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Measuring urinary 8-hydroxy-2'-deoxyguanosine and malondialdehyde levels in women with overactive bladder

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Purpose: In this study, we aimed to explain the role of oxidative stress in women with overactive bladder (OAB) by investigating the levels of 8-hydroxy-2'-deoxyguanosine (8-OHdG), a marker of oxidative DNA damage, and malondialdehyde (MDA), an indicator of lipid peroxidation.

Materials and Methods: A total of 90 women were included in the study: 45 female patients diagnosed with OAB at Hopa State Hospital Urology Polyclinic and 45 healthy women without any metabolic or neurologic disease. Levels of MDA and 8-OHdG were measured in 24-hour urine samples for all subjects.

Results: Urinary levels of MDA and 8-OHdG were significantly higher in the OAB group than in the control group ($p<0.001$). A significant positive correlation ($p<0.001$) was found between the measurements of 8-OHdG and MDA.

Conclusions: Oxidative stress may be important in the pathophysiology of OAB, because levels of 8-OHdG and MDA are increased. Increased levels of 8-OHdG may be due to damaged nuclear and mitochondrial DNA as a result of oxidative attacks caused by free radicals. Nevertheless, further randomized and prospective studies with larger patient populations are needed.

Keywords: Lipid peroxidation; Oxidative stress; Urinary bladder, overactive; 8OHdG

INTRODUCTION

Overactive bladder (OAB) is described by the International Continence Society as 'the feeling of urinary urgency, with or without urge incontinence, associated with increased urination frequency at day and night' [1]. OAB is defined by involuntary bladder contractions that occur during bladder filling despite the patient's attempt to compress them [2]. The incidence of OAB, which has negative effects on quality of life, sleep quality, and mental health, increases in parallel with advancing age. The prevalence of OAB is high; the disease is known to affect millions of people worldwide. Despite such a high prevalence, millions of patients with OAB are not diagnosed or treated [3-5].

In biological systems, the loss of balance between reactive oxygen species (ROS) and the antioxidant defense

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mechanism against them is defined as oxidative stress. An increase in ROS levels leads to damage on cell membranes, disruption of the structure and function of intracellular proteins, and structural damage to DNA, leading to cell damage. Lipid peroxidation plays a role in the pathogenesis of many diseases with tissue damage, and malondialdehyde (MDA), a product of lipid peroxidation, is used as a marker of oxidative stress [6,7]. ROS cause more than 20 oxidative base damage products in DNA. Among the bases that suffer such damage, 8-hydroxy-2'-deoxyguanosine (8-OHdG) is a highly sensitive and common marker of oxidative DNA damage [8,9]. 8-OHdG, which is formed as a result of hydroxyl radical attacks on the 8th carbon atom of guanine, is cleaved off by DNA repair enzymes, then passed to the peripheral circulation, and then excreted in the urine. 8-OHdG, which is found in the serum and urine, is a mutagen formed in DNA by endogenously produced ROS during normal oxidative metabolism or by exogenous ROS. For this reason, measurement of 8-OHdG is the most commonly used method for determining oxidative DNA damage [10,11].

Urine and serum levels of 8-OHdG can increase as the result of many pathological conditions, including aging, cancer, diabetes, and hypertension [12]. The need for a marker to clarify the diagnosis of OAB and to predict treatment success arose because OAB is a symptomatological description. There is no pathologic cause that explains these symptoms. In this study, we aimed to explain the role of oxidative stress in the pathophysiology of OAB in women by investigating the levels of 8-OHdG, a marker of oxidative DNA damage, and of MDA, an indicator of lipid peroxidation.

MATERIALS AND METHODS

1. Selection and evaluation of patients and healthy volunteers

The study was carried out after obtaining the required approvals from the Ethics Committee of Ordu University Medical Faculty Hospital (approval number: 2016/01). A total of 90 women were included in the study: 45 female patients diagnosed with OAB at Hopa State Hospital Urology Polyclinic and 45 healthy women without any metabolic disease. All patients and control women gave informed consent and the local ethics committee approval was obtained in accordance with the ethical standards of the Helsinki Declaration. The patients' medical history, physical examination findings, height, weight, and age were collected by means of signed standard forms. Women with a history of genitourinary operations, pelvic operations, pelvic radiotherapy, chronic neurological or endocrine disease, cystocele, stress urinary incontinence, urinary tract infection, drug use leading to urinary symptoms, smoking, or any other drug use were excluded from the study. Urine specimens taken from all subjects were centrifuged at 3,000 ×*g* for 10 minutes and the supernatants were separated and stored at -80°C until tested. MDA and 8-OHdG levels were measured in 24-hour urine samples for all subjects.

