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Psychosocial Factors as a Potential Trigger of Oxidative DNA Damage in Human Leukocytes

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Although numerous studies have been carried out on the stress-cancer linkage, the results are still inconclusive. One of the useful, but rarely applied, methods to assess this linkage is to examine the relationship between psychosocial stress and cancer-predisposing genetic alterations simultaneously. We investigated whether various psychosocial factors can be associated with the levels of 8-hydroxydeoxyguanosine (8-OH-dG), a biomarker of cancer-related oxidative DNA damage, in peripheral blood leukocytes in 362 healthy workers (276 males and 86 females). After adjustments for age, body mass index, cigarette smoking, and alcohol use, female subjects showed positive relationships between the amount of 8-OH-dG and the Tension-Anxiety, Depression-Rejection, Anger-Hostility, Fatigue, and Confusion scores of the Profile of Mood States, respectively. The levels of 8-OH-dG also increased reliably in the female subjects who had poor stress-coping behaviors, particularly wishful thinking strategy, in the NIOSH general job stress instrument. There were positive relationships of the 8-OH-dG levels to average working hours, a self-blame coping strategy, and recent loss of a close family member in male subjects. These findings in a nonclinical sample of healthy adults not only provide evidence of a stress-cancer linkage, but also suggest possible sex differences in the mechanisms of stress-related cancer initiation.

Key words: 8-Hydroxydeoxyguanosine — Stress coping — Life event — Working hours — Sex difference

The association between cancer risk and several psychosocial factors, such as emotions, stress-coping behaviors, personality traits, and life events still remains an unsolved question of general interest. Large numbers of epidemiological and experimental studies have been carried out to identify the possible relationship; however, inconsistent and often contradictory findings have been produced.[1–6] In general, the influence of psychological factors has been more convincingly demonstrated for cancer progression than cancer initiation.[5] Laboratory research with animals has also suggested that stress affects cancer progression, but has no effect on cancer initiation.[6] To investigate whether psychosocial factors are associated with cancer initiation, their effects on cancer cell transformation need to be clarified. Nevertheless, genetic alterations, which are important in the pathogenesis of cancer, have scarcely been investigated in relation to psychosocial factors. Very limited issues, such as poorer repair of DNA damage, an increase of sister chromatid exchange and alterations in apoptosis, have been reported to be linked causatively with stress.[4] In the present study, we examined the relationship between psychosocial factors and cancer-predisposing genetic alterations simultaneously in order to assess the stress-cancer linkage, particularly with respect to cancer initiation.

Reactive oxygen species (ROSs) such as superoxides, hydroxyl radicals, and hydrogen peroxide are produced in the ordinary course of human life.[7] The resultant oxidative stress is known to induce nuclear DNA injury.[7] One of the base modifications due to oxidative stress involves 8-hydroxydeoxyguanosine (8-OH-dG, 7,8-dihydro-8-oxodeoxyguanosine).[8] Such DNA damage has been confirmed to cause misreading of the DNA template and subsequent point mutations, G:C to T:A transversions,[9] which are frequently observed in the *p53* gene of lung and liver cancers.[10] Within the spectrum of damage to nuclear DNA produced by ROSs, 8-OH-dG is considered to represent the most useful marker, since it can be detected with high sensitivity.[11] The formation of 8-OH-dG following exposure to various carcinogens and other hazardous substances has been reported in many papers,[7, 12, 13] and 8-OH-dG is therefore regarded as one of the corroborative markers involved in carcinogenesis.

Psychological stress has been reported to raise the 8-OH-dG levels in rat liver.[14] We recently demonstrated that the formation of 8-OH-dG in renal tissues increased in response to a neutral stimulus using a conditioned taste

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aversion paradigm.[15] To our knowledge, however, no definitive evidence for such changes, including oxidative DNA damage other than 8-OH-dG, has been obtained in human subjects. Also, no reports on 8-OH-dG in relation to stress-coping behaviors and social support have yet been published. If psychological factors actually do promote oxidative damage to nuclear DNA in human subjects, they could be relevant to the enhancement of susceptibility to cancer.

The present study was undertaken to investigate the relationships between the levels of 8-OH-dG in peripheral blood leukocytes and various psychosocial factors that may be associated with this oxidative DNA damage, such as emotions, stress-coping behaviors, social support, working conditions, and lifestyle factors, in workers during usual stressful conditions. The relationships were examined adjusting for age,[16] body mass index (BMI),[17] and cigarette smoking[17, 18] and alcohol drinking habits,[19] because these factors have been reported to have associations with carcinogenicity and the formation of 8-OH-dG.

