Effects of Psychological and Physical Stress on Oxidative Stress, Serotonin, and Fatigue in Young Females Induced by Objective Structured Clinical Examination: Pilot Study of u-8-OHdG, u-5HT, and s-HHV-6

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ABSTRACT

BACKGROUND: To establish a method to prevent and manage fatigue caused by psychological and physical stress in young females, early detection factors, such as understanding of fatigue and causes of psychological and physical stress, as well as a review of early management of psychiatric disease, are important. With increasing knowledge regarding the diverse causes of stress, it is important to select biomarkers with consideration of the types of stress burden and mechanisms underlying the development of physical symptoms. The methods used to search for stress characteristics is an issue that needs to be addressed. However, consensus regarding objective assessment methods for impaired mental health is lacking.

METHODS: We examined the effects of an objective structured clinical examination (OSCE), considered to be a uniform source of psychological and physical stress, on biomarkers of oxidative stress and fatigue in 16 third-year female medical university students (21.3 ± 2.1 years old) in Japan with a normal menstrual cycle. A self-administered questionnaire consisting of Zung's Self-rating Depression Scale (SDS) and State-Trait Anxiety Inventory (STAI) was used to assess subjective stress. Furthermore, stress-related biomarkers (urinary 8-hydroxy-2′-deoxyguanosine [u-8-OHdG], urinary 5-hydroxytryptamine [u-5-HT], and salivary human herpesvirus-6 [s-HHV-6]) were examined at 1 month, 1 week, and 1 day before, and 1 week after the OSCE.

RESULTS: The results indicated that the OSCE did not have effects on u-8-OHdG, a biomarker of oxidative stress. However, u-5-HT and s-HHV-6 were found to be elevated in examinations performed prior to the OSCE.

CONCLUSIONS: The present findings suggest that u-5-HT and s-HHV-6 levels can be used for objective assessment of mental and physical fatigue in young females, including that produced not only by knowledge regarding an upcoming OSCE, but also by skill and attitude aspects related to that examination.

KEYWORDS: objective structured clinical examination, u-8-OHdG, u-5-HT, s-HHV-6, SDS, STAI

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Background

The state of impaired physical function due to continuing or accumulated stress is referred to as "fatigue," and in recent years that has been the focus in searches for biomarkers in the body that can be used as objective indices to assess mental health.[1] Fatigue is a phenomenon that features decreased mental and physical performance caused by a general physical or mental burden in the affected individual, accompanied by a desire for rest and feelings of discomfort.[2] Among the mechanisms involved, changes in oxidative stress biomarkers such as 8-isoprostane[3] and urinary 8-hydroxy-2′-deoxyguanosine (u-8-OHdG),[4-8] which are common to both physical and mental burdens, have been confirmed as underlying the manifestation of fatigue from findings showing that oxidative stress from reactive oxygen species (ROS) induces destruction of nerve cells, mainly those in autonomic nerves.[9-11]

In addition, examinations of mental fatigue from the perspective of autonomic nerve activity have shown that hyperactive sympathetic nerve activity caused by parasympathetic nerve activity suppression is common in cases of acute and

subacute fatigue.[12] According to the monoamine hypothesis of depression, accumulated fatigue is due to a decrease in the ratio of serotonin to dopamine, resulting in a decrease in performance when serotonin is increased.[13] One possible cause is thought to be alteration of intestinal flora by a stress response via the gut-brain axis, thus promoting 5-HT biosynthesis from enterochromaffin cells.[14,15] Aoki et al[1] examined the usefulness of human herpesvirus 6 (HHV-6), a known biomarker, in subjects with physiological or pathological fatigue, and found that an elevated level had a strong relationship with physiological fatigue. HHV-6 infection is latent, though when immunity is reduced, a virus reactivation is manifested, thus quantification of activated HHV-6 can be useful as an objective indicator of and used to measure fatigue.[16] However, though a direct association between HHV-6 and u-5HT has not been shown, it is possible that $CD4^+$ T cells indirectly activated by HHV-6B induce upregulation of 5-HT from intestinal EC cells.[17] This has been suggested and is considered to be a factor related to increased 5-TH in association with changes in intestinal flora. With fatigue caused by stress, the type and magnitude of decreased performance differs because of variations in causes for that burden. Therefore, it has been suggested that biomarkers used for fatigue quantification should be selected based on the type of burden and mechanism underlying manifestation of fatigue.[16] Fatigue is one of the big 3 alarms, along with pain and fever. With its accumulation, the immune system becomes weakened and susceptibility to infectious disease, such as respiratory infection and herpes labialis, is increased. In fact, a physiological response by the stress response system and immune system because of fatigue can be predicted, though there may be a time lag between awareness of accumulated fatigue and an associated response. Therefore, subjective evaluations of fatigue and biomarkers used for fatigue quantification, as well as their relationships over time are needed.

Ferreira et al[18] investigated psychological (anxiety) and physical (cortisol levels) factors in a cross-sectional study in subjects who underwent an objective structured clinical examination (OSCE), to examine the effects on specific skills and behaviors in a simulated work environment, and reported that no significant relationship was found. On the other hand, OSCE-related nervousness, stress, and anxiety are consistently reported.[19-22] Hence, the OSCE is assumed to be a psychological and physical stressor, and not only the hypothalamic-pituitary-adrenal axis including cortisol but also ROS or autonomic nerve activity may be involved. The male-to-female ratio for psychiatric diseases caused by psychological stress is reported to be 1:2.[23] In Japan, the incidence of psychiatric diseases in younger age groups has gradually increased during the past 20 years and recently been emphasized.[24] Mental health of young females is important for preventing psychiatric disease, though the manner in which stress is felt and its causes vary.

