, 25 min). All these steps were conducted under red light or without direct light in order to prevent additional DNA damage. After electrophoresis, the slides were stained with ethidium bromide (2 µ/ml in distilled H_2O; 70 µl/slide), covered with a coverslip, and analyzed using a epifluorescence microscope (Nikon, Japan) equipped with rhodamine filter (excitation wavelength 546 nm, barrier filter 580 nm). The images of 100 randomly chosen nuclei (50 cells from each of two replicate slides) were analyzed visually. Each image was classified according to the intensity of the fluorescence in the comet tail, which was rated from 0 to 4 (undamaged to maximally damaged) (Fig. 1), so that the total score of two replicate slides could be between 0 and 400 arbitrary units (AU). All procedures were performed by the same biochemistry staff and DNA damage was assessed by a single observer who was not aware of the diagnosis.

Measurement of TAS. Total antioxidant status of serum was determined using an automated measurement method,[27] whereby hydroxyl radical, the most potent biological radical, is produced. In this assay, ferrous ion solution, which is present in Reagent 1, is mixed with hydrogen peroxide, which is present in Reagent 2. The sequentially produced radicals such as brown-colored dianisidine radical cation, produced by the hydroxyl radical, are also potent radicals. This assay measures antioxidative effect of the sample against potent free radical reactions initiated by the produced hydroxyl radical. The assay has excellent precision values which are lower than 3%. The results were expressed as mmol Trolox equivalent/l.

Statistical analysis. The results were presented as mean ± standard deviation or frequency. Categorical

Table 1. Demographic, echocardiographic, and biochemical characteristics of the three groups

	White-coat hypertension (n=23)	Sustained hypertension (n=21)	Control (n=19)	*p**
Age (years)	46.2±5.5	45.3±6.5	47.4±3.5	NS
Body mass index (kg/m^2)	26.2±2.4	26.2±2.7	26.2±3.6	NS
Office systolic blood pressure (mmHg)	145.3±7.9	145.7±11.7	113.2±11.9**	<0.001
Office diastolic blood pressure (mmHg)	91.3±5.3	91.3±9.5	71.8±9.7**	<0.001
Heart rate (beat/min)	76.7±11.7	77.2±13.0	77.7±12.7	NS
Left ventricle end-systolic diameter (mm)	29.5±3.9	28.5±3.1	30.2±4.5	NS
Left ventricle end-diastolic diameter (mm)	45.0±4.2	43.6±4.3	45.7±4.6	NS
Interventricular septal thickness (mm)	10.3±1.5	10.6±1.7	9.4±1.0$^+$	0.020
Posterior wall thickness (mm)	9.4±1.5	9.7±1.6	8.7±1.2^{++}	0.087
Left ventricular mass index (g/m^2)	96.9±12.0	96.3±12.6	86.6±15.0^{+++}	0.028
Ejection fraction (%)	64.0±4.4	64.5±3.7	64.1±2.6	NS
Total cholesterol (mmol/l)	4.6±0.7	4.5±0.6	4.3±0.5	.NS
Triglyceride (mmol/l)	1.50±0.20	1.45±0.3	1.44±0.3	.NS
HDL-cholesterol (mmol/l)	1.2±0.2	1.2±0.1	1.2±0.1	.NS
LDL-cholesterol (mmol/l)	2.7±0.7	2.7±0.6	2.5±0.6	.NS
Glucose (mmol/l)	5.4±0.6	5.2±0.4	5.3±0.3	NS

*Chi-square test; NS: Not significant; **Comparison with the two hypertension groups; $^+$p=0.006 and $^{++}$p=0.031 *vs* SHT group; $^{+++}$p=0.024 *vs* SHT group and p=0.015 *vs* WCH group.

Table 2. Ambulatory blood pressure monitoring data of the study groups

	White-coat hypertension (n=23)	Sustained hypertension (n=21)	Control (n=19)	*p*
24 h systolic	111.5±8.2	138.5±5.3*	110.7±4.9	<0.001
24 h diastolic	65.9±5.4	85.4±6.3*	66.1±4.6	<0.001
Daytime systolic	115.4±9.4	145.2±4*	116.2±5.1	<0.001
Daytime diastolic	70.0±5.4	89.9±6.5*	71.9±5.5	<0.001
Nighttime systolic	107.5±10.4	131.7±8.5*	105.2±5.1	<0.001
Nighttime diastolic	61.7±11.3	81.0±7.0*	60.2±4.5	<0.001

*p<0.001 *vs* WCH and control groups.

variables were compared using the chi-square test. Comparison among multiple groups was performed by one-way analysis of variance (ANOVA) with the LSD post hoc test for continuous variables. Associations between other variables and lymphocyte DNA damage were assessed by Pearson correlation test. Independent relationships with lymphocyte DNA damage were assessed by multiple linear regression analysis. Standardized beta coefficients and their significance were derived. A two-tailed *p* value of less than 0.05 was considered statistically significant.

RESULTS

Baseline clinical, hemodynamic and echocardiographic parameters. Demographic features, heart rate and lipid measurements did not differ significantly between the three groups (p>0.05). Office systolic and diastolic BP were similar in the two hypertensive groups (p>0.05), whereas they were significantly lower in the control group (p<0.01) (Table 1). The two hypertensive groups did not differ significantly with regard to LVMI, IVSth, and PWth (p>0.05), whereas the control group had significantly lower values for these parameters (p<0.05). There were no significant differences between the three groups with regard to LVESD, LVEDD, and EF (p>0.05).