MATERIALS AND METHODS

Design and sample A total of 362 workers (276 males and 86 females) were recruited as participants for this study from two occupational settings. They ranged in age from 20 to 64 years (41.3±11.5, mean±SD) and consisted of 211 white-collar and 149 blue-collar workers (2 unknown). There were no significant differences in age and BMI between male and female subjects (Table I). There was a significantly higher ratio of male current smokers or drinkers than female counterparts (χ^2=66.9, P<0.01; χ^2= 37.0, P<0.01, respectively). Twenty-three out of 149 blue-collar workers were engaged in the handling of toluene, xylene, and normal-hexane, and an additional 27 were engaged in arc welding operations. After obtaining a signed informed consent form from each subject, an examination was performed with a self-rating questionnaire under the situation of a usual labor load during two moderately busy periods, July of 1997 and August of 1998. The items included in the questionnaire were: demographic factors; type of work; present, past, and family histories of illness; alcohol use; cigarette smoking; the 65-item Profile of Mood States (POMS)[20]; stress-coping items and social support items from the NIOSH general job stress instrument[21]; and recent death of a spouse, parent, sibling, or child. The POMS contains 6 factorially derived subscales (Tension-Anxiety, Depression-Rejection, Anger-Hostility, Fatigue, Confusion and Vigor) and is widely used in the study of psychological aspects of cancer.[22] Stress-coping items in the NIOSH general job stress instrument measure 6 dimensions: planning, "Make a plan to solve the problem and stick to it," neglect, "Go on as if nothing happened," self-blame, "Feel responsible for the problems," wishful thinking, "Daydream or wish that you could change the problem," consultation, "Talk to your boss or co-workers about the problem," and involvement, "Become more involved in activities outside of work." Social support consisted of 12 items which reflect overall support from a supervisor, other people at work, or other people excluding work. All of the indices indicate higher negative moods, except vigor, or poorer coping patterns and social support as the scores become higher. The subjects excluded from the present study included those with cancer, chronic hepatitis, various kinds of autoimmune diseases, diabetes mellitus, atopic dermatitis, and other diseases which were reported to raise the 8-OH-dG level.[12, 23] Subjects with a fever or common cold at the time of investigation were also excluded. Those subjects who had been diagnosed with psychiatric diseases, such as depression and schizophrenia, were not included in the present study. Smokers were instructed not to smoke after rising in the morning on the day of blood collection, and 12 smokers were omitted from the analysis as they had smoked after rising. At the times of obtaining the questionnaire, fasting blood samples (7 ml each) were collected early in the morning on weekdays. The blood

Table I. Comparisons of 8-OH-dG Levels and Characteristics between Male and Female Subjects

Variables	Males (n=276)	Females (n=86)
8-OH-dG (/10^5 dG)		
Mean±SD (Range)	0.36±0.20 (0.08–1.27)	0.36±0.24 (0.07–1.88)
Age (years)		
Mean±SD (Range)	41.6±11.7 (20–64)	40.4±10.8 (21–56)
Body mass index (kg/m^2)		
Mean±SD (Range)	23.1±2.8 (16.9–33.1)	21.6±3.0 (17.2–32.4)
Type of work		
White collar	155 (56.1)	56 (65.1)
Blue collar	120 (43.5)	29 (33.7)
Unknown	1 (0.4)	1 (1.2)
Cigarette smoking		
Current	170 (61.6)	15 (17.4)
Former	35 (12.7)	8 (9.3)
Never	70 (25.3)	63 (73.3)
Unknown	1 (0.4)	0 (0.0)
Alcohol drinking		
Current	217 (78.6)	40 (46.5)
Former	7 (2.6)	2 (2.3)
Never	52 (18.8)	43 (50.0)
Unknown	0 (0.0)	1 (1.2)

Numbers in table are subjects (%) with the relevant characteristic.

samples were immediately centrifuged (600*g* for 10 min), and the buffy coats were drawn off and stored at −80°C.

Chemicals The DNA Extractor WB Kit, standard deoxyguanosine, and standard 8-OH-dG were purchased from Wako Biochemicals (Osaka). Nuclease P_1 (from *Penicillium citrinum*) was from Yamasa Co. (Chiba). The acid phosphatase (type XA, P-1435) was from Sigma Chemical Co. (St. Louis, MO). Other chemicals were of the highest purity commercially available. Milli-Q water was used in all experiments.

Determination of 8-OH-dG levels The amount of 8-OH-dG was measured according to the method of Asami *et al.*[18] with minor modifications. In brief, the nuclear DNA from peripheral leukocytes was extracted using the DNA Extractor WB Kit (Wako Biochemicals). The samples were digested with nuclease P_1 (0.8 units, Yamasa Co.) and acid phosphatase (1 unit, Sigma Chemical Co.) in a solution of 1 m*M* EDTA, 10 m*M* sodium acetate (pH 4.5). This mixture was incubated at 37°C for 30 min, and then the iron exchange resin Muromac (Muromachi Kagaku Kogyo, Tokyo) was added to remove the NaI, which is harmful to an electrochemical detector (ECD). The mixture was centrifuged at 16 350*g* for 5 min. The supernatant was transferred to an Ultrafree-Probind filter (Millipore Co., Bedford, MA), then centrifuged at 7270*g* for 2 min. The filtered deoxynucleoside was injected onto a high-performance liquid chromatography column (Beckman; Ultrasphere-ODS; 5 μm, 4.6×250 nm; elution, 10 m*M* NaH_2PO_4 containing 8% methanol) coupled to the ECD (ESA Coulochem II; detector 1, 0.15 V; detector 2, 0.30 V). The 8-OH-dG was detected with the ECD, and deoxyguanosine (dG) was detected by ultraviolet absorbance measurement at 290 nm. Standard samples of dG (0.5 mg/ml) and 8-OH-dG (5 ng/ml) solutions (Wako Biochemicals) were used for comparison with the samples of the subjects. The titer of 8-OH-dG is shown as the number per 10^5 guanine residues.

