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.5) 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...
aversion paradigm. 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, body mass index (BMI), and cigarette smoking and alcohol drinking habits 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 (χ²=66.9, P<0.01; χ²=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); stress-coping items and social support items from the NIOSH general job stress instrument; 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. 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.

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

| Variables                        | Males (n=276) | Females (n=86) |
|----------------------------------|--------------|---------------|
| 8-OH-dG (/10¹² dG)               | 0.36±0.20    | 0.36±0.24     |
| Mean±SD (Range)                  | (0.08–1.27)  | (0.07–1.88)   |
| Age (years)                      | 41.6±11.7    | 40.4±10.8     |
| Mean±SD (Range)                  | (20–64)      | (21–56)       |
| Body mass index (kg/m²)          | 23.1±2.8     | 21.6±3.0      |
| Mean±SD (Range)                  | (16.9–33.1)  | (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 characteristics.
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 P₁ (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. 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 P₁ (0.8 units, Yamasa Co.) and acid phosphatase (1 unit, Sigma Chemical Co.) in a solution of 1 mM EDTA, 10 mM 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 deoxyribonucleoside was injected onto a high-performance liquid chromatography column (Beckman; Ultrasphere-ODS; 5 μm, 4.6×250 nm; elution, 10 mM NaH₂PO₄ 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 10⁵ guanine residues. **Statistical analysis** The SAS software 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 continuous variables. A regression analysis was also done with the influence of the four factors adjusted for continuous variables and several continuous 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 χ² 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/10⁷ 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).
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 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 (95% CI) | P    | No. | Regression coefficient (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 strategiesa                   | 242 | 0.005 (-0.013, 0.031)          | 0.416| 82  | 0.023 (0.002, 0.103)           | 0.047|
| Social support                            | 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 strategiesa                   | Rarely | Occasionally | Sometimes | Fairly often | Very often | Regression coefficient (95% CI) | P    |
|--------------------------------------------|--------|--------------|-----------|--------------|------------|-------------------------------|------|
|                                            | No.    | Mean (SE)    | No.       | Mean (SE)    | No.        | Mean (SE)                     | No.  |
| Planning                                  |        |              |           |              |            |                               |      |
| Males (n=273)                              | 14     | 0.42 (0.08)  | 51        | 0.34 (0.02)  | 103        | 0.37 (0.02)                   | 63   |
| Females (n=85)                             | 7      | 0.49 (0.10)  | 10        | 0.26 (0.04)  | 27         | 0.35 (0.04)                   | 19   |
| Neglect                                    |        |              |           |              |            |                               |      |
| Males (n=274)                              | 67     | 0.39 (0.03)  | 96        | 0.37 (0.02)  | 88         | 0.35 (0.02)                   | 16   |
| Females (n=85)                             | 28     | 0.35 (0.04)  | 24        | 0.35 (0.03)  | 23         | 0.30 (0.03)                   | 7    |
| Self-blame                                 |        |              |           |              |            |                               |      |
| Males (n=276)                              | 10     | 0.37 (0.05)  | 31        | 0.33 (0.04)  | 74         | 0.32 (0.02)                   | 94   |
| Females (n=86)                             | 8      | 0.52 (0.20)  | 22        | 0.34 (0.03)  | 26         | 0.32 (0.03)                   | 16   |
| Wishful thinking                           |        |              |           |              |            |                               |      |
| Males (n=276)                              | 64     | 0.35 (0.02)  | 55        | 0.38 (0.03)  | 102        | 0.35 (0.02)                   | 40   |
| Females (n=85)                             | 41     | 0.28 (0.02)  | 16        | 0.36 (0.03)  | 18         | 0.47 (0.10)                   | 6    |
| Consultation                               |        |              |           |              |            |                               |      |
| Males (n=274)                              | 26     | 0.39 (0.05)  | 62        | 0.38 (0.03)  | 96         | 0.37 (0.02)                   | 63   |
| Females (n=86)                             | 7      | 0.33 (0.03)  | 12        | 0.36 (0.04)  | 30         | 0.35 (0.04)                   | 15   |
| Involvement                                |        |              |           |              |            |                               |      |
| Males (n=274)                              | 39     | 0.35 (0.03)  | 59        | 0.37 (0.03)  | 106        | 0.36 (0.02)                   | 54   |
| Females (n=85)                             | 32     | 0.31 (0.02)  | 17        | 0.44 (0.10)  | 25         | 0.38 (0.05)                   | 7    |

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.
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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 \( 0.41 \pm 0.22 (n=52) \) vs. \( 0.35 \pm 0.20 (n=224), F(1, 262) = 5.29, P = 0.022 \), 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 \( 0.38 \pm 0.22 (n=156) \) vs. \( 0.34 \pm 0.18 (n=120), F(1, 261) = 4.78, P = 0.030 \), 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. Among several kinds of psychological factors, depression has often been reported to have an important role in cancer initiation. 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-
mental 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.\(^{32}\) 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.\(^{33}\) In contrast, no sex difference was observed in the relationship between depressive mood and cancer incidence in older persons.\(^{34}\) 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 disparate 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 signifi-

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the Social Readjustment Rating Scale of Holmes and Rahe. Some epidemiological studies have shown a positive association between widowhood and an increased disease mortality, including cancer, and an increased risk of cancer. Furthermore, widowed men were also reported to have higher excess disease mortality, including cancer, than widowed women. These findings suggest that cancer is likely to occur in spouses, particularly in widowers, although negative reports also exist. 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. 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. 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.

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. 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 oxidative DNA damage with carcinogenicity has been found, 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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