2. Analysis of malondialdehyde levels

The level of MDA, a measure of lipid peroxidation, was measured spectrophotometrically by the method described by Ohkawa et al. [13]. For each sample, 0.5 mL of 8.1% sodium dodecyl sulfate, 0.5 mL of 0.8% thiobarbituric acid, 1.0 mL of 10% of trichloroacetic acid, 1.0 mL of 2% glacial acetic acid/sodium hydroxide (pH=3.5), and 50 μL of 2% butyl hydroxytoluene were added to the urine sample (1.0 mL) and this mixture was thoroughly mixed and kept in a water bath at 95°C for 60 minutes. After the tubes were chilled, a mixture of 4.0 mL of butanol/pyridine (1:15) was added and the tubes were centrifuged at 4,000 ×*g* at 4°C for 10 minutes. After centrifugation, the upper organic phase was removed and the absorbance was read at 532 nm. The MDA results were reported as nmol/mL.

3. Measurement of urinary 8-hydroxy-2'-deoxyguanosine levels

Urinary 8-OHdG levels were determined by means of the enzyme-linked immunosorbent assay (ELISA) technique, using human ELISA kits (Cell Biolabs, Inc., San Diego, CA, USA) with the test procedure suggested by the manufacturer. The 8-OHdG results were reported as ng/mL.

4. Statistical analysis

All data analysis was performed by using the SPSS ver. 11.0 software (SPSS Inc., Chicago, IL, USA). The values were compared between the groups (patient and control groups) by using Student's t-test according to the results of the Levene test and the Shapiro Wilk test for equality of variances and the normality supposition, respectively ($p>0.05$). Also, Pearson correlation coefficients were estimated to explain the relationships among the analyzed traits (age, weight, height, body mass index, MDA, and 8-OHdG). Data were presented as sample size (n) and mean with standard deviations. Significance was evaluated at $p<0.05$ for all tests.

Table 1. Demographic characteristics of the patient and control groups

Characteristic	Control group	OAB group	p-value
Age (y)	52.49±9.55	55.83±11.59	NS
Height (cm)	160.86±4.33	160.23±4.85	NS
Weight (kg)	66.37±8.30	70.51±12.91	NS
BMI (kg/m^2)	25.65±6.30	27.47±8.91	NS

Values are presented as mean±standard deviation.
OAB, overactive bladder; BMI, body mass index; NS, not significant.

Table 2. Correlations between examined traits

Variable	MDA	8-OHdG	Age	Height	Weight
MDA	-	1.000[a]	0.132	0.187	0.203
8-OHdG	-	-	0.132	0.187	0.203
Age	-	-	-	-0.321[a]	0.452[a]
Height	-	-	-	-	0.027

MDA, malondialdehyde; 8-OHdG, 8-hydroxy-2'-deoxyguanosine.
[a]:p=0.01 (2-tailed).

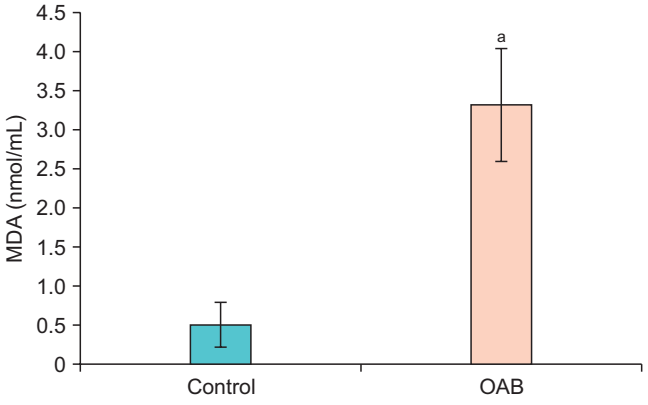

Fig. 1. Urinary MDA levels of the patient and control groups. MDA, malondialdehyde; OAB, overactive bladder. [a]:Significantly different from control group (p<0.001).

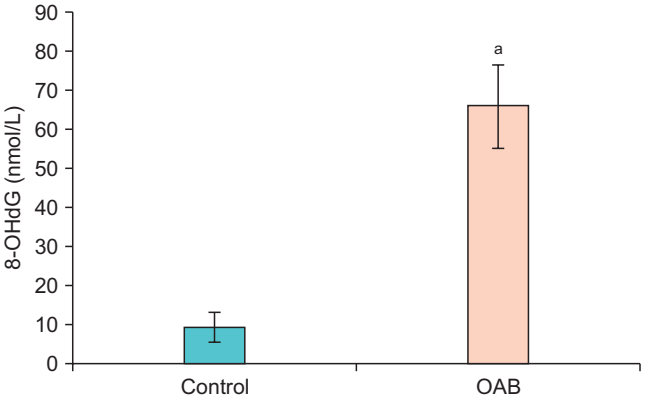

Fig. 2. Urinary 8-OHdG levels of the patient and control groups. 8-OHdG, 8-hydroxy-2'-deoxyguanosine; OAB, overactive bladder. [a]:Significantly different from the control group (p<0.001).

