Received: 18 November 2020 | Revised: 10 February 2021 | Accepted: 12 February 2021

DOI: 10.1002/1348-9585.12210

ORIGINAL ARTICLE

Journal of Occupational Health Open Access WILEY

Health examination results and work environment factors affecting urinary 8-hydroxy-2′-deoxyguanosine levels

Sintaroo Watanabe[1,2] | **Yun-Shan Li**[1] | **Yuya Kawasaki**[1] | **Yuko Ootsuyama**[1] | **Kazuaki Kawai**[1,3]

[1]Department of Environmental Oncology, Institute of Industrial Ecological Sciences, University of Occupational and Environmental Health Japan, Kitakyushu, Japan

[2]Japan Marine United Corporation Kure Shipyard, Hiroshima, Japan

[3]Center for Stress-related Disease Control and Prevention, University of Occupational and Environmental Health Japan, Kitakyushu, Japan

Correspondence
Kazuaki Kawai, Department of Environmental Oncology, Institute of Industrial Ecological Sciences, University of Occupational and Environmental Health, Kitakyushu, Japan.
Email: kkawai@med.uoeh-u.ac.jp

Funding information
JSPS, Grant/Award Number: JP17H01908

Abstract

Objective: Oxidative stress is considered to cause lifestyle-related diseases, including cancer. Urinary 8-hydroxy-2′-deoxyguanosine (8-OHdG) is widely analyzed as an oxidative stress marker. We extensively scrutinized the relationships between 8-OHdG levels and lifestyle choices as carcinogenic factors.

Methods: In this study, we investigated health examination results and working conditions affecting urinary 8-OHdG levels in 503 male workers.

Results: The urinary 8-OHdG level was positively associated with high blood sugar and leanness in smokers. In addition, urinary 8-OHdG tended to increase with organic solvent or hydrochloric acid exposure, as well as long working hours. On the other hand, the urinary 8-OHdG level was negatively associated with high plasma LDL-cholesterol levels in non-smokers and anemia.

Conclusion: According to the results, anemia decreased the oxidative stress, regardless of smoking status, while leanness or high blood sugar increased the oxidative stress in smokers, and the presence of plasma cholesterol contributed to the lower oxidative stress in non-smokers. Certain types of occupational exposure may cause oxidative stress. The measurement of urinary 8-OHdG at annual health checks may be a useful biomarker for preventing lifestyle- and work-related diseases.

KEYWORDS

8-hydroxy-2′-deoxyguanosine, health examination, lifestyle, oxidative stress, working environment

1 | INTRODUCTION

Oxidative stress is considered to cause lifestyle-related diseases, including cancer, diabetes, and cardiovascular disease.[1,2] In addition, oxidative stress increases with exposure to hazardous materials such as asbestos[3,4] and nanoparticles,[5] which are associated with work-related diseases. Measuring oxidative stress could clarify the mechanisms underlying the onsets of these diseases and their prevention. A representative oxidative stress marker is 8-hydroxy-2′-deoxyguanosine (8-OHdG), which reflects the oxidized state of a nucleobase.[6] This oxidized nucleobase has been widely analyzed using

Abbreviations: 8-OHdG, 8-hydroxy-2′-deoxyguanosine; ALT, alanine aminotransferase; AST, aspartic aminotransferase; BMI, body mass index; BS, blood sugar; DBP, diastolic blood pressure; ECD, electrochemical detector; GSH, glutathione; Hb, hemoglobin; HDL, high-density lipoprotein; HDL-Chol, HDL-cholesterol; HPLC, high-performance liquid chromatography; LDL, low-density lipoprotein; LDL-Chol, LDL-cholesterol; SBP, systolic blood pressure; SOD, superoxide dismutase; TG, triglyceride; UA, uric acid; γ-GTP, γ-glutamyl transpeptidase.

urine samples, which can be collected non-invasively. In addition, human urinary 8-OHdG levels are reportedly stable throughout the day.[7]

Cross-sectional studies have shed light on the relationship between lifestyle/work environment and urinary 8-OHdG levels. Urinary 8-OHdG levels were increased by smoking,[8,9] drinking,[8] exposure to cooking oil fumes,[10] long working hours,[11] and shift work.[8] On the other hand, urinary 8-OHdG levels were decreased by light-colored vegetable consumption[11] and moderate physical activity.[12] In addition, a recent longitudinal study has revealed a decrease in urinary 8-OHdG levels by smoking cessation.[13]

Previous studies also reported the negative correlation between body mass index (BMI) and urinary 8-OHdG levels.[8,11] The mechanism remains unclear, although obesity is a risk factor for lifestyle-related diseases.[14] Hyperuricemia associated with obesity may contribute to lower urinary 8-OHdG levels, because of the antioxidant effect of uric acid (UA).[15,16]

To the best of our knowledge, no investigations have considered the associations between working environment, BMI, and urinary 8-OHdG levels, with reference to health examinations, including blood tests. Identifying the factors that affect the urinary 8-OHdG levels will support the correct interpretation of urinary 8-OHdG results and facilitate the prevention of lifestyle- and work-related diseases.

The purpose of this study is to clarify the relationships between urinary 8-OHdG levels and health examination results, including blood tests, and between urinary 8-OHdG levels and the working environment, including metal fume, organic solvent, or hydrochloric acid exposure.

2 | MATERIALS AND METHODS

2.1 | Urine collection

A total of 602 male volunteers ages 18-64, from three companies in Japan, participated in the study. Urine samples (~10 ml) were collected in polypropylene tubes between 8:00 AM and 11:00 AM, stored in a cooler box containing ice during the sampling, and then frozen at −30°C until analysis. The study was approved by the University of Occupational and Environmental Health Ethics Committee (H26-239). Written informed consent was obtained from all subjects.

2.2 | Lifestyle/work environment survey and health examination

At the time of urine collection, a physical examination, anthropometry, and blood tests were performed, and the subjects answered lifestyle, disease, and working environment questionnaires.

We obtained information about smoking (smoker or non-smoker), daily alcohol intake (<20 or ≥20 g/d), and aerobic exercise for 30 minutes at least twice a week (yes or no) as lifestyle choices by the two-case method.

