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*Original Article*

# Comparison of Urinary Levels of 8-Hydroxy-2'-deoxyguanosine between Young Females with and without Depressive Symptoms during Different Menstrual Phases

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**This study aimed to clarify the association between depressive symptoms and a marker of oxidative stress-induced DNA damage in young females. Since the menstrual cycle may confound or modify this association, depressive symptoms and urinary levels of 8-hydroxy-2' deoxyguanosine (8-OHdG) were evaluated during each menstrual phase. A total of 57 female fourth-year students (aged 21.6 ± 0.8) from a Japanese health science university were studied. The menstrual cycle was divided into 3 phases: menstrual (days 1 to 3 after the onset of menses); proliferative (days 13 to 15); and secretory (days 24 to 26). Depressive symptoms were assessed by the self-rating depression scale (SDS). Positive depressive symptoms were defined as a score of 53 or more during 2 different menstrual phases. The association between the presence of depressive symptoms and 8-OHdG levels adjusting for the menstrual cycle was examined by two-way analysis of variance with the menstrual cycle (menstrual, proliferative, and secretory phases) as the within-individual factor. The menstrual cycle did not show a significant correlation with urinary 8-OHdG levels. On the other hand, the menstrual cycle-adjusted 8-OHdG level was significantly higher in those with depressive symptoms (7.01 ng/mL) than in those without them (3.98 ng/mL). The ROC curve analysis showed that urinary 8-OHdG levels had reasonably high discriminative performance throughout all the menstrual cycles (0.73–0.81; all $p < 0.05$). These results indicated the presence of oxidative stress in subjects with depressive symptoms independent of the menstrual cycle.**

**Key words:** depression, 8-OHdG, menstrual cycle

There is a growing interest in establishing physiological indices related to depression. Several biomarkers have been shown to be relevant to depression, including C-reactive protein, cytokines, neopterin, malondialdehyde, 8-hydroxy-2' deoxyguanosine and isoprostanes (for review see [1]). Recently, an association was reported between depression and uri-

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nary 8-OHdG levels, a marker of oxidative stress to DNA. Ishihara *et al.* examined the association between the prevalence of depression and urinary 8-OHdG levels in female nurses, and reported that urinary 8-OHdG levels were higher in depressive nurses [2]. Similarly, in a study conducted by Forlenza *et al.* involving 169 youths (32 males and 137 females), the depressive group showed significantly higher serum 8-OHdG levels than the control group, independently of sex and age [3]. On the other hand, in a study conducted by Yi *et al.* involving 210 female workers, no significant correlation was observed between depressive symptoms and urinary 8-OHdG levels [4]. In short, inconsistencies exist regarding the association between depression and 8-OHdG levels. One of the explanations may be that in these previous studies, the menstrual cycle was not examined despite its possible influences on the association between depression and oxidative stress markers. In fact, Matsumoto *et al.* compared the urinary 8-OHdG levels of 205 female workers during different menstrual phases, and found that the levels tended to increase during the proliferative phase, although the difference was not statistically significant [5]. On the other hand, it has been reported that 20% to 50% of healthy females experience mental symptoms such as depressed feelings, anxiety, and restlessness 7 to 10 days before the onset of menses [8]. We previously reported that stress influenced the secretion of female hormones during menstruation [6], that anxiety symptoms appeared before menstruation in females with depression, and that the anxiety symptoms were correlated with the urinary 8-OHdG and serum serotonin (s-serotonin) levels [7]. This correlation should be investigated further, with consideration paid to variation in the acceptance of stress among individuals and individuals' uniformity. However, few studies have evaluated stress/stress response biofactors with respect to the menstrual cycle, or investigated the association between anxiety, which is characteristic of depressive symptoms, and stress response biofactors. Depending on the findings of such analyses, it might be necessary to consider the menstrual cycle when examining the association between depression and urinary 8-OHdG levels in females.

Therefore, this study examined the association between depression and urinary 8-OHdG levels while taking the menstrual cycle into consideration.

## Materials and Methods

***Participants.*** Volunteers who self-reported having normal menstrual cycles were recruited. Among 60 fourth-year female students of a Japanese health science university who were provided with sufficient explanations regarding the study's objective and methods, 58 consented to participate. Informed consent was acquired from all the participants in written form. One student whose menstrual cycle did not meet the inclusion range of 26 to 37 days was excluded, leaving 57 students in the final study group [9, 10]. This study was conducted in accordance with the Helsinki Declaration with the approval of the Ethics Committee of Fujita Health University (approval number: 10-075).