Statistical analysis The SAS software[24] was used for all statistical analyses. An analysis of covariance (ANCOVA), including age, BMI, cigarette smoking, and alcohol use as covariates, was conducted for the uncontinuous variables. A regression analysis was also done with the influence of the four factors adjusted for continuous variables and several uncontinuous variables, which could be examined for the existence of a trend. We used the logarithms of 8-OH-dG to stabilize the variance of the dependent variable in both the covariance and regression analyses. Student's *t* test was used to determine if significant differences existed according to age, BMI and the 8-OH-dG levels between male and female subjects or bereaved and non-bereaved subjects. A χ^2 test was used to compare the smoking or drinking states between male and female subjects or bereaved and non-bereaved subjects. The Spearman correlation coefficient was calculated to investigate the relationships between the average number of working hours per weekday and the 8-OH-dG levels in white- and blue-collar male workers. A *P* value of <0.05 (2 tailed) was accepted as indicating statistical significance.

RESULTS

The 8-OH-dG levels in the subjects of this study ranged from 0.07 to 1.88, with a mean of 0.36±0.21 (8-OH-dG/10^5 dG, mean±SD). There were no significant differences in the levels of 8-OH-dG between male and female subjects (Table I). The 8-OH-dG levels in each sex showed a skewed distribution, in which only a few cases were observed at higher levels and more were found at lower levels. The 8-OH-dG levels of the males showed 1.90 skewness and 4.62 kurtosis while those of females were 3.61 and 19.90, respectively. These results necessitated a logarithmic conversion of the 8-OH-dG levels for analysis. After the conversion, the 8-OH-dG levels showed 0.45 skewness and 0.08 kurtosis in males and 0.29 skewness and 1.25 kurtosis in females, which demonstrated a nearly normal distribution.

There were no significant relations between any of the four factors, i.e., age, BMI, and cigarette smoking and alcohol drinking habits, and the 8-OH-dG levels in all subjects or when divided into male and female subjects. A regression analysis and/or ANCOVA were conducted separately in males and females, as the relationships between the 8-OH-dG levels and the question items differed in the two sexes.

Table II shows the relationships between the psychosocial variables of the continuous data and the 8-OH-dG levels. Even after adjustments for age, BMI, and cigarette smoking and alcohol drinking habits, there were significant relationships between the 8-OH-dG levels and the scores of the POMS items, except for Vigor in female subjects. In contrast, male subjects showed no significant relationships between the two factors. These results showed different relationships between the scores of the POMS items and the 8-OH-dG levels in both sexes, but the correlations among the scores of the POMS items were generally high for each sex and were quite similar between them. There were no significant differences between both sexes in the scores of the POMS items.

The levels of 8-OH-dG in female subjects showed a significantly positive association with a total score that is cumulative in the direction of worsening stress-coping behaviors (Table II). Among 6 coping strategies in the NIOSH general job stress instrument, the self-blame about occupational problems was significantly associated with the 8-OH-dG levels in the male subjects (Table III). Female subjects showed a significantly positive relationship between the wishful thinking to change occupational problems and the levels of 8-OH-dG (Table III).

Table II. Relationships between Stress-related Factors and the 8-OH-dG Levels in Leukocyte DNA in Male and Female Workers

Factors	Males			Females		
	No.	Regression coefficient[b] (95% CI)	*P*	No.	Regression coefficient[b] (95% CI)	*P*
Profile of mood states (POMS)						
Tension-Anxiety	274	−0.003 (−0.014, 0.008)	0.604	85	0.032 (0.010, 0.053)	0.005
Depression-Rejection	274	−0.005 (−0.012, 0.001)	0.085	85	0.023 (0.011, 0.035)	0.000
Anger-Hostility	274	−0.007 (−0.014, 0.001)	0.075	85	0.021 (0.006, 0.035)	0.006
Vigor	274	0.002 (−0.008, 0.011)	0.765	85	0.009 (−0.010, 0.028)	0.344
Fatigue	274	−0.005 (−0.015, 0.006)	0.376	85	0.031 (0.010, 0.052)	0.005
Confusion	274	−0.010 (−0.024, 0.004)	0.174	85	0.040 (0.012, 0.067)	0.007
Stress-coping strategies[a]	242	0.009 (−0.013, 0.031)	0.416	82	0.052 (0.002, 0.103)	0.047
Social support[a]	245	0.001 (−0.008, 0.010)	0.814	84	−0.016 (−0.035, 0.003)	0.101
Working hours per weekday	255	0.061 (0.021, 0.102)	0.003	81	0.020 (−0.119, 0.158)	0.780

Data shown are logarithmic values of 8-OH-dG.
a) The items used are from the NIOSH general job stress instrument.
b) Regression coefficients are adjusted for age, BMI, cigarette smoking, and alcohol use.