To effectively explore objective indices to help young females with mental health, an understanding of the time lags between a subjective evaluation for accumulating fatigue and biomarkers used for fatigue quantification is required. In the present study, we examined the effects of an objective structured clinical examination (OSCE),[25,26] a test that objectively evaluates skills and attitudes prior to clinical practicums, and generally understood as a uniform psychological and physical stressor, on biomarkers indicating oxidative stress, serotonin, and fatigue in young females.

Methods

Participants

The study participants were third-year female students at a medical university in Japan. A full explanation of the study contents and methods were provided in advance, and all enrolled participants provided informed written consent (Figure 1). Volunteers were recruited under the condition that their menstrual cycle was between 26 and 38 days, based on the cumulative pattern of menstrual cycles presented by Taylor et al .[27] Finally, 16 students who participated in a meeting explaining the study were chosen for inclusion (mean age ± standard deviation [SD]: 21.3 ± 2.1 years, height: 160.3 ± 4.3 cm, weight: 53.1 ± 5.3 kg). None were smokers, based on their self-report. The survey was conducted from December 2015 to January 2016 and time was divided into 4 periods: 1 month, 1 week, and 1 day before, and 1 week after the OSCE. The period of 1 month before was set as the baseline, as that was considered to be a time prior to stress related to the OSCE, while 1 day before was considered to be the time of maximum fatigue. Samples collected on the day of the OSCE might have affected the results of the test, so those were obtained 1 day before based on this ethical reason. The collection times 1 week before and after the OSCE were set as the same day of the week that the OSCE was conducted. The school timetable of a Japanese university typically varies according to the day of the week, thus the internal consistency between physical and psychological stress as part of the daily rhythm, such as wake-up and class times, was considered. In addition, the 1 week after period was set in consideration of previous reports showing that a higher level of u-8-OHdG occurs on the day after the national exam[28] as well as in individuals with depression symptoms,[29] and then remains elevated. Also, the 1 week before period was established so as to compare with the value on the day before the OSCE was conducted, the point at which stress was considered to be maximum. Heavy physical exercise and alcohol consumption the day before the sample collection were restricted. This study was conducted in accordance with the Declaration of Helsinki and approved by the ethical review board of the Faculty of Health and Welfare, Prefectural University of Hiroshima (approval No. 14MH045).

The objective structured clinical examination (OSCE)

For the OSCE, the students were divided into groups of 5 and each group visited 3 different testing stations. While at a