Regarding the working environment, we asked for responses by the two-case method about metal fume exposure, organic solvent, or hydrochloric acid (chemicals handled at these companies) exposure, shift work, sunlight exposure, and long working hours (60 hours or more overtime: defined as "long working hours" in these companies during the urine collection month). The survey on the working environment was based on self-reporting; therefore, not only those who directly handled hazardous substances but also the workplace managers and those who transported hazardous materials answered that they were exposed to hazardous substances.

Information on working environment measurements (Control Class-Ⅰ/Control Class-Ⅱ/Control Class-Ⅲ) of metal fume-exposed workers was obtained if available. In addition, information on urinary organic solvent metabolite levels (Level-Ⅰ/Level-Ⅱ/Level-Ⅲ) of organic solvent-exposed workers was obtained if available.[17]

The following information was collected from the anthropometry and blood tests. This information included height and weight (for BMI), systolic blood pressure (SBP), diastolic blood pressure (DBP), and the levels of blood sugar (BS), blood hemoglobin (Hb), plasma HDL-cholesterol (HDL-Chol), plasma LDL-cholesterol (LDL-Chol), plasma triglyceride (TG), plasma UA, plasma aspartic aminotransferase

TABLE 1 Assignment of dummy variables for lifestyle and working environment

Lifestyle and working environment factors	Category: assigned dummy variable
Smoking status	Non-smoker: 0
	Smoker: 1
Alcohol consumption (g/d)	<20:0
	≥20:1
Aerobics 30 min	No: 0
	Yes: 1
Fume exposure (self-reported)	No: 0
	Yes: 1
Organic solvents or hydrochloric acid exposure (self-reported)	No: 0
	Yes: 1
Shift work	No: 0
	Yes: 1
Sunlight exposure	No: 0
	Yes: 1
Long working hours	No: 0
	Yes: 1

TABLE 2 Lifestyle factors and health examination vs urinary 8-OHdG (N = 503)

		Total (N = 503)		Non-smoker (N = 290)		Smoker (N = 213)	
Factors	**Category**	**N**	**8-OHdG**	**N**	**8-OHdG**	**N**	**8-OHdG**
Smoking status	Non-smoker	290	3.30 (2.60-4.10)				
	Smoker	213	3.60 (2.80-4.70)**				
Age (y)	<50	424	3.40 (2.70-4.30)	238	3.30 (2.60-4.10)	186	3.60 (2.80-4.70)
	≥50	79	3.40 (2.85-4.60)	52	3.30 (2.70-4.15)	27	4.30 (3.00-5.10)
Alcohol consumption (g/d)	<20	376	3.35 (2.70-4.30)	227	3.20 (2.60-4.05)	149	3.60 (2.80-4.70)
	≥20	127	3.50 (2.90-4.55)	63	3.40 (2.85-4.30)	64	3.75 (2.95-4.80)
Aerobics 30 min	No	432	3.40 (2.70-4.40)	251	3.20 (2.60-4.05)	181	3.60 (2.90-4.80)
	Yes	71	3.30 (2.65-4.30)	39	3.40 (3.05-4.45)	32	3.20 (2.45-4.15)*
BMI (kg/m^2)	<18.5	23	3.70 (3.05-4.70)*, a	9	3.20 (3.00-3.60)	14	4.35 (3.30-5.60)*, a
	18.5-25	341	3.40 (2.80-4.40)*, b	203	3.40 (2.70-4.20)	138	3.60 (2.80-4.70)
	>25	139	3.20 (2.45-4.10)	78	3.10 (2.30-4.10)	61	3.50 (2.70-4.70)
BP (mmHg)	SBP: <140 and DBP: <90	466	3.40 (2.70-4.40)	272	3.35 (3.10-3.80)	194	3.60 (2.90-4.70)
	SBP: ≥140 or DBP: ≥90	37	3.30 (2.70-4.20)	18	3.30 (2.60-4.10)	19	3.00 (2.20-4.30)
BS (mg/dL)	<110	472	3.40 (2.70-4.30)	270	3.25 (2.60-4.10)	202	3.60 (2.80-4.50)
	≥110	31	3.40 (2.85-4.85)	20	3.30 (2.60-4.10)	11	4.80 (4.45-6.55)**
TG (mg/dL)	<300	486	3.40 (2.70-4.40)	285	3.30 (2.60-4.10)	201	3.60 (2.90-4.70)
	≥300	17	2.80 (2.50-4.10)	5	2.80 (2.70-3.30)	12	3.10 (2.35-4.35)
HDL-Chol (mg/dl)	<40	15	3.20 (2.80-4.75)	9	3.10 (2.80-3.60)	6	3.90 (2.80-5.50)
	≥40	488	3.40 (2.70-4.35)	281	3.30 (2.60-4.10)	207	3.60 (2.85-4.70)
LDL-Chol (mg/dl)	<140	403	3.60 (2.80-4.45)	220	3.40 (2.80-4.30)	183	3.70 (2.80-4.80)
	≥140	100	3.05 (2.50-3.60)**	70	2.90 (2.50-3.40)*	30	3.40 (2.90-3.80)
AST (U/L)	<36	453	3.40 (2.70-4.30)	258	3.30 (2.60-4.10)	195	3.60 (2.85-4.70)
	≥36	50	3.15 (2.70-4.50)	32	3.05 (2.60-4.10)	18	4.35 (2.80-5.60)
ALT (U/L)	<41	430	3.40 (2.70-4.30)	248	3.30 (2.60-4.10)	182	3.60 (2.90-4.70)
	≥41	73	3.30 (2.70-4.40)	42	3.10 (2.80-4.10)	31	3.60 (2.70-4.85)
γ-GTP (U/L)	<81	441	3.40 (2.70-4.30)	255	3.30 (2.60-4.20)	186	3.60 (2.80-4.70)
	≥81	62	3.30 (2.70-4.40)	35	3.30 (2.60-4.20)	27	3.50 (2.85-4.75)
UA (mg/dl)	<8	458	3.40 (2.70-4.40)	262	3.30 (2.60-4.10)	196	3.60 (2.80-4.80)
	≥8	45	3.20 (2.90-3.60)	28	3.15 (2.80-3.35)	17	3.40 (3.00-3.80)
Hb (g/dl)	≤13	5	1.40 (1.10-2.30)**	3	1.40 (1.25-2.30)*	2	1.55 (0.80-2.30)*
	>13	498	3.40 (2.70-4.40)	287	3.30 (2.60-4.10)	211	3.60 (2.90-4.70)

Note: Urinary 8-OHdG (ng /mg creatinine) levels were median (25th quartile-75th quartile).