Table III. Relationships between Stress-coping Strategies and the Levels of 8-OH-dG ($/10^5$ dG) in Leukocyte DNA in Male and Female Workers

Stress-coping strategies[a]	Rarely		Occasionally		Sometimes		Fairly often		Very often		Regression coefficient[b] (95% CI)	*P*
	No.	Mean (SE)	No.	Mean (SE)	No.	Mean (SE)	No.	Mean (SE)	No.	Mean (SE)		
Planning												
Males (*n*=273)	14	0.42 (0.08)	51	0.34 (0.02)	103	0.37 (0.02)	63	0.37 (0.03)	42	0.35 (0.03)	0.002 (−0.050, 0.055)	0.940
Females (*n*=85)	7	0.49 (0.10)	10	0.26 (0.04)	27	0.35 (0.04)	19	0.42 (0.09)	22	0.33 (0.02)	−0.014 (−0.111, 0.083)	0.774
Neglect												
Males (*n*=274)	67	0.39 (0.03)	96	0.37 (0.02)	88	0.35 (0.02)	16	0.36 (0.06)	7	0.27 (0.11)	−0.050 (−0.107, 0.008)	0.094
Females (*n*=85)	28	0.35 (0.04)	24	0.35 (0.03)	23	0.30 (0.03)	7	0.58 (0.22)	3	0.50 (0.24)	0.081 (−0.023, 0.185)	0.133
Self-blame												
Males (*n*=276)	10	0.37 (0.05)	31	0.33 (0.04)	74	0.32 (0.02)	94	0.41 (0.02)	65	0.37 (0.02)	0.056 (0.002, 0.109)	0.043
Females (*n*=86)	8	0.52 (0.20)	22	0.34 (0.03)	26	0.32 (0.03)	16	0.42 (0.07)	13	0.32 (0.04)	−0.031 (−0.129, 0.067)	0.535
Wishful thinking												
Males (*n*=276)	64	0.35 (0.02)	55	0.38 (0.03)	102	0.35 (0.02)	40	0.42 (0.04)	12	0.36 (0.03)	0.036 (−0.014, 0.087)	0.162
Females (*n*=85)	41	0.28 (0.02)	16	0.36 (0.03)	18	0.47 (0.10)	6	0.52 (0.11)	4	0.41 (0.06)	0.162 (0.066, 0.258)	0.001
Consultation												
Males (*n*=274)	26	0.39 (0.05)	62	0.38 (0.03)	96	0.37 (0.02)	63	0.35 (0.03)	27	0.35 (0.03)	−0.027 (−0.079, 0.025)	0.309
Females (*n*=86)	7	0.33 (0.03)	12	0.36 (0.04)	30	0.35 (0.04)	15	0.35 (0.05)	21	0.40 (0.08)	0.009 (−0.088, 0.106)	0.861
Involvement												
Males (*n*=274)	39	0.35 (0.03)	59	0.37 (0.03)	106	0.36 (0.02)	54	0.36 (0.02)	16	0.41 (0.07)	0.033 (−0.020, 0.087)	0.222
Females (*n*=85)	32	0.31 (0.02)	17	0.44 (0.10)	25	0.38 (0.05)	7	0.33 (0.04)	4	0.40 (0.08)	0.059 (−0.047, 0.164)	0.281

a) The items used are from the NIOSH general job stress instrument.
b) Regression coefficients using logarithmic values of 8-OH-dG are adjusted for age, BMI, cigarette smoking, and alcohol use.

There were 15 male subjects who had experienced the death of a close family member, such as a spouse (4), parent (10), or sibling (1), within one year of the present study. On the other hand, only 3 female subjects had lost one of their parents within one year. No differences were found regarding demographic and lifestyle characteristics between bereaved and non-bereaved subjects, irrespective of gender (Table IV). The male subjects who had experienced the death in the past year showed significantly higher values of 8-OH-dG, as compared with those who

Table IV. Comparisons of 8-OH-dG Levels and Characteristics between Bereaved Subjects due to the Death of a Close Family Member within One Year and Non-bereaved Subjects

Variables	Males		Females	
	Bereaved (n=15)	Non-bereaved (n=261)	Bereaved (n=3)	Non-bereaved (n=83)
8-OH-dG (/10^5 dG)				
Mean±SD (Range)	0.49±0.30 (0.17−1.18)	0.36±0.01 (0.08−1.27)	0.33±0.08 (0.27−0.42)	0.36±0.03 (0.07−1.88)
Age (years)				
Mean±SD (Range)	46.9±13.9 (20−63)	41.3±11.5 (22−64)	48.7±1.5 (47−50)	40.1±10.9 (21−56)
Body mass index (kg/m^2)				
Mean±SD (Range)	22.7±3.5 (16.9−33.1)	23.1±2.7 (17.6−29.4)	25.1±6.4 (20.6−32.4)	21.4±2.8 (17.2−32.0)
Type of work				
White collar	7 (46.7)	148 (56.7)	0 (0.0)	56 (67.5)
Blue collar	8 (53.3)	112 (42.9)	3 (100.0)	26 (31.3)
Unknown	0 (0.0)	1 (0.4)	0 (0.0)	1 (1.2)
Cigarette smoking				
Current	7 (46.7)	163 (62.4)	0 (0.0)	15 (18.1)
Former	3 (20.0)	32 (12.3)	0 (0.0)	8 (9.6)
Never	5 (33.3)	65 (24.9)	3 (100.0)	60 (72.3)
Unknown	0 (0.0)	1 (0.4)	0 (0.0)	0 (0.0)
Alcohol drinking				
Current	13 (86.7)	204 (78.2)	1 (33.3)	39 (47.0)
Former	0 (0.0)	7 (2.7)	0 (0.0)	2 (2.4)
Never	2 (13.3)	50 (19.1)	2 (66.7)	41 (49.4)
Unknown	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)

Numbers in table are subjects (%) with the relevant characteristic.

had not [F(1, 262)=4.21, P=0.041, by ANCOVA, Table IV]. The male subjects bereaved within 3 years still showed significantly higher values of 8-OH-dG than non-bereaved subjects [0.41±0.22 (n=52) vs. 0.35±0.20 (n=224), F(1, 262)=5.29, P=0.022, by ANCOVA]; however, there were no significant differences in the 8-OH-dG levels between the male subjects who had experienced the bereavement 1−2 years previously and the male subjects who had never had such an experience or had had it more than 2 years previously.