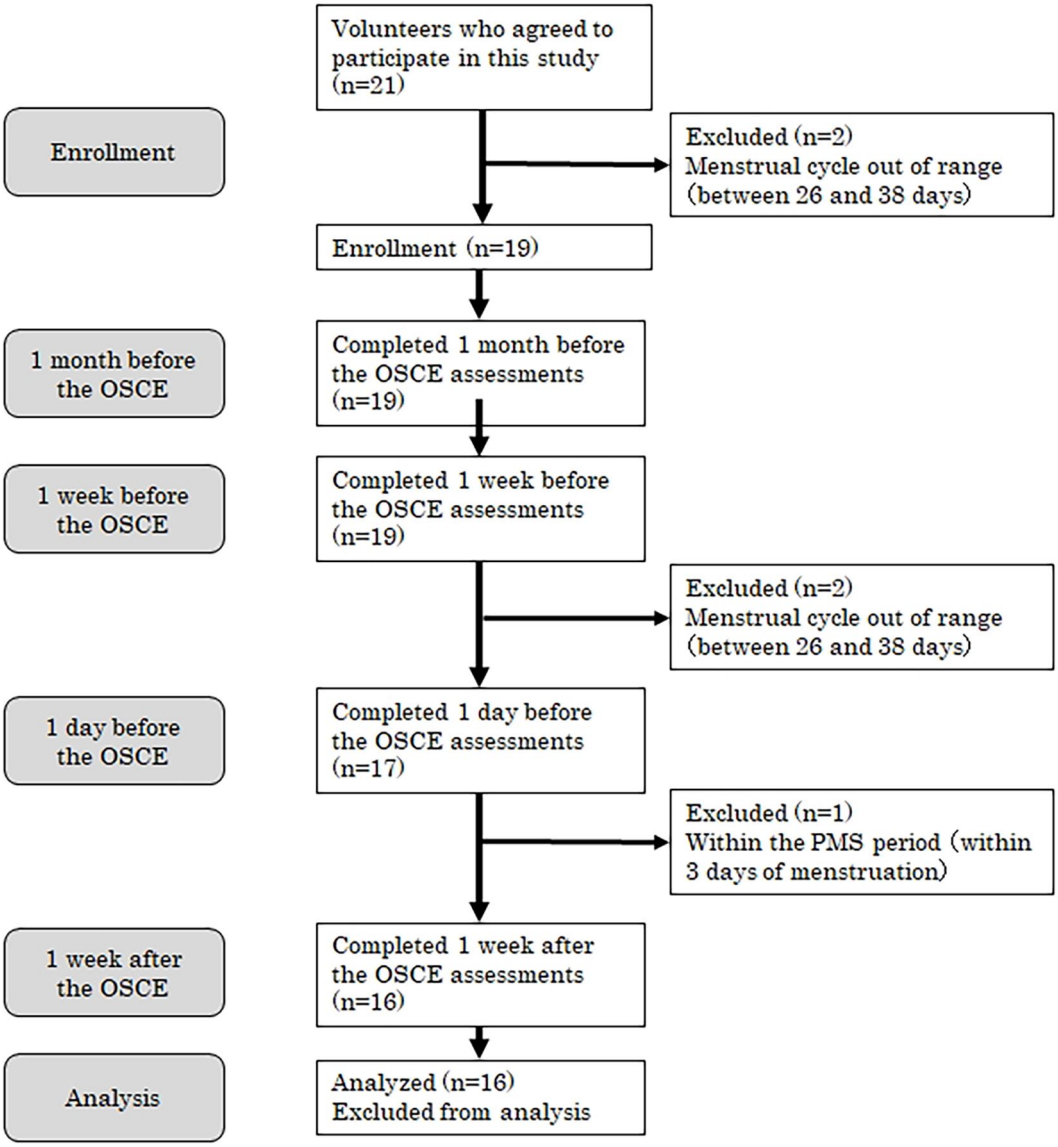

Figure 1. Subject enrollment flow diagram.

station, each student took the practical test for a total of 10 minutes. Specifically, the examinee was first given 1 minute to read the case description and then 9 minutes to demonstrate the role of a therapist. A physical therapist working at an outside facility and not acquainted with the students took the role of the patient. Two faculty members who were licensed physiotherapists observed each student's performance and evaluated it according to a pre-prepared checklist. The other 4 students in the same group also observed the examinee student by surrounding them nearby. During the 10-minute practical test, the examinee was not allowed to interact with anyone other than the patient-role physical therapist. The examination was recorded by a video camera for use as feedback after the OSCE.

The OSCE was used as a final exam given after taking a series of courses. Students were required to attend 15 lectures of 90 minutes each and then pass the OSCE to receive credit for the course. Passing the OSCE requires communication skills, and professional evaluation and treatment skills, which the students had studied for approximately 15 weeks. They were given a large number of possible questions in advance, and based on these questions, practiced intensively during and outside of the lecture times to prepare for the OSCE. Therefore, on the day of the examination, each student was expected to be in a state of chronic physical fatigue due to preparation for the examination as well as mental tension from anticipation. Variables of autonomic modulation based on analysis of heart rate variability in the temporal, frequency, and non-linear domains, subjective perception of distress train, and academic performance were determined before and after the 2 different evaluations that composed the OSCE. Additionally, decreasing sympathetic activity was measured.[30]

Survey measures

A self-administered questionnaire survey consisting of Zung's Self-rating Depression Scale (SDS) and the State-Trait Anxiety

Inventory (STAI) was conducted as a measure of subjective stress, and stress-related biomarkers (urine and saliva samples) were collected at 1 month, 1 week, and 1 day before, and 1 week after the OSCE. In consideration of the effects of premenstrual syndrome, the 3 days before the next menstruation (self-reported) were avoided.[31] The u-8-OHdG level may be elevated during the proliferation period,[32] and a significantly low SDS score in the secretory phase and high level of u-8-OHdG in young females with depression symptoms in comparison to those in subjects without depression symptoms have been reported.[28] From the above, the 3 days before the next menstruation (self-reported) were avoided. The SDS contains 20 items and its design is based on the diagnostic criteria for depression. The participants rated each item with regard to how they have felt during the past several days using a 4-point Likert scale. The raw sum score of the SDS ranges from 20 to 80, though the results are usually presented as SDS index, which is obtained by expressing the raw score converted to a 100-point scale.[33] The self-evaluation STAI questionnaire,[34] developed by Spielberger in 1983, includes separate scales for measuring State anxiety and Trait anxiety.[35] The self-evaluation STAI questionnaire, an internationally validated questionnaire related to levels of test anxiety,[36] has been shown to have good internal consistency and test-retest reliability.[37] The anxiety items used for the present study were scaled from 1 to 4, with higher scores indicating the presence of greater levels of anxiety. On the other hand, anxiety-absent items were scaled in reverse from 4 to 1. The total score for the STAI ranged from a minimum of 20 to a maximum of 80. The levels of u-8-OHdG, urinary 5-HT (u-5HT), and salivary HHV-6 (s-HHV-6) were measured as stress-related biomarkers. All urine and saliva samples were collected between 12:00 and 13:00, before the participant had lunch. Prior to collecting the samples, it was confirmed orally that the participant had not engaged in any strenuous physical activity the day before. Urine specimens used for measuring u-8-OHdG levels were centrifuged at 1500 rpm for 5 minutes after collection, with the supernatant then frozen and stored at −20°C. u-8-OHdG levels were measured using a Highly Sensitive ELISA kit for 8-OHdG (Japan Institute for the Control of Aging, NIKKEN SEIL. Co., Ltd. Shizuoka, Japan). For urine 5-HT measurements, urine samples were examined with an All Species Serotonin ELISA kit (LifeSpan BioSciences, Inc., Seattle, WA, USA). For each sample, the calculated value was obtained by dividing by the amount of creatinine (u-8-OHdG: ng/mgCr, u-5-HT: ng/mgCr). All concentration measurements were triple-checked for maintaining accuracy (R^2 = 0.91-0.96, CV = 0.022-0.025). Saliva samples were used to measure s-HHV-6 and collected with a Salivette® (SARSTEDT AG & Co. KG, Nümbrecht, Germany). They were then centrifuged for 15 minutes at 1500 rpm, and frozen and stored at −20°C. s-HHV-6 reaction was assessed by extracting all DNA from the saliva sample, then quantifying the amount of s-HHV-6 DNA using a real-time quantitative polymerase chain reaction (PCR) assay. The number of HHV-6 copies in 1 mL of saliva (copies/mL) was obtained using an *in vitro* real-time amplification test (HHV-6 Real-TM Quant; Sacace Biotechnologies Srl, Como, Italy).

