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# Effects of Psychological Stress from a National License Examination on the Urine 8-Hydroxy-Deoxyguanosine Levels in Young Female Students, Taking into Account the Menstrual Cycle

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## Abstract

Objective: Urine 8-hydroxy-deoxyguanosine (U8-OHdG) was a marker of oxidative stress-induced DNA damage; it was increased by psychological stress. Since the menstrual cycle may confound or modify the association between psychological stress and U8-OHdG. The aim of this study was to verify the effect of psychological stress from a national license examination on levels of U8-OHdG, which is a biomarker of oxidative stress. And the effects of women's menstrual cycles, which should be considered in mental and physical assessments, on U8-OHdG, were evaluated. Methods: The subjects were 18 female university students at a medical university in whom Self-rating Depression Scale (SDS) scores, State-Trait Anxiety Inventory (STAI) scores, and U8-OHdG levels were measured in 3 phases of the menstrual cycle, the follicular phase, ovulatory phase, and luteal phase. The mean values were taken for the group during a time of classroom learning. The same measurements were also made one week before and the day after a national license examination and the measurements were compared among the three periods. Results and Conclusion: State anxiety and U8-OHdG levels were significantly higher in those with a week before the national license examination than in those with classroom lecture (State anxiety: $p = 0.002$, U8-OHdG levels: $p = 0.007$). The menstrual cycle did not show a significant correlation with U8-OHdG le-

vels. This study demonstrated that levels of the oxidative stress biomarker U8-OHdG are not affected by changes in the menstrual cycle. It also showed that U8-OHdG levels increased with the psychological stress of a national license examination.

## Keywords

8-OHdG, Oxidative Stress, Menstrual Cycles, Psychological Stress

## 1. Introduction

The percentage of people with work- and life-related anxiety, worry, and stress is increasing every year, and it is said to exceed 60%. The relationship between psychological stress and oxidative stress has become a focus of interest, and the mechanism by which psychological stress increases reactive oxygen species (ROS) [1] [2] [3] [4] is slowly being elucidated [5] [6] [7]. Reports in recent years have also indicated that oxidative stress markers are effective as biomarkers for the assessment of psychological stress. Reactive oxygen species oxidize the major biological constituents of DNA, lipids, and proteins, increasing oxidative stress in the body [8]. Oxidized DNA, lipids, and proteins are also produced in the blood and urine, and so it is possible that these biomarkers in the body can be used as oxidative stress markers [9]. Among them, urine 8-hydroxy-2'-deoxyguanosine (U8-OHdG), which is a marker of DNA oxidative damage, can be measured non-invasively using urine. In this study, therefore, the focus was on the relationship between psychological stress and U8-OHdG, with the aim of objectively assessing psychological stress with a biomarker.

Adachi *et al.* reported that U8-OHdG increases with psychological stress [3]. Urita *et al.* also demonstrated that the Profile of Mood States (POMS), used as a measure of psychological stress, improved as a result of giving a bifidum YIT 10347 lactic acid bacteria drink for 4 weeks to patients with a functional gastrointestinal disorder, which is a factor in psychological stress, and that U8-OHdG decreased, suggesting a relationship between POMS and U8-OHdG [10]. However, these studies were limited in terms of uniformity of stress and menstrual cycle confounding, and solid proof is lacking [11]. Twenty to fifty percent of healthy women reportedly experience mental symptoms such as depression, anxiety, or irritation from about 10 days to 1 week before menstruation [12]. We have previously reported that psychological stress inhibits female hormone secretion during menstruation [13] that depressed women show symptoms of anxiety prior to menstruation, and that this anxiety and U8-OHdG are correlated [14]. However, there are few reported assessments of stress and stress response substances in relation to the menstrual cycle and examinations of the relationship between anxiety symptoms that are characteristic to depressive conditions and stress response substances. Therefore, the menstrual cycle needs to be considered when examining the relationship between psychological stress

in women and oxidative stress as an objective means of assessment.

There are also various ways to apply psychological stress. Jemmott and colleagues proposed the concept of academic stress for psychological stress due to an examination, and they reported a decrease in salivary secretory immunoglobulin A (sIgA) and high stress levels in dental students facing examinations [15]. From this, it may be inferred that undergraduate students who will soon take a national license examination are exposed to high stress levels.