There was a significant positive relationship between the average number of working hours and the levels of 8-OH-dG in male subjects (Table II). White-collar male workers showed significantly higher levels of 8-OH-dG, when compared with those of blue-collar workers [0.38±0.22 (n=156) vs. 0.34±0.18 (n=120), F(1, 261)=4.78, P=0.030, by ANCOVA]. The Spearman correlation coefficient between the average number of working hours per weekday and the 8-OH-dG values in white-collar male workers was higher than that of blue-collar ones [r=0.181 (P=0.030) vs. r=0.077 (P=0.426)]. The exposure to organic solvents and arc welding operations in the workplace was not associated with the levels of 8-OH-dG. Twenty users of organic solvents were examined for the amounts of hippuric acid, methylhippuric acid, and 2,5-hexanedione in their urine at their periodic health examinations, performed three months after the present research, but none of these products showed a significant relationship with the 8-OH-dG levels.

DISCUSSION

We found that negative or unfavorable emotions and inadequate stress-coping strategies, particularly wishful thinking, are positively related to the 8-OH-dG levels in female subjects. In turn, there are positive relationships of the 8-OH-dG levels to average working hours, a self-blame coping strategy, and recent loss of a close family member in the male counterparts. These results were almost unchanged when the data were unadjusted for age, BMI, cigarette smoking, and alcohol use.

It is noteworthy that negative emotions were associated with potential carcinogenicity due to oxidative DNA damage in female subjects, as compared with other various well-known risk factors.[16−19] Among several kinds of psychological factors, depression has often been reported to have an important role in cancer initiation.[25, 26] In agreement with such findings, the highest association was found between the Depression-Rejection score of the POMS and the 8-OH-dG levels in female subjects. This study was conducted in a nonclinical sample of healthy adults, so that another investigation incorporating patients with clini-

cal depression is required in order to examine the findings further.

More evidence for a relationship between psychological stress and the synthesis of ROSs has recently been obtained. For example, superoxide production in peripheral blood neutrophils increased in association with examination stress.[27] Synthesis of lipid peroxidation in several organs occurred under acute cold-immobilization stress.[28] Furthermore, it was suggested that the practice of stress reduction contributed to diminishing lipid peroxide levels.[29] These observations seem consistent with a possible association between negative emotions and oxidative DNA damage. The underlying mechanism linking the two factors is not yet clearly understood. It was suggested that psychological stress stimulates the hypothalamic-pituitary-adrenal (HPA) axis and this avenue increases the formation of ROSs relevant to cytotoxicity[30]; however, this possibility has not been tested. Stress has been reported to increase the numbers and percentages of blood neutrophils,[31] and this may play a role as another possible mechanism, since examination stress was found to elevate superoxide production in blood neutrophils.[27] Psychological stress has been reported to decrease DNA repair in lymphocytes exposed to X-irradiation in psychiatric inpatients and to inhibit apoptosis in leukocytes exposed to γ-irradiation in medical students.[4] These findings appear to result in the persistence of oxidative DNA damage, and this may also contribute to the association between psychological stress and oxidative DNA damage.

In the present study, we found sex differences in the relationships between negative emotions and the 8-OH-dG levels. Sex differences have received little attention in the previous, particularly prospective, studies which focused on the stress-cancer linkage, since the study population was generally only male or only female.[3] In earlier studies, it was reported that female subjects who had a low degree of emotional control showed a higher cancer incidence when compared with the expected number, whereas male subjects did not show such a tendency.[32] In contrast, no sex difference was observed in the relationship between depressive mood and cancer incidence in older persons.[26] Our results seem to support the earlier findings, although the methods of investigation differ. Females have generally been found to report psychological distress more frequently than males,[33] and this may result in the associations. However, we could not find any significant difference between the sexes in the scores of the POMS items. Since the reason for the desparate results in both sexes is unclear, further investigations are needed in order to confirm our findings.

Sex differences in the relationships between the psychosocial factors and the 8-OH-dG levels were also recognized in the stress-coping behaviors. In a regression analysis, the 8-OH-dG levels in the male subjects significantly rose with more self-blame about occupational problems, while those in the female subjects were significantly higher with more wishful thinking to change problems. A positive association was also found between the inadequacy of total coping strategies and the levels of 8-OH-dG in female subjects. In both sexes, however, no significant relation was observed between the 8-OH-dG levels and the total score of social support, or the individual items. Emotion-focused coping strategies, such as self-blame, expression of emotions, and wishful thinking, were reported to be associated with various psychosomatic symptoms.[34] In particular, self-blame is often discussed in patients with cancer.[35] Self-blame and wishful thinking were considered to have a stronger influence on carcinogenicity than were the other coping skills in the present study.