Abbreviations: 8-OHdG, 8-hydroxy-2′-deoxyguanosine; ALT, alanine aminotransferase; AST, aspartic aminotransferase; BMI, body mass index; BP, blood pressure; BS, blood sugar; DBP, diastolic blood pressure; Hb, hemoglobin; HDL-Chol, high-density lipoprotein-cholesterol; LDL-Chol, low-density lipoprotein-cholesterol; SBP, systolic blood pressure; TG, triglyceride; UA, uric acid;γ-GTP, γ-glutamyl transpeptidase.

[a]BMI: <18.5 kg/m^2 versus > 25 kg/m^2.

[b]BMI: 18.5-25 kg/m^2 versus > 25 kg/m^2.

*P < .05, by Mann-Whitney test.

**P < .01by Mann-Whitney test.

(AST), plasma alanine aminotransferase (ALT), and plasma γ-glutamyl transpeptidase (γ-GTP).

2.3 | Analysis of urinary 8-OHdG

We determined the urinary 8-OHdG levels according to the method of Kasai et al.[8] Briefly, urine samples were thawed and centrifuged, and then 50 μL of the supernatant was mixed with an equal volume of a dilution solution containing the ribonucleoside marker 8-hydroxyguanosine. A 20-μL portion of this mixture was injected into the high-performance liquid chromatography (HPLC)-1 column (MCI GEL CA08F, 1.5 × 150 mm; Mitsubishi Chemical). The chromatograph was recorded with a UV detector (Gilson UV/VIS-151, 235 nm). The fraction containing 8-OHdG was automatically injected into the HPLC-2 column (Inertsil™ ODS-3, 3 μm, 4.6 × 250 mm; GL Sciences, Inc). The 8-OHdG was detected by an electrochemical detector (Coulochem II, ESA). The 8-OHdG levels were expressed as the ratios to the urinary creatinine contents (UV detection at 235 nm).

2.4 | Statistical analysis

From 602 male participants, we excluded subjects from this study with malignancies, cerebrovascular disease, and cardiovascular disease, as well as subjects with missing data on their questionnaires, and those who had consumed meals within 10 hours. Finally, 503 male subjects were selected for the analysis. All statistical analyses were performed with the EZR statistical software[18] and Exploratory for Windows 10, which is a graphical user interface for R (R Foundation for Statistical Computing, Vienna, Austria).

The urinary 8-OHdG levels did not have a normal distribution (Shapiro-Wilk test: $P < .001$); therefore, a nonparametric method was used for statistical analysis. The values are shown as the median with the 25th-75th quantiles. In the univariate analysis, the health examination results were classified as follows: BMI (<18.5, 18.5-25, and >25 kg/m^2), blood pressure (SBP: <140 mmHg and DBP: <90 mmHg; SBP: ≥140 mmHg or DBP: ≥90 mmHg), BS (<110 and ≥110 mg/dL), Hb (≤13.0 and >13.0 g/dL), HDL-Chol (<40 and ≥40 mg/dL), LDL-Chol (<140 and ≥140 mg/dL), TG (<300 and ≥300 mg/dL), UA (<8.0 and ≥8.0 mg/dL), AST (<36 and ≥36 U/L), ALT (<41 and ≥41 U/L), and γ-GTP (<81 and ≥81 U/L). These cutoffs were based on the criteria of the Japan Society of Ningen Dock.[19] The Mann-Whitney test (between two groups) was used to compare 8-OHdG levels with these categorical variables. Because it is well known that urinary 8-OHdG levels are elevated in smokers, we also conducted separate analyses for non-smokers and smokers.

In the multiple regression analysis, we used the forced entry method. The logarithmic levels of 8-OHdG were used

TABLE 3 Multiple regression analysis of (logarithmic 8-OHdG) versus health examination results

Health examination results	Total[a] (N = 503, R^2 = 0.10, $P < .001$)				Non-smoker (N = 290, R^2 = 0.10, $P = .02$)				Smoker (N = 213, R^2 = 0.16, $P = .002$)			
	Partial *r*	SE	*β*	*P*	Partial *r*	SE	*β*	*P*	Partial *r*	SE	*β*	*P*
Hb	0.029	0.008	0.157	<0.001	0.025	0.012	0.143	0.040	0.03	0.014	0.176	0.017
LDL-Chol	−0.001	0.000	−0.136	0.007	−0.001	0.000	−0.201	0.003	0.000	0.000	−0.040	0.612
BS	0.002	0.001	0.112	0.026	0.000	0.001	0.007	0.918	0.003	0.001	0.228	0.004
BMI	−0.005	0.003	−0.112	0.048	−0.003	0.003	−0.068	0.379	−0.010	0.004	−0.203	0.022
TG	0.000	0.000	−0.075	0.176	0.000	0.000	0.054	0.498	0.000	0.000	−0.142	0.091
UA	−0.008	0.006	−0.060	0.204	−0.003	0.007	−0.025	0.689	−0.011	0.010	−0.080	0.281
AST	0.001	0.001	0.099	0.224	−0.002	0.002	−0.153	0.212	0.003	0.001	0.227	0.064
γ-GTP	0.000	0.000	−0.043	0.449	−0.001	0.000	−0.127	0.103	0.000	0.000	−0.102	0.286
SBP	0.001	0.001	0.045	0.504	0.001	0.001	0.061	0.481	0.001	0.002	0.054	0.634
DBP	−0.001	0.001	−0.049	0.521	−0.001	0.002	−0.063	0.528	0.000	0.002	−0.028	0.815
HDL-Chol	0.000	0.001	−0.039	0.466	0.000	0.001	−0.027	0.715	−0.001	0.001	−0.045	0.585
ALT	0.000	0.001	−0.042	0.601	0.001	0.001	0.183	0.178	−0.001	0.001	−0.080	0.481

Note: Analyses were adjusted for age, alcohol consumption, aerobics, and health examination results.