The purpose of this study was to clarify the effects of the menstrual cycle on the levels of the oxidative stress biomarker U8-OHdG and to verify the effects of psychological stress on U8-OHdG levels. The relationship between subjective stress and U8-OHdG levels was also studied in subjects who were planning to take a national license examination, which is projected to convey uniform psychological stress.

## 2. Materials and Methods

### 2.1. Subjects

The subjects were fourth year female medical university students in Japan. The study content and methods were explained fully in advance, and written consent was obtained. Nineteen of the 22 students who attended the explanation meeting gave consent, but 1 whose menstrual cycle was not within the range of 26 to 38 days was excluded, and the remaining 18 (mean age 21.6 ± 0.9 years) were the subjects of the statistical analysis [14] [16]. This study was conducted in accordance with the Declaration of Helsinki and approved by the Fujita Health University Ethics Committee (approval number 10-075).

### 2.2. Survey Items

Surveys were conducted during the menstrual cycle and before and after the national license examination. The menstrual cycle survey was conducted between July and September 2011. The menstrual period during the survey period was divided into 3 self-reported phases, the follicular phase (within 3 days from the start of menstruation), the ovulatory phase (13 - 15 days), and the luteal phase (24 - 26 days), and surveys were conducted during each phase. The mean values of measurements during these 3 phases were used as the measurement values for the time of classroom learning, and they were compared with measurements at the time of stress from the national license examination. The surveys before and after the national license examinations were divided into 2 time periods, the period of 1 week before the national license examination held in February 2012, and the day after the national license examination. Self-rating Depression Scale (SDS) [17] and State-Trait Anxiety Inventory (STAI) [18] surveys were conducted with self-completed questionnaires to assess psychological stress in each of the 3 phases of the menstrual cycle and the 2 time periods before and after the national license examination. Urine samples were also taken from 12:00 to 13:00, before taking lunch. The NEO Five-Factor Inventory was used for personality

characteristics. It was conducted once during one of the 3 phases of the menstrual cycle.

### 2.3. Urine 8-OHdG Measurements

Urine samples for measurement were collected between 12:00 and 13:00 before lunch in each period, taking into account circadian variations. Urine samples were collected for the certain period in consideration of the possibility of the U8-OHdG levels fluctuating within the day. When urine was collected, it was confirmed orally whether the individual had performed strenuous exercise the day before. After collection, the urine was centrifuged at 1500 rpm for 5 min, and the supernatant was frozen and stored at −20˚C. An 8-OHdG Check (Japan Institute for the Control of Aging (JaICA), Shizuoka, Japan) was used in the measurements, and the values obtained by dividing the calculated values by creatinine (ng/mg creatinine) were used. Triple checks were conducted, and measurement accuracy was R2 = 0.92 - 0.96 and cv = 0.021 - 0.023.

### 2.4. Statistical Analysis

Spearman's rank correlation coefficient was obtained for the relationship between the NEO-FFI and the SDS or STAI. The NEO-FFI N factor showed significant correlations with the SDS score and the STAI scores (trait anxiety and state anxiety) ((SDS r = 0.660, $p = 0.003$; STAI (trait anxiety) r = 0.671, $p = 0.002$; STAI (state anxiety) r = 0.575, $p = 0.013$). Therefore, subjects' SDS score, STAI scores (trait anxiety and state anxiety), and U8-OHdG levels are expressed as means ± standard deviation adjusted for age, BMI, and NEO-FFI N factor. Repeated measures ANOVA was conducted with menstrual cycle (intra-individual levels in the follicular phase, ovulatory phase, and luteal phase) as a factor and SDS score, STAI scores, and U8-OHdG levels as dependent variables. Then, multiple comparisons were performed with Bonferroni's method. Repeated measures ANOVA was also used with the time of classroom learning and before and after the national license examination (intra-individual levels at the time of classroom learning and one week before and the day after the national license examination) as individual factors and SDS score, STAI score, and U8-OHdG levels as dependent variables. Then, multiple comparisons were done with Bonferroni correction method. The normality of the SDS score, STAI scores, and U8-OHdG levels was confirmed with the Kolmogorov-Smirnov test ($p = 0.065$ - 0.200). Statistical analyses were performed using SPSS 21.0J (IBM Japan, Tokyo), and the level of significance was taken to be $p < 0.05$.