With regard to work, the average number of working hours per weekday in male subjects was significantly associated with the 8-OH-dG levels. Their 8-OH-dG levels were higher with longer working hours. Our findings are consistent with the results of Watabe and co-workers,[36] who reported that long working hours were a risk factor for stomach cancer. However, this does not necessarily indicate that cumulative physical tasks cause oxidative DNA damage, since white-collar male workers had higher levels of 8-OH-dG and a higher correlation coefficient between the average number of working hours and the 8-OH-dG values than blue-collar workers. The exposure to organic solvents and arc welding operations in the workplace was unrelated to the levels of 8-OH-dG in the present study, possibly due to protection systems. It is therefore suggested that work-associated stress, other than physical and environmental working conditions, has a stronger influence on oxidative DNA damage. There have been some other reports on the impact of work stress and psychosocial factors in the workplace on cancer development. For example, Persky *et al.*[25] identified depression as a prognostic indicator of cancer mortality in a middle-aged male cohort of a case-controlled epidemiologic study of Western Electric Company workers. Spiegelman and Wegman[37] showed that jobs with high demand and low control increased the risk of colon cancer in male workers with high exposure to solvents, abrasives, and fuel oil. In addition, Jahn and co-workers[38] reported that the job-changing histories of male workers were related to the causes of lung cancer. These reports suggest that high work stress is associated with cancer development. Our findings seem to substantiate these previous positive findings from a genetic viewpoint.

As for life events, the male subjects who had experienced the death of a close family member within one year showed a significantly higher value of 8-OH-dG than did the others. The loss of a spouse was categorized as the most stressful life change and the death of a close family member was regarded as the fifth most stressful event on

the Social Readjustment Rating Scale of Holmes and Rahe.[39] Some epidemiological studies have shown a positive association between widowhood and an increased disease mortality, including cancer, and an increased risk of cancer.[40–42] Furthermore, widowed men were also reported to have higher excess disease mortality, including cancer, than widowed women.[41] These findings suggest that cancer is likely to occur in spouses, particularly in widowers, although negative reports also exist.[43, 44] In addition to these epidemiological studies, several psychoneuroimmunological studies have demonstrated that spouses had decreased immune functions, such as lymphocyte stimulation responses to concanavalin A, phytohemagglutinin, and pokeweed mitogen, and natural killer cell activity, a defense against cancer progression, following the loss of their partners.[45–47] Insofar as we are aware, however, no definitive evidence has been found concerning genetic changes relevant to cancer initiation. Our results appear to provide support for the previous epidemiological findings from the genetic point of view, although the death of a close family member was not limited to a spouse, due to the small number of subjects. We could not conclude whether or not a relationship existed in the female subjects, because too few subjects had experienced the death of a close family member.

The positive relationships of the 8-OH-dG levels to average working hours, a self-blame coping strategy, and recent loss of a close family member in male subjects could well induce a positive association between negative emotions and the value of 8-OH-dG. However, the 8-OH-dG levels in male subjects had a tendency to have either no or a rather inverse relationship with negative emotions in the present study. Thus, we cannot eliminate the possibility that carcinogenicity is induced through a failure to perceive, express or relieve stress in male subjects. Lower expressions of negative emotions and conflicts in cancer patients have often been regarded as representing cancer-prone personality traits, possibly resulting from psychological defense mechanisms, such as repression or denial.[1–3] In spite of the positive associations between negative emotions and the 8-OH-dG levels, females may be more resistant to cancer initiation due to oxidative DNA damage than males, since female rats produce less 8-OH-dG than male rats when treated with carcinogens.[48]

Several limitations must also be considered in discussing our findings. First, it has been reported that some aspects of lifestyle, such as physical exercise and dietary habits, are also related to the formation of 8-OH-dG, in addition to cigarette smoking and alcohol use.[7, 12] These lifestyle factors might confound the associations between psychosocial factors and the 8-OH-dG levels. Second, we investigated the relationship between the 8-OH-dG levels and the most stressful life event, the death of a close family member, in the present study, so that the effect of other life events on the levels of 8-OH-dG was uncertain. Third, the number of female subjects in comparison with male counterparts might limit the ability to draw reliable conclusions regarding sex differences in the stress-related formation of 8-OH-dG. Fourth, our findings might include a possible selection bias: good communicators or those who had a high interest in stress or cancer were more likely to participate in this study. In addition, well-known risk factors for cancer, such as age, BMI, cigarette smoking, and alcohol use, were unrelated to the 8-OH-dG levels, possibly due to the rather selected population of generally healthy workers who may have a low intake of meat, and less obesity, in comparison to the population of Western industrialized countries. Fifth, the putative causation could not be verified, in view of the cross-sectional design of the study. Consequently, further investigations, particularly incorporating prospective studies and stress management intervention studies, are required in order to confirm the stress-cancer linkage via oxidative DNA damage.

Despite the above limitations, the results obtained strongly suggest that psychosocial factors are associated with the levels of 8-OH-dG. The 8-OH-dG levels were related to subjective psychosocial factors in female subjects, but objective ones in male subjects. Since growing evidence for an association of this oxidative DNA damage with carcinogenicity has been found,[7–9, 11–13] it seems possible that stressful conditions may play an etiological role in the pathogenesis of cancer. An examination of oxidative DNA damage in peripheral leukocytes may be useful in estimating the cancer risk easily and to make a follow-up survey, although longer observation periods are needed to verify whether cancer in humans is actually caused by higher leukocyte 8-OH-dG levels. We must note the fact that this examination was conducted during a period when the male and female workers were not particularly busy. Therefore, the oxidative DNA damage may become more prominent when the subjects are busier or under more stressful conditions. We believe this study offers valuable suggestions concerning stress-related cancer initiation.