Abbreviations: 8-OHdG, 8-hydroxy-2′-deoxyguanosine; ALT, alanine aminotransferase; AST, aspartic aminotransferase; BMI, body mass index; BP, blood pressure; BS, blood sugar; DBP, diastolic blood pressure; Hb, hemoglobin; HDL-Chol, high-density lipoprotein-cholesterol; LDL-Chol, low-density lipoprotein-cholesterol; SBP, systolic blood pressure; TG, triglyceride; UA, uric acid;γ-GTP, γ-glutamyl transpeptidase.

[a]Smoking status was entered into the multiple regression analysis model only when analyzing total subjects. Smoking ($\beta = 0.128$, $P = .004$) was positively associated with (logarithmic 8-OHdG).

TABLE 4 Health examination factors and urinary 8-OHdG levels in obese (BMI: >25 kg/m^2) subjects

		Total (N = 139)		Non-smoker (N = 78)		Smoker (N = 61)	
Factors	**Category**	**N**	**8-OHdG**	**N**	**8-OHdG**	**N**	**8-OHdG**
BP (mmHg)	SBP: <140 and DBP: <90	118	3.20 (2.40-4.10)	66	3.00 (2.30-4.10)	52	3.40 (2.70-4.50)
	SBP: ≥140 or DBP: ≥90	21	3.40 (3.00-4.30)	12	3.35 (2.80-3.65)	9	4.30 (3.00-5.10)
BS (mg/dL)	<110	123	3.20 (2.40-4.10)	68	3.10 (2.30-4.10)	55	3.30 (2.65-4.05)
	≥110	16	4.25 (2.85-5.10)	10	3.25 (2.20-3.80)	6	5.90 (4.70-7.10)**
TG (mg/dL)	<300	133	3.20 (2.50-4.10)	75	3.10 (2.30-4.05)	58	3.45 (2.70-4.70)
	≥300	6	3.70 (2.20-4.30)	3	3.30 (2.60-3.70)	3	4.30 (3.25-4.55)
HDL-Chol (mg/dL)	<40	7	2.90 (2.75-4.65)	4	2.85 (2.75-4.20)	3	3.80 (3.00-5.80)
	≥40	132	3.25 (2.40-4.10)	74	3.15 (2.30-4.10)	58	3.45 (2.70-4.70)
LDL-Chol (mg/dL)	<140	92	3.35 (2.55-4.35)	48	3.30 (2.55-3.4.25)	44	3.60 (2.55-4.80)
	≥140	47	3.00 (2.40-3.55)	30	2.90 (2.20-3.40)*	17	3.40 (3.00-3.60)
AST (U/L)	<36	113	3.20 (2.40-4.10)	59	3.30 (2.25-3.85)	54	3.50 (2.70-4.30)
	≥36	26	3.20 (2.80-4.50)	19	3.30 (2.85-4.15)	7	3.00 (2.60-5.05)
ALT (U/L)	<41	89	3.40 (2.40-4.30)	47	3.20 (2.20-4.10)	42	3.50 (2.80-4.30)
	≥41	50	3.15 (2.70-4.10)	31	3.10 (2.75-3.60)	19	3.30 (2.55-4.70)
γ-GTP (U/L)	<81	111	3.20 (2.50-4.10)	61	3.10 (2.40-4.10)	50	3.50 (2.70-4.30)
	≥81	28	3.30 (2.30-4.25)	17	3.30 (2.20-3.70)	11	3.40 (2.85-4.75)
UA (mg/dL)	<8	111	3.30 (2.40-4.30)	60	3.15 (2.25-4.10)	51	3.50 (2.75-4.80)
	≥8	28	3.10 (2.80-3.40)	18	3.10 (2.90-3.30)	10	3.05 (2.50-3.60)
Hb (g/dL)	≤13	1	1.10	1	1.10	—	—
	>13	138	3.25 (2.50-4.10)	77	3.10 (2.30-4.10)	61	3.50 (2.70-4.70)

Note: Urinary 8-OHdG (ng/mg creatinine) levels were median (25th quartile-75th quartile).

Abbreviations: 8-OHdG, 8-hydroxy-2′-deoxyguanosine; ALT, alanine aminotransferase; AST, aspartic aminotransferase; BMI, body mass index; BP, blood pressure; BS, blood sugar; DBP, diastolic blood pressure; Hb, hemoglobin; HDL-Chol, high-density lipoprotein-cholesterol; LDL-Chol, low-density lipoprotein-cholesterol; SBP, systolic blood pressure; TG, triglyceride; UA, uric acid;γ-GTP, γ-glutamyl transpeptidase.

*P <.05, by Mann-Whitney test.

**P <.01, by Mann-Whitney test.

as the dependent variable. Lifestyle factors, working environment factors, and health examination results were used as the independent variables. Age and health examination results were fed directly into the multiple regression analysis models as numerical data. Regarding lifestyle and working environment, we created dummy variables as shown in Table 1 and used them for the multiple regression analysis models.

3 | RESULTS

The coefficient of variation, calculated based on the 8-OHdG standard solution, was within 5%. The 8-OHdG level (ng/mg creatinine) in 503 male subjects aged 18-64 years was 3.65 ± 1.37 (mean ± SD); minimum: 0.80; 25th quartile: 2.70; median: 3.40; 75th quartile: 4.35; maximum: 10.8.

Table 2 shows the relationships between lifestyle-related factors and health examination results with the urinary 8-OHdG levels. As a whole, the urinary 8-OHdG levels were significantly higher in the groups of smokers, low BMI subjects, and low LDL-Chol subjects, while on the other hand, the urinary 8-OHdG levels were significantly lower in the low Hb groups.

In smokers, the urinary 8-OHdG levels were significantly higher in lean individuals, as compared with obese individuals. In addition, urinary 8-OHdG levels were significantly elevated in those with high BS. In non-smokers, the urinary 8-OHdG levels were significantly lower in the high LDL-Chol groups.