Statistical analysis

The sample size was determined according to the change of u-8-OHdG level by the national license examination using unified power. Based on the results of a prior study conducted by Iida et al[28] on the relationship between before and after the national license examination and after considering M1 = 7.5 (mean u-8-OHdG level of before the national license Examination), SD 1 = 1.95, M2 = 11.4 (mean u-8-OHdG level of after the national license Examination), SD2 = 1.30, correlation coefficient = .36, two-sided α = .05, and power = 95%, the minimum number of participants was determined to be 6, which was increased to 16 to take account for potential attrition.[38,39] The effects of psychological stress on subjective stress and stress-related biomarkers were investigated using one-way repeated-measures analysis of variance (ANOVA) and Friedman's test. One-way repeated-measures ANOVA was conducted by considering the 4 periods before and after the OSCE (intra-individual level at 1 month, 1 week, 1 day before, and 1 week after) as a single factor, and subjective stress (SDS and STAI scores), and u-8-OHdG and u-5-HT levels as dependent variables. Next, multiple comparisons were done using the Bonferroni method. Friedman's test was also performed with the 4 periods before and after the OSCE as a single factor, and s-HHV-6 level as the dependent variable. For multiple comparison analysis, Bonferroni correction for the mean rank obtained from Friedman's test and the number of pairs was performed. The normality of SDS and STAI scores, and u-8-OHdG and u-5-HT levels was confirmed with a histogram together with a Shapiro-Wilk test for normality ($P > .20$). The normality of s-HHV-6 was shown to be less than $P = .05$ with a histogram and the Shapiro-Wilk test, thus no normality was seen. SDS and STAI scores, and u-8-OHdG and u-5-HT levels of the participants are presented as the mean ± SD. For s-HHV-6, median and quartile values are shown. SPSS 25.0 J (IBM Japan, Tokyo) was used for the analyses, with the level of significance set at $P < .05$.

Results

Subjective stress

SDS scores. A significant relationship was noted between SDS scores and the 4 periods before and after the OSCE ($P = .002$, one-way repeated measures ANOVA) (Figure 2). The value seen at 1 week before was significantly higher than that at 1 month before the OSCE ($P = .019$), while a significantly lower value was seen at 1 week after as compared with 1 week before the OSCE ($P = .018$).

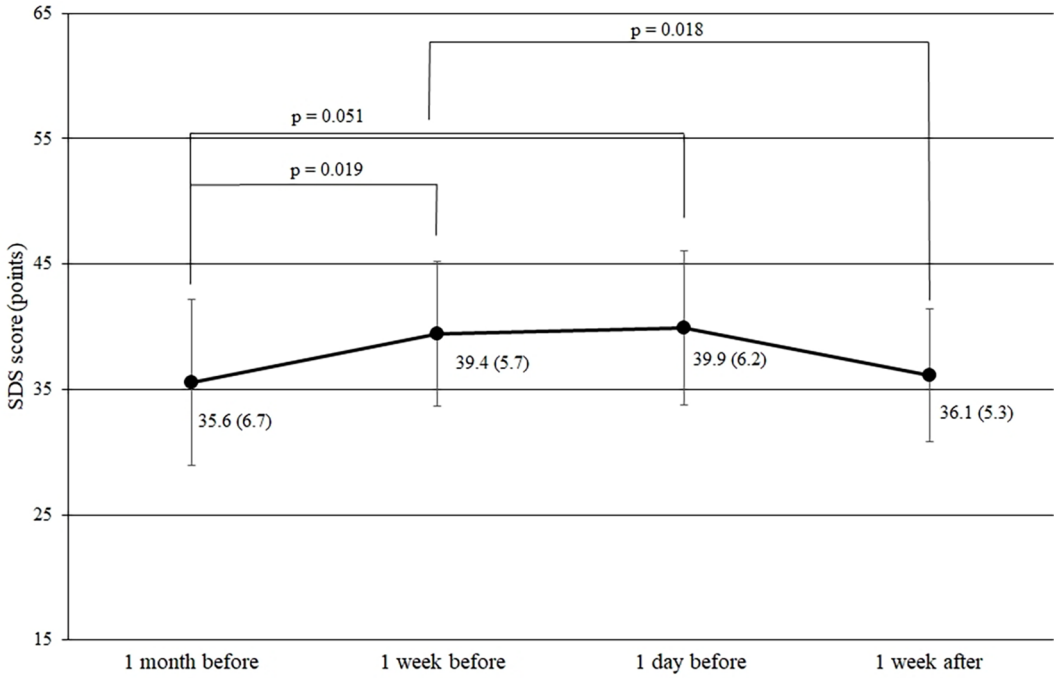

Figure 2. Relationship between SDS scores and 4 periods before and after objective structured clinical examination (OSCE). One-way repeated-measures analysis of variance: $P = .002$. Values are expressed as the mean ± standard deviation (error bars). Bonferroni correction was conducted for each period.
Abbreviations: SDS, self-rating depression scale.

STAI scores. A significant relationship was noted between STAI scores and the 4 periods before and after the OSCE ($P < .001$, one-way repeated measures ANOVA) (Figure 3). The score was significantly higher at 1 week ($P = .004$) and 1 day ($P < .001$) before as compared to 1 month before the OSCE. In contrast, it was significantly lower at 1 week after than 1 day before the OSCE ($P = .004$). Furthermore, the trait anxiety score was significantly higher at 1 week ($P = .031$) and 1 day ($P = .017$) before as compared to 1 month before the OSCE.