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REFERENCES

1) Fox, B. H. Premorbid psychological factors as related to cancer incidence. *J. Behav. Med.*, **1**, 45–133 (1978).
2) Greer, S. and Watson, M. Towards psychobiological model of cancer: psychological considerations. *Soc. Sci. Med.*, **20**, 773–777 (1985).
3) Gross, J. Emotional expression in cancer onset and progression. *Soc. Sci. Med.*, **28**, 1239–1248 (1989).
4) Kiecolt-Glaser, J. K. and Glaser, R. Psychoneuroimmunology and cancer: fact or fiction? *Eur. J. Cancer*, **35**, 1603–1607 (1999).
5) Garssen, B. and Goodkin, K. On the role of immunological factors as mediators between psychosocial factors and cancer progression. *Psychiatry Res.*, **85**, 51–61 (1999).
6) Justice, A. Review of the effects of stress on cancer in laboratory animals: importance of time of stress application and type of tumor. *Psychol. Bull.*, **98**, 108–138 (1985).
7) Loft, S. and Poulsen, H. E. Cancer risk and oxidative DNA damage in man. *J. Mol. Med.*, **74**, 297–312 (1996).
8) Kasai, H. and Nishimura, S. Hydroxylation of deoxyguanosine at the C-8 position by ascorbic acid and other reducing agents. *Nucleic Acids Res.*, **12**, 2137–2145 (1984).
9) Cheng, K. C., Cahill, D. S., Kasai, H., Nishimura, S. and Loeb, L. A. 8-Hydroxyguanine, an abundant form of oxidative DNA damage, causes G→T and A→C substitution. *J. Biol. Chem.*, **267**, 166–172 (1992).
10) Hollstein, L., Sidransky, D., Vogelstein, V. and Harris, C. C. P53 mutations in human cancers. *Science*, **253**, 49–53 (1991).
11) Floyd, R. A., Watson, J. J., Wong, P. K., Altmiller, D. H. and Rickard, R. C. Hydroxyl free radical adduct of deoxyguanosine: sensitive detection and mechanisms of formation. *Free Radic. Res. Commun.*, **1**, 163–172 (1986).
12) Kasai, H. Analysis of a form of oxidative DNA damage, 8-hydroxyguanosine, as a marker of cellular oxidative stress during carcinogenesis. *Mutat. Res.*, **387**, 147–163 (1997).
13) Helbock, H. J., Beckman, K. B. and Ames, B. N. 8-Hydroxydeoxyguanosine and 8-hydroxyguanine as biomarkers of oxidative DNA damage. *Methods Enzymol.*, **300**, 156–166 (1999).
14) Adachi, S., Kawamura, K. and Takemoto, K. Oxidative damage of nuclear DNA in liver of rats exposed to psychological stress. *Cancer Res.*, **53**, 4153–4155 (1993).
15) Irie, M., Asami, S., Nagata, S., Miyata, M. and Kasai, H. Classical conditioning of oxidative DNA damage in rats. *Neurosci. Lett.*, **288**, 13–16 (2000).
16) Ames, B. N. Endogenous oxidative DNA damage, aging, and cancer. *Free Radic. Res. Commun.*, **7**, 121–128 (1989).
17) Loft, S., Vistisen, K., Ewertz, M., Tjonneland, A., Overvad, K. and Poulsen, H. E. Oxidative DNA damage estimated by 8-hydroxydeoxyguanosine excretion in humans: influence of smoking, gender and body mass index. *Carcinogenesis*, **13**, 2241–2247 (1992).
18) Asami, S., Hirano, T., Yamaguchi, R., Tomioka, Y., Itoh, H. and Kasai, H. Increase of a type of oxidative DNA damage, 8-hydroxyguanine, and its repair activity in human leukocytes by cigarette smoking. *Cancer Res.*, **56**, 2546–2549 (1996).
19) Nakajima, M., Takeuchi, T., Takeshita, T. and Morimoto, K. 8-Hydroxydeoxyguanosine in human leukocyte DNA and daily health practice factors: effects of individual alcohol sensitivity. *Environ. Health Perspect.*, **104**, 1336–1338 (1996).
20) McNair, D. M., Lorr, M. and Doppelman, L. F. Profile of mood states instrument. *In* "Manual for the Profile of Mood States," ed. D. M. McNair, M. Lorr and L. F. Doppelman, pp. 3–29 (1971). Educational and Industrial Testing Service, San Diego.
21) Hurrell, J. J. and McLaney, M. A. Exposure to job stress: a new psychometric instrument. *Scand. J. Work Environ. Health*, **14** (Suppl. 1), 27–28 (1988).
22) Cella, D. F., Tross, S., Orev, E. J., Holland, J. C., Silberfarb, P. M. and Rafla, S. Mood states of patients after the diagnosis of cancer. *J. Psychosoc. Oncol.*, **7**, 45–54 (1989).
23) Tsuboi, H., Kouda, K., Takeuchi, H., Takigawa, M., Masamoto, Y., Takeuchi, M. and Ochi, H. 8-Hydroxydeoxyguanosine in urine as an index of oxidative damage to DNA in the evaluation of atopic dermatitis. *Br. J. Dermatol.*, **138**, 1033–1035 (1998).
24) SAS Institute. SAS User's Guide: Statistics. Version 6.12 (1996). SAS Institute Inc., Cary, NC.
25) Persky, V. W., Kempthorne-Rawson, J. and Shekelle, R. B. Personality and risk of cancer: 20-year follow-up of the Western Electric Study. *Psychosom. Med.*, **49**, 435–449 (1987).
26) Penninx, B. W. J. H., Guralnik, J. M., Pahor, M., Ferrucci, L., Cerhan, J. R., Wallace, R. B. and Havlik, R. J. Chronically depressed mood and cancer risk in older persons. *J. Natl. Cancer Inst.*, **90**, 1888–1893 (1998).
27) Kihara, H., Teshima, H., Sogawa, H. and Nakagawa, T. Stress and superoxide production by human neutrophils. *Ann. N. Y. Acad. Sci.*, **15**, 307–310 (1992).
28) Kovacs, P., Juranek, I., Stankovicova, T. and Svec, P. Lipid peroxidation during acute stress. *Pharmazie*, **51**, 51–53 (1996).
29) Schneider, R. H., Nidich, S. I., Salerno, J. W., Sharma, H. M., Robinson, C. E., Nidich, R. J. and Alexander, C. N. Lower lipid peroxide levels in practitioners of the transcendental meditation. *Psychosom. Med.*, **60**, 38–41 (1998).
30) Busciglio, J., Andersen, J. K., Schipper, H. M., Gilad, G. M., McCarty, R., Marzatico, F. and Toussaint, O. Stress, aging, and neurodegenerative disorders. Molecular mechanisms. *Ann. N. Y. Acad. Sci.*, **851**, 429–443 (1998).
31) Dhabhar, F. S., Miller, A. H., McEwen, B. S. and Spencer, R. L. A. D. Effects of stress on immune cell distribution. Dynamics and hormonal mechanisms. *J. Immunol.*, **154**, 5511–5527 (1995).