## 3. Results

### 3.1. During the Menstrual Cycle

SDS score and STAI scores (trait anxiety and state anxiety) were measured in the follicular phase (43.7 ± 1.4, 50.9 ± 1.6, 46.8 ± 1.9), ovulatory phase (41.8 ± 1.3,

50.7 ± 1.7, 44.6 ± 2.1), and luteal phase (42.8 ± 1.1, 50.9 ± 1.8, 47.2 ± 2.1), respectively, and no significant differences were seen in the 3 items among the 3 phases during the menstrual cycle (Table 1: repeated measures 1-way ANOVA, $p = 0.170$ - 0.947). There was also no significant difference in U8-OHdG levels among the 3 phases during the menstrual cycle (Table 1).

### 3.2. Before and after the National License Examination

For the SDS score, no significant difference was seen 1 week before the national license examination (45.6 ± 2.0) compared with the time of classroom learning (42.7 ± 2.5) (Figure 1(A)). No significant difference was seen the day after the national license examination (39.3 ± 3.7) compared with 1 week before the examination (45.6 ± 2.0). On the STAI score (trait anxiety), no significant difference was seen 1 week before the national license examination (53.2 ± 3.4) compared with the time of classroom learning (50.9 ± 3.4). In contrast, it showed significantly lower scores on the day after the examination (46.7 ± 3.4) than during classroom learning and 1 week before the examination (Figure 1(B)). On the STAI score (state anxiety), the score was significantly higher 1 week before the national license examination (54.9 ± 40) compared with classroom learning (46.2 ± 3.1), showing that state anxiety was increased 1 week before the national license examination. In contrast, the score on the day after the national license examination (34.1 ± 4.0) was significantly lower compared with the time of classroom learning and 1 week before the examination, showing that state anxiety had decreased (Figure 1(C)). The U8-OHdG levels was significantly higher 1 week before the national license examination (11.4 ± 1.2 ng/mg creatinine) than at the time of classroom learning (7.5 ± 1.9 ng/mg creatinine) ($p = 0.007$). No difference was seen on the day after the national license examination (10.8 ± 1.5 ng/mg creatinine) compared with 1 week before the examination, and it was significantly higher than during classroom learning (Figure 1(D)).

Table 1. SDS score, STAI scores (trait anxiety and state anxiety) and U8-OHdG levels among the 3 phases during the menstrual cycle (n = 18).

| | Follicular phase | | | Ovulatory phase | | | Luteal phase | | | *p* value |
|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | SD | 95% CI | Mean | SD | 95% CI | Mean | SD | 95% CI | |
| SDS (score) | 43.7 | (1.4) | 40.6 - 46.7 | 41.8 | (1.3) | 39.0 - 44.6 | 42.8 | (1.1) | 40.4 - 45.2 | 0.170 |
| STAI: trait anxiety (score) | 50.9 | (1.6) | 47.6 - 54.3 | 50.7 | (1.7) | 47.1 - 54.3 | 50.9 | (1.8) | 47.1 - 54.8 | 0.947 |
| STAI: state anxiety (score) | 46.8 | (1.9) | 42.9 - 50.8 | 44.6 | (2.1) | 40.2 - 49.0 | 47.2 | (2.1) | 42.7 - 51.7 | 0.670 |
| U8-OHdG (g/mg creatinine) | 6.6 | (0.9) | 4.8 - 8.4 | 7.9 | (1.0) | 5.9 - 10.0 | 7.9 | (1.3) | 5.1 - 10.7 | 0.299 |

*P* value: One-factor repeated measures analysis of variance; SD: standard deviation; 95% CI: 95% confidence interval; SDS: self-rating depression scale; STAI: State-Trait Anxiety Inventory; U8-OHdG: urine 8-hydroxy-2'-deoxyguanosine.