Oxidative stress and fatigue biomarkers

U-8-OHdG levels. No significant relationship was noted between u-8-OHdG levels and the 4 periods before and after the OSCE ($P = .300$, one-way repeated measures ANOVA) (Figure 4).

U-5-HT levels. A borderline significant relationship was noted between u-5-HT levels and the 4 periods before and after the OSCE ($P = 0.097$, one-way repeated measures ANOVA) (Figure 5). The level of u-5-HT showed a gradual increase prior to the OSCE, with a tendency for the greatest increase at 1 week after the examination. In addition, the value was significantly higher at 1 week after as compared to 1 week before the OSCE ($P = .076$, adjusted Bonferroni correction).

S-HHV-6 levels. A significant relationship was noted between s-HHV-6 levels and the 4 periods before and after the OSCE ($P = .022$, Friedman's test) (Figure 6). The highest value for s-HHV-6 level was seen at 1 day before the OSCE and that was significantly higher as compared to the value at 1 month before the OSCE ($P = 0.037$, adjusted Bonferroni correction).

Discussion

In the present study, the effects of the OSCE, a uniform source of psychological and physical stress, on biomarkers of oxidative stress and fatigue were examined. The results showed that the level of the oxidative stress biomarker u-8-OHdG was not affected, whereas u-5-HT level (a biomarker of serotonin) and s-HHV-6 (a biomarker of fatigue) levels were elevated before the OSCE. As for u-5-HT, that was gradually increased as the examination approached, with a borderline significant increase observed at 1 week after as compared to 1 week before the OSCE. The level of s-HHV-6 in saliva was below the lower limit of detection at 1 month before the OSCE in nearly all participants, then was significantly increased in more than half at 1 week and 1 day before the OSCE. Based on these results, we considered that the practical skills training conducted on consecutive days leading up to the OSCE facilitated accumulation of physical and psychological stress.[1]

HHV-6 is characterized by its readiness to reactivate during a latent infection and responds even to the relatively weak reactivation stimulus of accumulated fatigue, at which time viruses are reported to be released into saliva.[31,40] In association with accumulation of psychological stress or fatigue, it is known that respiratory infections or herpes labialis outbreaks develop, while immune strength is reported to decrease.[2] Mechanisms

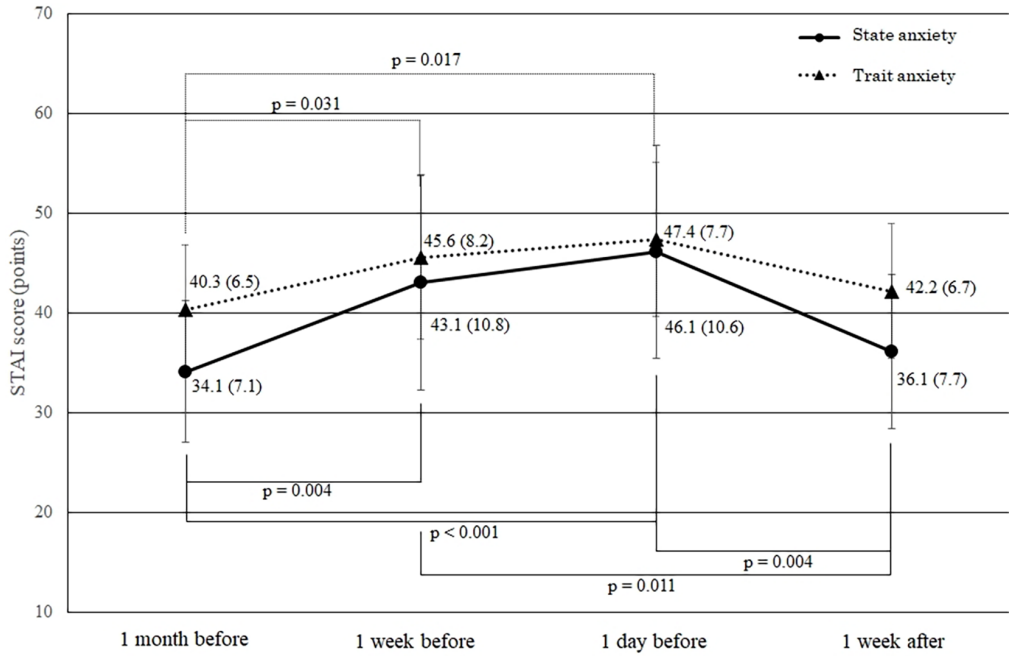

Figure 3. Relationship between STAI scores and 4 periods before and after objective structured clinical examination (OSCE). One-way repeated-measures analysis of variance: $P < .001$ (state and trait anxiety). Solid line indicates STAI scores (state anxiety). Dotted line indicates STAI scores (trait anxiety). Values are expressed as the mean ± standard deviation (error bars). Bonferroni correction was conducted for each period.
Abbreviations: STAI, state–trait anxiety inventory.