Table 3 shows the multiple regression analysis of the health examination factors associated with the urinary 8-OHdG levels. The urinary 8-OHdG levels were positively associated with smoking ($\beta = 0.128$, $P = .004$), Hb ($\beta = 0.157$, $P < .001$), and BS ($\beta = 0.112$, $P = .026$). On the other hand, the urinary 8-OHdG levels were negatively associated with LDL-Chol ($\beta = -0.136$, $P = .007$) and BMI ($\beta = -0.112$,

$P = .048$), independently from age, smoking status, alcohol consumption, exercise, and other health examination items. In smokers, positive associations were found between the urinary 8-OHdG levels and BS ($\beta = 0.228$, $P = .004$) and Hb ($\beta = 0.176$, $P = .017$), no association was found between the urinary 8-OHdG level and LDL-Chol ($\beta = -0.004$, $P = .612$), and a negative association was found between the urinary 8-OHdG level and BMI ($\beta = -0.203$, $P = .002$).

In non-smokers, a positive association was found between the urinary 8-OHdG level and Hb ($\beta = 0.143$, $P = .040$), no associations were found between the urinary 8-OHdG levels and BS ($\beta = 0.007$, $P = .918$) and BMI ($\beta = -0.068$, $P = .379$), and a negative association was found between the urinary 8-OHdG level and LDL-Chol ($\beta = -0.201$, $P = .003$).

The relationships between health examination results and urinary 8-OHdG levels in obese (BMI: >25 kg/m^2) subjects are shown in Table 4. High BS subjects have significantly higher urinary 8-OHdG levels, especially in smokers.

In terms of working environment and urinary 8-OHdG levels (Table 5), subjects exposed to organic solvents or hydrochloric acid had significantly higher urinary 8-OHdG levels, as compared with subjects with no exposure. Among them, 15 subjects had known urinary metabolite levels of organic solvents, but we did not compare the urinary 8-OHdG levels because all metabolites were Level-Ⅰ.

Table 6 shows the multiple regression analysis of the working environment associated with the urinary 8-OHdG levels. The urinary 8-OHdG levels were positively associated with shift work ($\beta = 0.103$, $P = .028$), and marginally positively associated with exposure to organic solvents or hydrochloric acid ($\beta = 0.092$, $P = .051$) and long working hours ($\beta = 0.064$, $P = .137$), independently from age, smoking status, alcohol consumption, exercise, and other health examination items. When analyzed by smoking status, no significant results were found.

4 | DISCUSSION

The levels of urinary 8-OHdG as an oxidative stress marker were related to lifestyle choices, medical examination results and work environments in this study. The urinary 8-OHdG levels were increased due to smoking, in both the univariate and multiple regression analyses. Other studies have likewise shown that the urinary 8-OHdG levels in smokers are higher than those in non-smokers.[7,8,20] In addition, the levels of 8-hydroxyguanine (8-OHGua), the oxide of a free guanine base, in leukocyte DNA[21] and saliva[22] reportedly rise after smoking.

In the present study, we evaluated the relationship between health examination results, work environment, and urinary 8-OHdG in non-smokers and smokers, and obtained some interesting findings.

Our results demonstrated that urinary 8-OHdG levels are positively associated with Hb. It is possible that iron deficiency, which is one cause of anemia, contributed to the

TABLE 5 Working environment and urinary 8-OHdG (N = 503)

		Total (N = 503)		Non-smoker (N = 290)		Smoker (N = 213)	
Factors	**Category**	**N**	**8-OHdG**	**N**	**8-OHdG**	**N**	**8-OHdG**
Fume exposure (self-reported)	No	273	3.40 (2.70-4.30)	163	3.20 (2.55-4.10)	110	3.70 (2.90-4.70)
	Yes	230	3.45 (2.80-4.40)	127	3.30 (2.65-4.10)	103	3.60 (2.80-4.80)
Fume levels in the work environment[a]	Control Class-Ⅰ	13	3.60 (3.30-4.00)	7	3.80 (3.05-4.00)	6	3.60 (2.90-3.65)
	Control Class-Ⅱ	41	3.55 (2.90-4.35)	21	3.50 (2.85-4.05)	20	3.60 (3.30-4.55)
Organic solvent or hydrochloric acid exposure (self-reported)	No	449	3.40 (2.70-4.30)	267	3.20 (2.60-4.00)	182	3.60 (2.80-4.70)
	Yes	54	3.75 (3.00-4.80)*	23	3.90 (3.20-4.35)*	31	3.70 (2.90-4.95)
Urinary metabolite levels of organic solvents[b]	Level-Ⅰ	15	3.40 (2.70-4.40)	8	3.40 (2.50-3.55)	7	3.95 (3.05-4.70)
Shift work	No	464	3.40 (2.70-4.30)	270	3.20 (2.60-4.10)	194	3.60 (2.80-4.70)
	Yes	39	4.00 (3.10-4.50)	20	4.00 (3.10-4.30)*	19	3.40 (3.10-5.10)
Sunlight exposure	No	314	3.40 (2.70-4.30)	188	3.20 (2.60-4.00)	126	3.60 (2.90-4.70)
	Yes	189	3.50 (2.70-4.50)	102	3.40 (2.60-4.10)	87	3.70 (2.80-4.85)
Long working hours	No	501	3.40 (2.70-4.30)	289	3.30 (2.60-4.10)	212	3.60 (2.80-4.70)
	Yes	2	5.05 (4.80-5.30)	1	4.80	1	5.30

Note: Urinary 8-hydroxy-2′-deoxyguanosine (8-OHdG: ng/mg creatinine) levels were median (25th quartile-75th quartile).

[a]Subjects with a known level of fume exposure in their working environment.

[b]Subjects with known levels of organic solvent metabolites in urine.

*$P < .05$, by Mann-Whitney test.