The mean daytime and nighttime systolic and diastolic pressures were significantly higher in the SHT group than in both WCH and control groups (p<0.05). Patients with WCH, on the other hand, had similar daytime and nighttime systolic and diastolic pressures compared to the controls (p>0.05, Table 2).

Analysis of lymphocyte DNA damage and TAS. The mean lymphocyte DNA damage in patients with SHT was higher than both WCH and control groups (p<0.001). However, there were no significant differences with regard to lymphocyte DNA damage between patients with WCH and the controls (p=0.052, Table 3).

The mean TAS level of patients with SHT was significantly lower than both patients with WCH (p=0.012) and the controls (p<0.001). Unlike the extent of lymphocyte DNA damage, patients with WCH exhibited a significantly lower TAS compared to the controls (p<0.001).

Correlation analysis. In the SHT group, lymphocyte DNA damage was correlated with TAS (r= -0.818, p<0.001), age (r=0.453, p=0.039), total cholesterol (r=0.550, p=0.010) and LDL-cholesterol (r=0.539, p=0.012). In multiple linear regression analysis, lymphocyte DNA damage was independently correlated with only serum TAS level (ß= -0.717, p<0.001).

In the WCH group, bivariate analysis showed that lymphocyte DNA damage was only correlated with serum TAS level (r= -0.458, p=0.028). This relationship was not observed in the control group (p>0.05).

DISCUSSION

To the best of our knowledge, the present study is the first to evaluate lymphocyte DNA damage and TAS in patients with WCH.

Oxidative stress due to excessive production of ROS outstripping antioxidant defense mechanisms has been implicated in many pathophysiological

Table 3. Analysis of lymphocyte DNA damage and total antioxidant status (TAS)

	White-coat hypertension	Sustained hypertension	Control	*p*
DNA damage (arbitrary unit)	14.2±5.4	31.4±12.1*	9.2±4.4	<0.001
TAS (mmol Trolox equivalent/l)	1.64±0.12**	1.55±0.10***	1.79±0.11	<0.001

*p<0.001 *vs* WCH and control groups; **p=0.012 *vs* SHT group and p<0.001 *vs* control group; ***p<0.001 *vs* control group.

conditions that affect the cardiovascular system, such as smoking, hypercholesterolemia, diabetes, and hypertension.[28,29] Decreased antioxidant capacity[13] and increased ROS release[14] were shown to be associated with essential HT. Hypertension is also considered a state of oxidative stress that can contribute to the development of atherosclerosis[30] and other HT-induced organ damage.[31]

It is well-known that DNA damage frequently occurs in cells exposed to oxidative stress.[8] Increased oxidative status may initiate lipid peroxidation in cell membranes, damage membrane proteins or cause DNA fragmentation. These processes may lead to severe myocardial cell damage resulting in the loss of cardiac contractile function.[32] In our study, a significantly increased lymphocyte DNA damage was shown in the SHT group and it was accompanied by a major decrease in TAS level. These findings suggest that increased lymphocyte DNA damage may be related to increased oxidative stress in patients with SHT. The presence of an inverse relationship between lymphocyte DNA damage and TAS support this hypothesis. Plasma TAS level is an accurate index of oxidative stress and provides a measure of total plasma defenses against ROS. Relationship between TAS and lymphocyte DNA damage was demonstrated in previous studies.[19,20] Antioxidant systems (such as TAS) prevent DNA damage.[8,33] Honda et al.[33] reported that reduced activities of antioxidant enzymes were associated with increased levels of oxidative DNA damage.

Our finding of increased DNA damage in patients with SHT was consistent with the study of Lee et al.[15] who showed that DNA damage caused by ROS occurs more commonly in hypertensive patients than in normotensives. They reported that DNA damage was reduced with the use of an antioxidant beta-blocking drug (carvedilol). However, in their study, lymphocyte DNA damage was not investigated in patients with WCH, and TAS was not evaluated in hypertensive patients.

Data on the relationship between oxidative stress markers and WCH are very limited.[16,17] In addition, TAS level has not been investigated in patients with WCH. Some authors did not find any difference in oxidative stress markers between WCH cases and normotensives.[17] In contrast, Uzun et al.[16] demonstrated that oxidative stress was increased in WCH and suggested that reduction in PON1 activity might be one of the factors leading to increased oxidative stress in WCH. However, it is not yet clear whether increased oxidative stress occurs before or after the development of WCH. In our study, the mean TAS level in the WCH group was significantly lower compared to the control group. Decreased antioxidant levels may be interpreted as the evidence for increased oxidative stress associated with HT. Therefore, patients with WCH may exhibit increased oxidative stress.

Compared to the controls, patients with WCH had significantly decreased TAS levels and insignificant increases in lymphocyte DNA damage. On the other hand, there was a significant correlation between increased lymphocyte DNA damage and decreased TAS level in patients with SHT. These imply that patients with SHT may be standing between SHT and normotensives with respect to oxidative stress and coexistent lymphocyte DNA damage, which may represent a novel pathophysiological mechanism for increased risk of cardiovascular diseases and target organ damage in patients with WCH.

Study limitations. In the present study, the size of the study sample may be insufficient. Studies with a larger sample might yield significantly increased lymphocyte DNA damage in cases with WCH. In our study, TAS was the only oxidative stress marker evaluated. Other oxidative stress markers and their relationship with lymphocyte DNA damage may be investigated in the future.

In conclusion, albeit insignificant, increased lymphocyte DNA damage and significantly decreased plasma TAS levels were detected in patients with WCH compared with the control group. Although lymphocyte DNA damage and oxidative stress were of lower degree in the WCH group compared to patients with SHT, WCH seems to be an important clinical situation requiring a close follow-up.

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