RESULTS

The demographic characteristics of the study groups are presented in Table 1. There were no statistically significant differences between the control and patient groups in terms of age, height, weight, or body mass index (p>0.05). Urinary MDA (3.30±1.29 nmol/L vs. 0.46±0.29 nmol/mL) and urinary 8-OHdG (66.03±16.49 nmol/L vs. 9.22±5.75 nmol/L) levels were significantly higher in the OAB group than in the control group (p<0.001) (Figs. 1, 2).

A significant positive correlation (r=1.000; p<0.001) was found between the measurements of 8-OHDG and MDA. There was also a significant positive correlation of 45.2% between age and weight (p<0.001) and a significant negative correlation of 32.1% between age and height (p=0.007). There was no statistically significant relationship between the other measurement values (p>0.05; Table 2).

DISCUSSION

OAB is a common condition characterized by a symptom complex (urgency to urinate, frequent urination, nocturia, and urge incontinence) that affects and limits patients' quality of life. Treatment of this condition requires an understanding of the underlying pathophysiology. Oxidative stress may be one possible factor in the pathophysiology of OAB. Previous studies offered the hypothesis that a state of oxidative stress may be present in a condition of chronic obstruction, and that this results in the production of metabolic end products that injure the phospholipids of the lipid membrane of the muscle cells. However, insufficient evidence has been provided to support such a

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hypothesis [14-16]. In the present study, we measured two of the most commonly used and accepted markers of the presence of oxidative stress in the urine: MDA and 8-OHdG. Biomarkers, such as 8-OHdG and MDA are considered to be significant indicators of the oxidative stress status in organisms. In another study, Dambros et al. [17] used hypochlorous acid in pig detrusor to model overactivity and found that 8-iso-PGF2α levels were increased in the overactive group in relation to oxidative stress. Huang et al. [18] studied kaempferol, which is a flavonoid compound that is suppressed in oxidative stress and that attenuated bladder overactivity in a rat model.

MDA, one of the most important products of lipid peroxidation, acts on ion exchange from cell membranes, leading to cross-linking of the compounds within the membrane and causing negative consequences, such as changes in ion permeability and enzyme activity. This occurs because the polyunsaturated fatty acids of cell membranes are degraded by this process, with the result being the disruption of membrane unity. Lipid peroxidation can lead to changes in membrane mobility and permeability and can also increase the rate of protein deterioration and cell lysis [19,20]. In the present study, the MDA concentration in the OAB group was significantly higher than that in the control group. Alexandre et al. [21] reported that increased oxidative stress plays an important role in bladder dysfunction. They suggested that mild, chronic ischemia in the bladder enhances oxidative stress, leading to detrusor overactivity and storage symptoms, which progress to detrusor underactivity when bladder ischemia becomes severe. Topol et al. [22] reported that OAB is associated with oxidative damage in the bladder and the resultant production of free radicals. The formation of high amounts of oxygen-derivative free radicals has an adverse effect on the constituents of biological systems. Chemical and physical changes resulting from various internal and external factors cause mutations in the structure of DNA, through lethal or direct or indirect mutagenesis, by ceasing replication and transcription. ROS cause various lesions containing 8-oxoguanine [23,24]. The level of DNA 8-OHdG and urinary 8-OHdG is frequently measured in humans as a marker of oxidative stress. Repair of oxidative DNA damage and repair of the cells in the organism is mostly reflected by the excretion of 8-OHdG [25]. In the literature, functional changes in bladder ischemia have been reported to be associated with impairment of mitochondrial respiration, cellular and mitochondrial stress, and activation of cell survival signaling via the PI3K/Akt pathway [26].

Nocchi et al. [27] found that oxidative stress is related to increased bladder nerve activity and intravesical pressure with H_2O_2 in a rat model. They also found that increased 8-OHdG levels in an aged rat group were related to increased oxidative stress in the bladder with aging. Matsui et al. [28] found that levels of both 8-OHdG and MDA were increased in a rat model of atherosclerosis-induced chronic bladder ischemia in relation to oxidative stress. They also found that Eviprostat, a phytotherapeutic agent, decreased MDA and 8-OHdG levels, which was also related to a normalized micturition interval.

Important limitations of the present study were the number of patients and the lack of urodynamic examinations or further neurologic examinations. As a second-stage hospital of our clinic, we could not perform magnetic resonance imaging (MRI) or urodynamic investigations. Last, an antioxidant therapy could provide a positive control with which to compare 8-OHdG and MDA levels after therapy.

CONCLUSIONS

In our study, levels of both MDA and 8-OHdG were higher in patients with OAB than in the control group. Although further examinations, such as urodynamic investigation, cranial MRI, or electroneuromyography were not done, our study showed that oxidative stress is related to OAB as a symptom complex (urgency with or without incontinence, nocturia, frequency) in women. Additional randomized and prospective studies with larger patient populations and further examinations are needed.

CONFLICTS OF INTEREST

The authors have nothing to disclose.

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