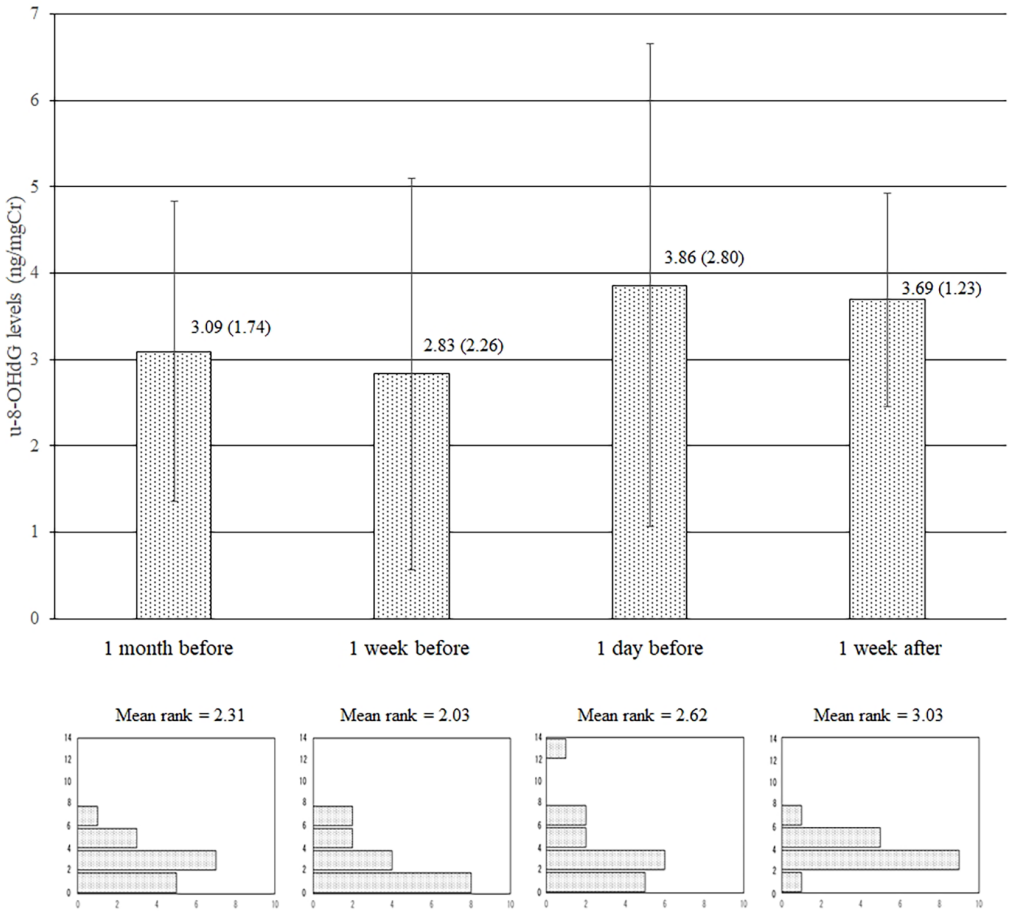

Figure 4. Relationship between u-8-OHdG levels and 4 periods before and after objective structured clinical examination (OSCE). One-way repeated-measures analysis of variance: $P = .300$. Values are expressed as the mean ± standard deviation (error bars).
Abbreviations: u-8-OHdG, urinary 8-hydroxy-2′-deoxyguanosine.

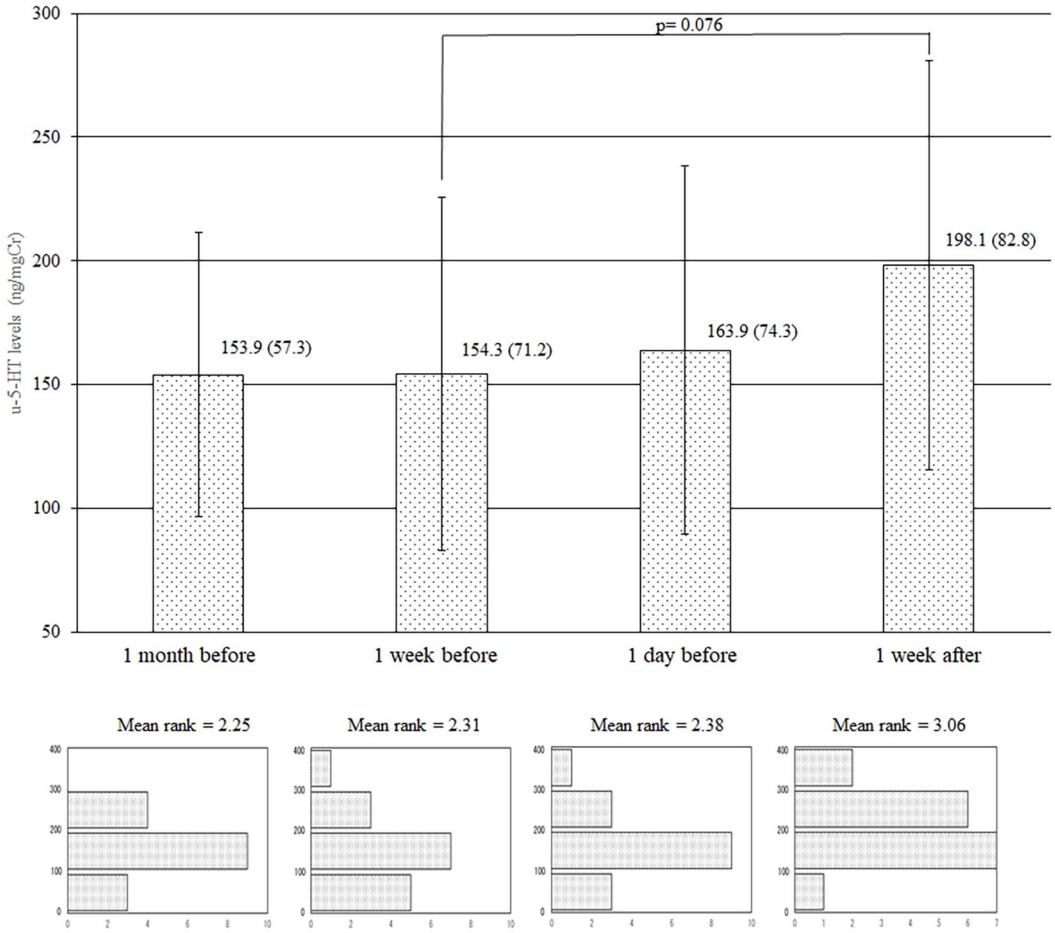

Figure 5. Relationship between u-5-HT levels and 4 periods before and after objective structured clinical examination (OSCE). Upper: u-5-HT levels in 4 periods before and after OSCE. Lower: Two-way analysis of variance by Friedman ranking for 4 periods before and after the OSCE. One-way repeated-measures analysis of variance: $P = .097$. Values are expressed as the mean ± standard deviation (error bars).
Abbreviations: u-5-HT, urinary 5-hydroxytryptamine.