32) Hagnell, O. The premorbid personality of persons who develop cancer in a total population investigated in 1947 and 1957. *Ann. N. Y. Acad. Sci.*, **125**, 846–855 (1966).
33) Mirowsky, J. and Ross, C. E. Sex differences in distress: real or artifact? *Am. Soc. Rev.*, **60**, 449–469 (1995).
34) Vingerhoets, A. J. and Van-Heck, G. L. Gender, coping and psychosomatic symptoms. *Psychol. Med.*, **20**, 125–135 (1990).
35) Malcarne, V. L., Compas, B. E., Epping-Jordan, J. E. and Howell, D. C. Cognitive factors in adjustment to cancer: attributions of self-blame and perceptions of control. *J. Behav. Med.*, **18**, 401–417 (1995).
36) Watabe, K., Nishi, M., Miyake, H. and Hirata, K. Lifestyle and gastric cancer: a case-control study. *Oncol. Rep.*, **5**, 1191–1194 (1998).
37) Spiegelman, D. and Wegman, D. H. Occupation-related risks for colorectal cancer. *J. Natl. Cancer Inst.*, **75**, 813–821 (1985).
38) Jahn, I., Becker, U., Jockel, K. H. and Pohlabeln, H. Occupational life course and lung cancer risk in men. Findings from a socio-epidemiological analysis of job-changing histories in a case-control study. *Soc. Sci. Med.*, **40**, 961–975 (1995).
39) Holmes, T. H. and Rahe, R. H. The social readjustment rating scale. *J. Psychosom. Res.*, **11**, 213–218 (1967).
40) Helsing, K. J., Szklo, M. and Comstock, G. W. Factors associated with mortality after widowhood. *Am. J. Public Health*, **71**, 802–809 (1981).
41) Martikainen, P. and Valkonen, T. Mortality after the death of a spouse: rates and causes of death in a large Finnish cohort. *Am. J. Public Health*, **86**, 1087–1093 (1996).
42) Courtney, J. G., Longnecker, M. P., Theorell, T. and Gerhardsson-de-Verdier, M. Stressful life events and the risk of colorectal cancer. *Epidemiology*, **4**, 407–414 (1993).
43) Jones, D. R., Goldblatt, P. O. and Leon, D. A. Bereavement and cancer: some data on deaths of spouses from the longitudinal study of Office of Population Censuses and Surveys. *BMJ*, **289**, 461–464 (1984).
44) Ewertz, M. Bereavement and breast cancer. *Br. J. Cancer*, **53**, 701–703 (1986).
45) Bartrop, R. W., Luckhurst, E., Lazarus, L. and Kiloh, L. G. Depressed lymphocyte function after bereavement. *Lancet*, **i**, 834–836 (1977).
46) Schleifer, S. J., Keller, S. E., Camerino, M., Thornton, J. C. and Stein, M. Suppression of lymphocyte stimulation following bereavement. *JAMA*, **250**, 374–377 (1983).
47) Irwin, M., Daniels, M., Smith, T. L., Bloom, E. and Weiner, H. Impaired natural killer cell activity during bereavement. *Brain Behav. Immun.*, **1**, 98–104 (1987).
48) Guo, N., Conaway, C. C., Hussain, N. S. and Fiala, E. S. Sex and organ differences in oxidative DNA and RNA damage due to treatment of Sprague-Dawley rats with acetoxime or 2-nitropropane. *Carcinogenesis*, **11**, 1659–1662 (1990).