TABLE 6 Multiple regression analysis of (logarithmic 8-hydroxy-2′-deoxyguanosine [8-OHdG]) versus working environment

	Total[a] (N = 503, R^2 = 0.12, P < .001)				Non-smoker (N = 290, R^2 = 0.12, P = .020)				Smoker (N = 213, R^2 = 0.18, P = .004)			
Working environment	**Partial *r***	**SE**	***β***	***P***	**Partial *r***	**SE**	***β***	***P***	**Partial *r***	**SE**	***β***	***P***
Shift work	0.061	0.028	0.103	0.028	0.067	0.037	0.110	0.074	0.071	0.124	0.043	0.097
Organic solvents or hydrochloric acid exposure (self-reported)	0.048	0.024	0.092	0.051	0.053	0.038	0.094	0.161	0.035	0.076	0.034	0.298
Long working hours	0.163	0.110	0.064	0.137	0.160	0.154	0.061	0.302	0.159	0.066	0.159	0.319
Fume exposure (self-reported)	−0.015	0.016	−0.048	0.342	−0.006	0.022	−0.018	0.799	−0.030	0.025	0.009	0.906
Sunlight exposure	−0.002	0.016	−0.007	0.884	−0.006	0.021	−0.019	0.767	0.003	0.024	0.025	0.228

Note: Analyses were adjusted for age, alcohol consumption, aerobic exercise, health examination results, and working environment factors.

[a]Smoking status was entered into the multiple regression analysis model only when analyzing total subjects.

oxidative stress reduction. In a rat experimental system, the levels of superoxide dismutase (SOD) and glutathione (GSH) in renal cells were decreased, due to an increase in oxidative stress by iron administration.[23] Further investigations are needed to reveal the mechanism by which anemia causes a decrease in urinary 8-OHdG levels.

Overall, obese subjects had lower urinary 8-OHdG levels, consistent with previous reports.[8,10,20] In general, oxidative stress is increased in obesity.[24] On the other hand, several studies about the inverse relationship between BMI and urinary 8-OHdG have been reported.[25,26] In this study, the inverse relationship between BMI and urinary 8-OHdG was observed in the smokers. Weight loss is attributed to an increase in the basal metabolism. Consequently, reactive oxygen species are increased in intracellular mitochondria, and thereby, higher levels of oxidative stress were observed with leanness. Exposure to nicotine in cigarettes may increase energy-producing metabolism,[27] thus amplifying the reactive oxygen species production and contributing to the increased urinary 8-OHdG level. In connection with that, leanness is associated with the induction of cancers in the lung[28-30] and esophagus.[31] In the case of another oxidative stress biomarker, 8-isoprostane, although the urinary levels of 8-isoprostane (oxidative damage marker of phospholipids) were elevated in obese children,[32] they were not elevated in the adults (37-88 years, male, non-smoker) up to a BMI of 29.1 kg/m^2.[33] In the recent systematic review of urinary 8-isoprostane measurements,[34] no differences in the 8-isoprostane levels were detected between the BMI: ≤25 kg/m^2 group and BMI: >25 kg/m^2 group in the non-smokers, as determined by an accurate chromatographic analysis. Further studies of the relationship between BMI and oxidative stress, including the mechanism, are needed.

In non-smokers, the LDL-Chol and urinary 8-OHdG levels showed an inverse relationship, but this relationship was not found in smokers. In non-smokers, cholesterol may contribute to the stabilization of cell membranes and the absorption of vitamin E, an intracellular antioxidant, thereby contributing to the reduction of oxidative stress.[35] In smokers, on the other hand, cholesterol may be oxidized by smoking and thus not capable of performing these functions.[36] No association between plasma UA and urinary 8-OHdG was observed, although UA is generally believed to have an antioxidant effect. Therefore, the effect of plasma UA itself on urinary 8-OHdG was considered to be limited.

This study revealed that high BS subjects had higher urinary 8-OHdG levels, only in the smoking subjects. This may be due to the effect of smoking, which contributes to increased oxidative stress in pancreatic beta cells.[37] As a result, glucose intolerance may have occurred.

The urinary 8-OHdG levels were generally higher in shift workers, as compared with non-shift workers, in this analysis. In a preceding study of steel-manufacturing workers,

day-night shift workers tended to have higher urinary 8-OHdG levels than daytime workers.[8] Another study demonstrated that the levels of SOD and catalase were lower in shift workers.[38] This study did not distinguish between the day and night shift workers. The effect of the latest night shift on urinary 8-OHdG should be investigated in a future study.

Salimi et al reported that xylene-exposed leukocytes had increased reactive oxygen species.[39] Another study demonstrated that exposure to low doses of toluene causes DNA damage.[40] El-Metwaly et al reported that the SOD and GSH levels in lung tissues were reduced by the endotracheal infusion of hydrochloric acid, in a rat experiment.[41] In this study, the urinary 8-OHdG levels were higher in the subjects exposed to organic solvents or hydrochloric acid, perhaps because this exposure caused oxidative stress. The relationship between quantified indicators of toxic substance exposure and urinary 8-OHdG needs to be elucidated in the future because only a few subjects in this study had known levels of urinary metabolites of organic solvents.

Our results showed that the urinary 8-OHdG levels tended to be higher in subjects with long working hours and are consistent with previous studies.[10,42] Further investigations will be needed to clarify the detailed mechanism.

The point of this study was to investigate the effects of health examination results and working environment on urinary 8-OHdG levels. We observed that leanness and high BS were associated with high urinary 8-OHdG levels in smokers, whereas high LDL-Chol was associated with low urinary 8-OHdG levels. Smoking, leanness, and diabetes increase the risk of cardiovascular disease death, all-cancer death, and all-cause death[43]; therefore, the results of this study may contribute to quantifying the risks of these deaths.

We also revealed that shift work, organic solvent or hydrochloric acid exposure, and long working hours raised urinary 8-OHdG levels. Shift work and long working hours are reportedly risk factors for cardiovascular disease,[44,45] and thus, the results of this study may be useful in quantifying these risks. The relationship between health problems due to metal fume and organic solvent/acid exposure and urinary 8-OHdG should be further investigated by quantifying the exposure indices.

One way to use the results of urinary 8-OHdG for health management is to observe the changes in urinary 8-OHdG levels within an individual, because urinary 8-OHdG levels can vary from person to person.[7] Changes in urinary 8-OHdG levels can occur due to changes in lifestyle, health examination results, and the work environment. The urinary 8-OHdG level is a typical oxidative stress marker, but not a specific disease marker. In addition, each subject had a characteristic 8-OHdG level reflecting their personal lifestyle factors, such as stress status, exercise, sleep time, alcohol consumption, and diet.[7] Therefore, it is useful to measure 8-OHdG levels for each individual and evaluate the changes over a certain period, rather than trying to manage the levels within a normal range. The results obtained by analyzing an individual's 8-OHdG levels during a health examination would be helpful to improve their lifestyle and working environment, for the prevention of oxidative stress-related diseases and for the risk assessment of occupational and environmental exposure.