underlying decreased immune strength caused by stress are reported to include activation of immune cells, such as macrophages, granulocytes, and natural killer cells, and increases in cytokines including interleukin (IL), interferon, and tumor necrosis factor (TNF).[41,42] Overproduction of cytokines has been shown to induce reactivation of HHV-6,[40] with immune involvement also suggested to have a relationship with increased detection of s-HHV-6 due to the OSCE.

Urinary 5-HT is mainly produced in enterochromaffin cells of the intestinal mucosa and excreted in blood, and is not highly correlated with serotonin in central nervous system cerebrospinal fluid.[43] Previous reports have shown that urinary 5-HT is rarely increased by psychological stress.[44,45] The present findings as well did not indicate an increase in urinary 5-HT levels 1 day before the OSCE, the period shown to have the greatest increase in psychological stress. However, 1 week after the OSCE, the level of urinary 5-HT showed an increase similar to that seen at the peak SDS and STAI scores. Possible reasons are stress-induced changes in intestinal flora[15,46] and associated regulation by intestinal flora of enterochromaffin cells.[47,48] In addition, it has been reported that 5-HT has a function of suppressing inflammatory cytokines (TNF-alfa, IL-6, etc).[17,49]

The present results showed that the level of s-HHV-6 at 1 day before the OSCE, as well as that and the level of u-5-HT at 1 week after the OSCE were elevated. The s-HHV-6 level was increased in association with high psychological stress (SDS and STAI) scores, while the level of u-5-HT level remained elevated even after the SDS and STAI scores showed decrease. It has been reported that fatigue due to overworking causes elevation of s-HHV-6B level,[1] which is supported by the present data. Also, it is known that elevated s-HHV-6 is related to psychological stress, especially that which can cause depression. Such stress stimulates secretion of HHV-6B from the parotid gland and generates the SITH-CAML complex, which then induces increased CRH, Fkbp5, and REDD1, and a reduction in hippocampal neurogenesis, leading to activation of the HPA axis and inducing a stress response such as depression.[50] HHV-6B has an affinity for CD4+ T cells,[51] and invades the host via the specific human receptor CD134 and the gH/gL/gQ1/gQ2 complex.[52-54] In a previous mouse study, activation of CD4+ T-cells was found to upregulate