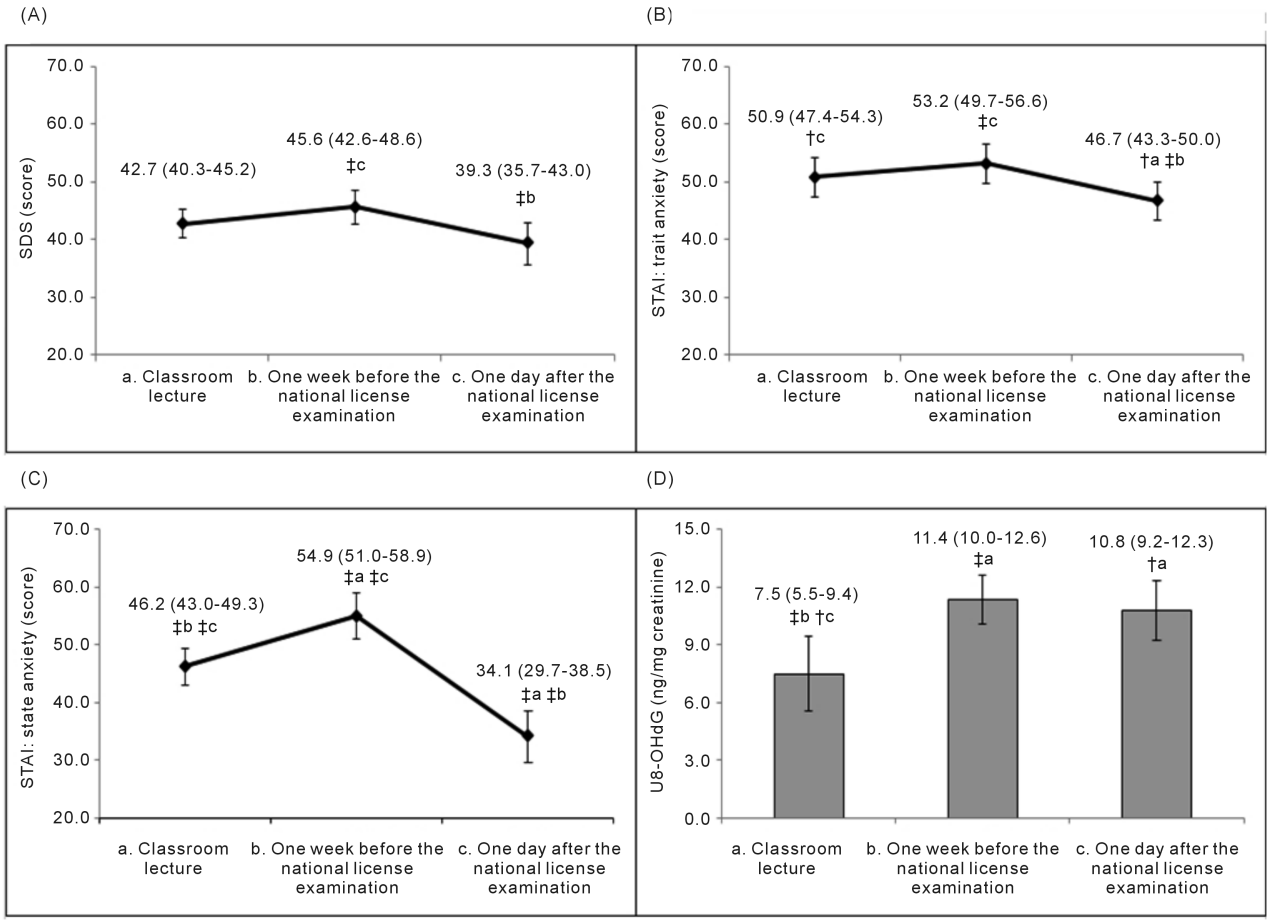


**Figure 1.** Comparisons of SDS score, STAI scores (trait anxiety and state anxiety) and U8-OHdG levels between before and after the national license examination. (A) SDS score in the upper left; (B) STAI score (trait anxiety) in the upper right; (C) STAI score (trait anxiety) in the lower left; (D) U8-OHdG levels in the lower right. Figures showed mean and SD error bar, and Numbers indicated mean (−1SD - +1SD). One-factor repeated measures analysis of variance: SDS score ($p = 0.006$), STAI score (trait anxiety) ($p = 0.003$), STAI score (state anxiety) ($p < 0.001$), U8-OHdG levels ($p = 0.010$). Bonferroni correction: †$p < 0.05$, ‡$p < 0.01$; a (vs a. Classroom lecture); b (vs b. One week before the national license examination); c (vs c. One day after the national license examination).

## 4. Discussion

It was demonstrated in this study that the oxidative stress biomarker U8-OHdG levels is not affected by changes in the menstrual cycle. U8-OHdG levels were shown to increase with the psychological stress from a national license examination. The results of this study suggest the possibility that U8-OHdG may be a biomarker of psychological stress in mental health care evaluations in women.

On the STAI score (state anxiety), a tool that has often been used as an indicator to evaluate psychological stress, the results showed that state anxiety increased significantly before the national license examination compared with the time of normal classroom learning, and that after the examination, it decreased significantly compared with the time of classroom learning and before the examination. This is similar to the results of a previous study [15] and suggests that national license examinations are valid as uniform psychological stressors.