In conclusion, our results suggested that leanness or high blood sugar contributes to higher urinary 8-OHdG levels in smokers, while anemia or high plasma LDL-Chol contributes to lower urinary 8-OHdG levels in non-smokers. At the same time, our results suggested that a harmful working environment increases the urinary 8-OHdG levels. The measurement of urinary 8-OHdG levels during regular health examinations may provide significant value toward preventing lifestyle- and work-related diseases.

ACKNOWLEDGMENTS

This work was supported by JSPS KAKENHI (grant number JP17H01908).

We thank Dr Hiroshi Kasai for helpful advice with the 8-OHdG measurement and Ms Megumi Taketomi for assistance with the urine collection and survey questionnaire.

DISCLOSURE

Approval of the research protocol: The study was approved by the University of Occupational and Environmental Health Ethics Committee (No. H26-239). *Informed consent:* Written informed consent was obtained from all participants. *Registry and the registration no. of the study/trial:* N/A. *Animal studies:* N/A. *Conflict of interest:* N/A.

AUTHOR CONTRIBUTIONS

S. W., K. Y., and K. K. collected the urine samples and survey questionnaires. Y.-S. L. and Y. O. analyzed urinary 8-OHdG levels. S. W. statistically analyzed the data. S. W. and K. K. designed and critically discussed the study. All authors read and approved the final manuscript.

ORCID

Kazuaki Kawai https://orcid.org/0000-0002-4724-2395

REFERENCES

1. Reuter S, Gupta SC, Chaturvedi MM, Aggarwal BB. Oxidative stress, inflammation, and cancer: how are they linked? *Free Radic Biol Med*. 2010;49(11):1603-1616.
2. Liguori I, Russo G, Curcio F, et al. Oxidative stress, aging, and diseases. *Clin Interv Aging*. 2018;13:757-772.
3. Pelclová D, Fenclová Z, Kačer P, Kuzma M, Navrátil T, Lebedová J. Increased 8-isoprostane, a marker of oxidative stress in exhaled breath condensate in subjects with asbestos exposure. *Ind Health*. 2008;46(5):484-489.