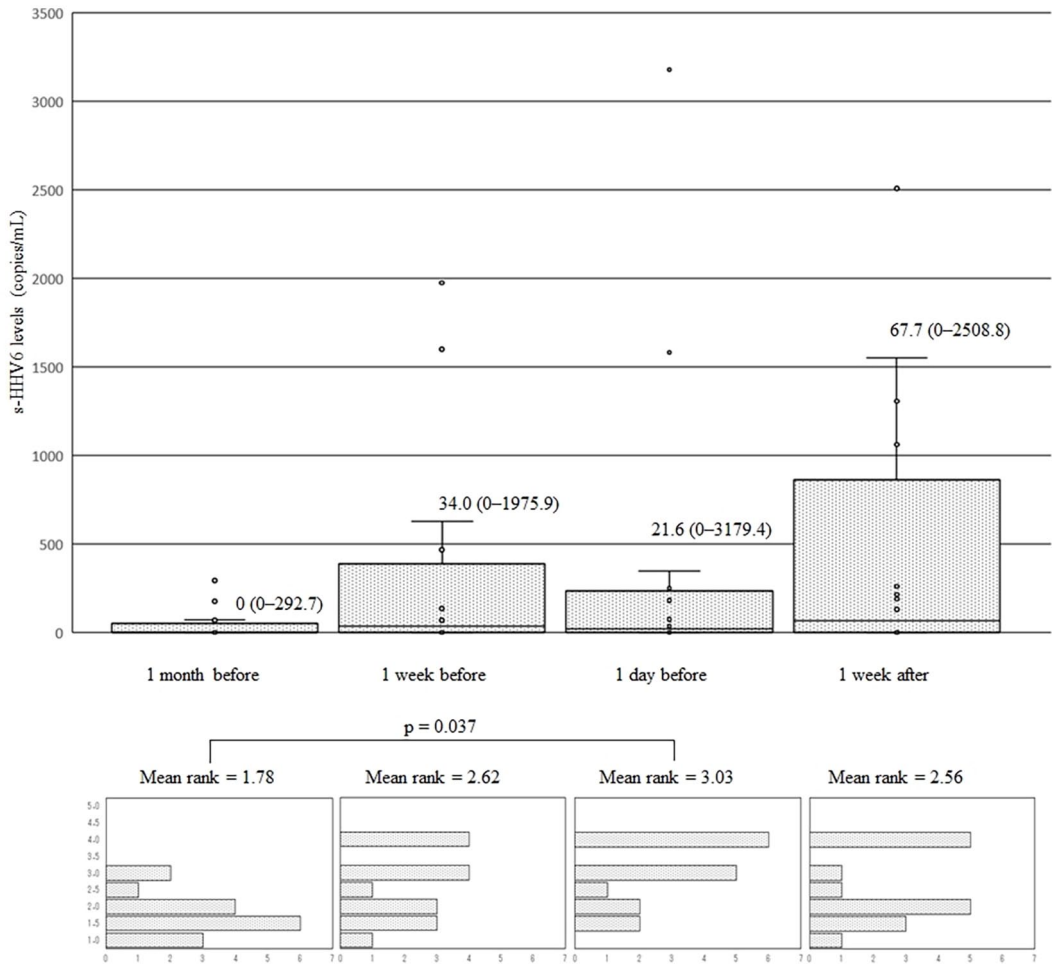

Figure 6. Relationship between s-HHV-6 levels and 4 periods before and after objective structured clinical examination (OSCE). Upper: s-HHV6 levels in 4 periods before and after OSCE. Lower: Two-way analysis of variance by Friedman ranking for 4 periods before and after OSCE. Friedman test: $P = .022$. Horizontal background indicates median –75%, dotted background indicates median –25%. Median and max values are shown. Numbers indicate the median (min–max).
Abbreviations: s-HHV6, salivary human herpesvirus-6.

enterochromaffin (EC) cell-derived 5-HT, though that process in humans has yet to be reported.[47] u-5-HT is mainly generated by and released from EC-cells, with virus infection or intestinal flora considered to be involved in its regulation.[47,48] That then increases the sense of temporal depression via the increase in the s-HHV-6 axis caused by fatigue from testing, such as the OSCE. At the same time, it is considered that s-HHV-6 activates T-cells and the interaction with EC cells of the intestinal mucosa generates 5-HT. Based on these factors, there may be time lags between psychological stress scores (SDS, STAI), and s-HHV-6 and u-5-HT level changes.

The present findings showed that the OSCE is a psychological stressor, based on SDS and STAI, both of which are psychological stress tests, though it did not cause increases in u-8-OHdG levels. Psychological stress is reported to induce ROS increases,[9-11] while it has also been shown that u-8-OHdG levels rise together with increased oxidative stress in the body.[28] We found a significant correlation between self-rated depression scale (SDS) and u-8-OHdG,[29] suggested that young females with depressive symptoms are in a state of increased oxidative stress. Shimanoe et al[55] reported a weak positive association between perceived stress and urinary excretion of u-8-OHdG, and detected high levels of u-8-OHdG in participants with high levels of perceived (psychological) stress. However, since the present female students showed normal limits in SDS and STAI results, psychological stress caused by the OSCE had no effect to increase urinary 8-OHdG levels. Similar to national examinations and other types of written tests,[49] the OSCE is both a psychological and physical stressor due to the performance examination of attitudes and skills. However, it might be difficult to detect the effects of physical psychological stress caused by the OSCE.

This study has several limitations. First, the subject cohort was comprised of female students from a single university, thus it would therefore be inappropriate to generalize the findings, though their height and weight were similar to those found in a national survey conducted in Japan.[56] In addition, the lifestyles of the students were likely quite similar, unlike the general population, though that may also be related to a higher internal validity of the results. Also, smoking history relied on

self-reporting and nicotine levels in urine may need to be measured in a future study. Furthermore, in consideration of the influence of premenstrual syndrome, no survey was conducted within 3 days before menstruation. However, since premenstrual syndrome can begin up to 10 days before menstruation, its influence cannot be excluded. Although full consideration was given to the confidentiality of the survey content, psychological stress, smoking, and premenstrual syndrome are known to be related.[23,57] Finally, psychological stressors were assessed only with the SDS and STAI, and the participants were not examined by a psychiatrist, thus it cannot be ruled out that some may have been affected by depression. And self-efficacy might have attenuated the SDS and STAI.[58] In a future study, it may be necessary to make judgments about psychological stress together with results from a psychiatric examination. Nevertheless, the findings suggest that s-HHV-6 can be used for objective assessment of psychological and physical stress in young females, including that produced not only by knowledge regarding, but also skill and attitude aspects of the OSCE. Additionally, the relationships of severity of depression and anxiety with levels of biological markers, as well as the correlation of their change rates can vary among individuals and each investigated item. Therefore, the present results cannot be used to show a relationship of depression and anxiety severity with levels of biological markers. In the future, we intend to examine the relationship over time, including the time lag for increased rates of biomarker levels found in the present study, and scales indicating the severity of depression and anxiety.

Conclusion

This study of 16 healthy young females revealed responses by s-HHV-6 related to the OSCE, but no significant oxidative stress responses. Psychological factors (depression, anxiety) are known to be sensitive to stress. Although the OSCE did not show effects on u-8-OHdG levels, a biomarker of oxidative stress, the levels of s-HHV-6, a biomarker of fatigue, were elevated. In addition, an increase in the levels of u-5-HT was detected at 1 week after the examination. Our results suggest that s-HHV-6 can be used for objective assessment of fatigue caused by psychological and physical stress in young females, including that produced not only by knowledge of, but also skill and attitude aspects of the OSCE. In addition, they provide evidence that fatigue biomarkers can be utilized for considering the status of students under psychological and physical stress conditions.

Author Contributions

TI conceived the original idea for the study, performed analyses of the data, and drafted the manuscript. YI and TM measured the biomarkers, and provided comments regarding the manuscript. MK, SM, and YY contributed to interpretation of data and critical comments, and revised the manuscript. AT, TH and SE commented on findings from a psychological perspective, and revised the manuscript. All authors have approved the final version.

Consent for Publication

All respondents provided consent to publish the findings prior to completing the questionnaires.

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