There are many reports on the relationships between psychological stress and physical symptoms, including that psychological stress increases the risk of acute myocardial infarction [19] and diabetes [20], and that psychological stress is related to dysmenorrhea [21]. At the same time, psychological stress is also reported to be difficult to assess objectively. Tools to test psychological stress are mainly questionnaires like the STAI and SDS, which rely on the subjective evaluations of subjects. In the search for objective assessment measures, the relationship between oxidative stress biomarkers and psychological stress has attracted attention in recent years [6] [7].

Psychological stress is said to increase the production of ROS and increase oxidative stress in the body [1] [2] [3] [4]. Oxidative stress is defined as a state in which ROS production exceeds the body's antioxidant capacity, and the balance between oxidation and antioxidation in the body is lost. Oxidative stress, by increasing the oxidative damage in the body, is said to facilitate the development of various diseases and aging. The body has a large antioxidant capacity, but excessive stress that exceeds the accommodation limit (threshold) weakens biological homeostasis. Disrupting the balance between ROS production and antioxidant defense leads to a state of oxidative stress, causing oxidative damage to biomolecules and then to cell and tissue damage. The psychological stress of national license examinations is thought to cause U8-OHdG levels to increase through these mechanisms.

Women have a menstrual cycle affected by sex hormones, in which various physiological reactions in the body [12] [22] and mental changes [23] [24] [25] occur. Sex hormones lead to the production of ROS via neutrophils, which have immune functions, and these ROS are reported to be involved in the occurrence of ovulation in the ovaries and subsequent follicle destruction [22]. After ovulation, the corpus luteum is formed and progesterone is secreted, but if pregnancy is not established, a process of functional corpus luteum regression begins. ROS are also said to play a role in promoting this functional corpus luteum regression [22], and ROS production may be considered essential in maintaining homeostasis in ovarian function. The sex hormone estrogen is considered to have an antioxidant effect [26], and the menstrual cycle is also thought to affect the antioxidant activity in the body. Consequently, since changes in the balance of ROS production and antioxidant activity occur through the secretion of menstrual cycle-dependent sex hormones, the menstrual cycle may be said to affect oxidative stress. Superoxide dismutase (SOD), glutathione reductase (GSHR), and glutathione peroxidase (GPx) are taken to be the main biomarkers of oxidative stress. However, tests using these biomarkers have confirmed that they increase in the ovulatory phase and decrease in the luteal phase, and the menstrual cycle has been reported to be involved in oxidative stress [27]. U8-OHdG is also commonly used as a biomarker of oxidative stress, and in a test of premenopausal women with uterine fibroids and healthy premenopausal women, the fluctuations in estrogen and progesterone levels accompanying the menstrual

cycle in healthy women was not reported to interact with U8-OHdG levels [28]. The U8-OHdG used as a biomarker of oxidative stress in this study is a marker of DNA oxidative damage that is released extracellularly in the process of gene DNA repair and excreted in urine via the blood [29]. There are many types of oxidative damage to DNA, but U8-OHdG is excreted into urine without being subjected to secondary metabolism. This suggests the possibility that U8-OHdG reflects oxidative stress that is due to psychological stress by a mechanism that differs from the one for the production of oxidative stress accompanying the menstrual cycle.

Our study has some limitations. The subjects in this study were female students from a single university, and there may be a problem in generalizing the study results. However, their physical measurements were similar to the mean values for the representative national population [30]. Conversely, they were similar in terms of lifestyle and other factors, and so the results are thought to have a high level of internal validity. In this study, smoking history was self-reported. The confidentiality of the survey content was duly considered, but there is known to be a relationship between psychological stress and smoking [31], and a study that measures urine cotinine levels may be needed in the future. Moreover, psychological stress was assessed only with the SDS and STAI. The subjects were not diagnosed by a psychiatrist. Since there was no psychiatric diagnosis, the possibility that the study included subjects with depression cannot be ruled out. A future investigation that determines depression together with a physician's diagnosis may also be necessary.

## 5. Conclusion

In summary, U8-OHdG levels were shown to increase with the psychological stress from a national license examination, unaffected by the menstrual cycle. This result suggests the possibility that U8-OHdG may be a useful biomarker for the objective assessment of psychological stress. Many things are still not understood about the mechanism for the occurrence of oxidative stress from psychological stress. Thus, from the perspective of the prevention and treatment of oxidative stress as well, elucidation of the relationship between psychological stress and oxidative stress may be a topic for future study.

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