4. Milić M, Neri M, Ceppi M, et al. DNA damage and genomic instability among workers formerly and currently exposed to asbestos. *Scand J Work Environ Health*. 2018;44(4):423-431.
5. Manke A, Wang L, Rojanasakul Y. Mechanisms of nanoparticle-induced oxidative stress and toxicity. *Biomed Res Int*. 2013;942916.
6. Kasai H, Nishimura S. Hydroxylation of deoxyguanosine at the C-8 position by ascorbic acid and other reducing agents. *Nucleic Acids Res*. 1984;12(4):2137-2145.
7. Li YS, Kawasaki Y, Watanabe S, Ootsuyama Y, Kasai H, Kawai K. Diurnal and day-to-day variation of urinary oxidative stress marker 8-hydroxy-2'-deoxyguanosine. *J Clin Biochem Nutr*. 2021;68(1):18-22.
8. Kasai H, Iwamoto-Tanaka N, Miyamoto T, et al. Life style and urinary 8-hydroxydeoxyguanosine, a marker of oxidative DNA damage: effects of exercise, working conditions, meat intake, body mass index, and smoking. *Jpn J Cancer Res*. 2001;92(1):9-15.
9. Kawasaki Y, Li YS, Watanabe S, Ootsuyama Y, Kawai K. Urinary biomarkers for secondhand smoke and heated tobacco products exposure. *J Clin Biochem Nutr*, in press.
10. Pan CH, Chan CC, Wu KY. Effects on Chinese restaurant workers of exposure to cooking oil fumes: a cautionary note on urinary 8-hydroxy-2'-deoxyguanosine. *Cancer Epidemiol Biomarkers Prev*. 2008;17(12):3351-3357.
11. Irie M, Tamae K, Iwamoto-Tanaka N, Kasai H. Occupational and lifestyle factors and urinary 8-hydroxydeoxyguanosine. *Cancer Sci*. 2005;96(9):600-606.
12. Hara M, Nishida Y, Shimanoe C, et al. Intensity-specific effect of physical activity on urinary levels of 8-hydroxydeoxyguanosine in middle-aged Japanese. *Cancer Sci*. 2016;107(11):1653-1659.
13. Kawasaki Y, Li Y-S, Ootsuyama Y, Nagata K, Yamato H, Kawai K. Effects of smoking cessation on biological monitoring markers in urine. *Genes and Environ*. 2020;42:26.
14. Rössner S. Obesity: the disease of the twenty-first century. *Int J Obes Relat Metab Disord*. 2002;26(Suppl 4):S2-S4.
15. Ames BN, Cathcart R, Schwiers E, Hochstein P. Uric acid provides an antioxidant defense in humans against oxidant- and radical-caused aging and cancer: a hypothesis. *Proc Natl Acad Sci USA*. 1981;78(11):6858-6862.
16. Waring WS, Webb DJ, Maxwell SR. Systemic uric acid administration increases serum antioxidant capacity in healthy volunteers. *J Cardiovasc Pharmacol*. 2001;38(3):365-371.
17. Japan Organization of Occupational Health and Safety. https://www.research.johas.go.jp/sanchu/seijo.html. Accessed December 16, 2020 (in Japanese).
18. Kanda Y. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. *Bone Marrow Transplant*. 2013;48(3):452-458.
19. Japan Society of Ningen Dock. Criteria category; 2018. https://www.ningen-dock.jp/wp/wp-content/uploads/2018/06/Criteria-category.pdf. Published April 2018. Accessed November 16, 2020.
20. Loft S, Vistisen K, Ewertz M, Tjønneland A, Overvad K, Poulsen HE. Oxidative DNA damage estimated by 8-hydroxydeoxyguanosine excretion in humans: influence of smoking, gender and body mass index. *Carcinogenesis*. 1992;3(12):2241-2247.
21. Asami S, Hirano T, Yamaguchi R, Tomioka Y, Itoh H, Kasai H. Increase of a type of oxidative DNA damage, 8-hydroxyguanine, and its repair activity in human leukocytes by cigarette smoking. *Cancer Res*. 1996;56(11):2546-2549.
22. Watanabe S, Kawasaki Y, Kawai K. Salivary 8-hydroxyguanine as a lifestyle-related oxidative stress biomarker in workers. *J Clin Biochem Nutr*. 2020;66(1):57-61.
23. Ige AO, Ongele FA, Adele BO, Emediong IE, Odetola AO, Adewoye EO. Pathophysiology of iron overload-induced renal injury and dysfunction: Roles of renal oxidative stress and systemic inflammatory mediators. *Pathophysiology*. 2019;26(2):175-180.
24. Matsuda M, Shimomura I. Increased oxidative stress in obesity: implications for metabolic syndrome, diabetes, hypertension, dyslipidemia, atherosclerosis, and cancer. *Obes Res Clin Pract*. 2013;7(5):e330-e341.
25. Mizoue T, Kasai H, Kubo T, Tokunaga S. Leanness, smoking, and enhanced oxidative DNA damage. *Cancer Epidemiol Biomarkers Prev*. 2006;15(3):582-585.
26. Mizoue T, Tokunaga S, Kasai H, Kawai K, Sato M, Kubo T. Body mass index and oxidative DNA damage: a longitudinal study. *Cancer Sci*. 2007;98(8):1254-1258.
27. Blauw LL, Boon MR, Rosendaal FR, et al. Smoking is associated with increased resting energy expenditure in the general population: the NEO study. *Metabolism*. 2015;64(11):1548-1555.
28. Knekt P, Heliövaara M, Rissanen A, et al. Leanness and lung-cancer risk. *Int J Cancer*. 1991;49:208-213.
29. Kabat GC, Wynder EL. Body mass index and lung cancer risk. *Am J Epidemiol*. 1992;135:769-774.
30. Kark JD, Yaari S, Rasooly I, Goldbourt U. Are lean smokers at increased risk of lung cancer? The Israel civil servant cancer study. *Arch Intern Med*. 1995;155:2409-2416.
31. Gallus S, La Vecchia C, Levi F, Simonato L, Dal Maso L, Franceschi S. Leanness and squamous cell oesophageal cancer. *Ann Oncol*. 2001;12:975-979.
32. Selvaraju V, Ayine P, Fadamiro M, Babu JR, Brown M, Geetha T. Urinary biomarkers of inflammation and oxidative stress are elevated in obese children and correlate with a marker of endothelial dysfunction. *Oxid Med Cell Longev*. 2019;2019:1-10.
33. Keaney JF, Larson MG, Vasan RS, et al. Obesity and systemic oxidative stress: clinical correlates of oxidative stress in the Framingham Study. *Arterioscler Thromb Vasc Biol*. 2003;23(3):434-439.
34. Graille M, Wild P, Sauvain JJ, Hemmendinger M, Guseva Canu I, Hopf NB. Urinary 8-isoprostane as a biomarker for oxidative stress. A systematic review and meta-analysis. *Toxicol Lett*. 2020;328:19-27.
35. Kikuchi H, Nanri A, Hori AI, et al. Lower serum levels of total cholesterol are associated with higher urinary levels of 8-hydroxydeoxyguanosine. *Nutr Metab*. 2013;10:59.
36. Linna MS, Ahotupa M, Irjala K, et al. Smoking and low serum testosterone associates with high concentration of oxidized LDL. *Ann Med*. 2008;40(8):634-640.
37. Tong X, Chaudhry Z, Lee C-C, et al. Cigarette smoke exposure impairs β-cell function through activation of oxidative stress and ceramide accumulation. *Mol Metab*. 2020;37:100975.
38. Teixeira KRC, dos Santos CP, de Medeiros LA, et al. Night workers have lower levels of antioxidant defenses and higher levels of oxidative stress damage when compared to day workers. *Sci Rep*. 2019;9(1). https://doi.org/10.1038/s41598-019-40989-6
39. Salimi A, Talatappe BS, Pourahmad J. Xylene induces oxidative stress and mitochondria damage in isolated human lymphocytes. *Toxicol Res*. 2017;33(3):233-238.
40. Moro AM, Brucker N, Charão M, et al. Evaluation of genotoxicity and oxidative damage in painters exposed to low levels of toluene. *Mutat Res*. 2012;746(1):42-48.

41. El-Metwaly S, El-Senduny FF, El-Demerdash RS, Abdel-Aziz AF. Mesenchymal stem cells alleviate hydrochloric acid-induced lung injury through suppression of inflammation, oxidative stress and apoptosis in comparison to moxifloxacin and sildenafil. *Heliyon*. 2019;5(12):e02710.
42. Irie M, Asami S, Nagata S, Ikeda M, Miyata M, Kasai H. Psychosocial factors as a potential trigger of oxidative DNA damage in human leukocytes. *Jpn J Cancer Res*. 2001;92(3):367-376.
43. Yano Y, Kario K, Ishikawa S, et al. Associations between diabetes, leanness, and the risk of death in the Japanese general population: the Jichi Medical School Cohort Study. *Diabetes Care*. 2013;36(5):1186-1192.
44. Torquati L, Mielke GI, Brown WJ, Kolbe-Alexander T. Shift work and the risk of cardiovascular disease. A systematic review and meta-analysis including dose-response relationship. *Scand J Work Environ Health*. 2018;44(3):229-238.
45. Kivimäki M, Jokela M, Nyberg ST, et al. Long working hours and risk of coronary heart disease and stroke: a systematic review and meta-analysis of published and unpublished data for 603,838 individuals. *Lancet*. 2015;386(10005):1739-1746.

How to cite this article: Watanabe S, Li Y-S, Kawasaki Y, Ootsuyama Y, Kawai K. Health examination results and work environment factors affecting urinary 8-hydroxy-2′-deoxyguanosine levels. *J Occup Health*. 2021;63:e12210. https://doi.org/10.1002/1348-9585.12210