***Study items.*** This study was conducted from June to September 2009. Participants' menstrual cycles were self-reported during the study period, and were divided into 3 phases: menstrual (days 1 to 3 after the onset of menses); proliferative (days 13 to 15); and secretory phase (days 24 to 26). self-rating depression scale (SDS) scores and urinary samples were evaluated during each phase.

***Definition of depressive symptoms.*** Those with an SDS score of 53 or more were defined as having depressive symptoms [11]. According to the definition of a major depressive episode in the DSM-IV, which requires the presence of symptoms lasting for at least 2 weeks [12], we considered those who had an SDS score of 53 or more during 2 different menstrual phases to be depressive, and considered the remaining participants as normal in the present study. The SDS and collection of urinary samples each menstrual phase were performed between 12 : 00 and 13 : 00 before lunch.

***Measurement of urinary 8-OHdG levels.*** The collected urinary samples were extracted between 12 : 00 and 13 : 00 before lunch in consideration of the daily fluctuation of each menstrual phase. Oral reports were checked on the day before each collection, and collections were omitted on the days following an intense menstrual flow. The collected urinary samples were centrifuged at 1,500 rounds per minute for 5 min, and the supernatant was stored in a freezer at −20 degrees Celsius until analysis. The urinary 8-OHdG was measured in triplicate (8-OHdG Check Kit; Japan Institute for the Control of Aging

(JaICA); $R^2 = 0.92–0.96$ and CV = 0.021–0.023).

***Data analysis.*** The association between depression and 8-OHdG levels was examined by partial correlation analysis with adjustment for age and BMI. In addition, the association between depression and 8-OHdG levels was examined by a two-way analysis of variance with the presence/absence of depression and the menstrual cycle (menstrual, proliferative, and secretory phases) as factors, and urinary 8-OHdG levels as the dependent variable. A histogram and Kolmogorov-Smirnov test ($p = 0.200$) were used to confirm that the urinary 8-OHdG levels were in the normal range. In addition, an area under the ROC curve (AUC) analysis was performed to determine the overall accuracy of 8-OHdG as an index of the presence/absence of depression in each of the 3 phases. Analysis was performed using SPSS 21.0 J (IBM Japan, Tokyo, Japan), and the significance level was set at $p < 0.05$.

## Results

No significant differences in age, height, or body weight were observed between the depressive and normal groups (Table 1). SDS scores did not vary significantly with the 3 menstrual phases in either group. SDS showed the subjects which was a high value about each menstrual phases and 2 or more time of them. (Table 2). The subjects who the SDS by the classification of menstrual phases was a high value did not vary.

Partial correlation analysis of the relation between urinary 8-OHdG levels and the SDS scores adjusted for age and BMI revealed a significantly positive correlation between the 2 parameters in each of the menstrual phases (Table 3). Similarly, there were no significant differences in urinary 8-OHdG levels by the menstrual phases in either group (Fig. 1; $p = 0.529$, *p for interaction* = 0.863). On the other hand, the mean urinary 8-OHdG level adjusted for the menstrual cycle was significantly higher in the depressive group (7.01 ng/mL) compared to the normal group (3.98 ng/mL) ($p = 0.040$).

**Table 2** Characteristics of the subjects of the Normal (SDS < 53) and Depressive (53 ≦) groups with respect to the menstrual cycle

| | SDS | |
|---|---|---|
| | < 53 | 53 ≦ |
| Menstrual phase | 49 | 8 |
| Proliferative phase | 50 | 7 |
| Secretory phase | 48 | 9 |
| Combination of menstrual and proliferative phases | 55 | 2 |
| Combination of proliferative and secretory phases | 55 | 2 |
| Combination of menstrual and secretory phases | 54 | 3 |
| Combination of all phases | 54 | 3 |

SDS, self-rating depression scale: < 53, SDS scale is less than 53 scores: 53 ≦, SDS scale is 53 or more scores.

**Table 3** Relationship between the urinary 8-OHdG levels and SDS scores by menstrual phase (adjusted for age and BMI)

| | Partial correlation coefficient | *p* value |
|---|---|---|
| Menstrual phase | 0.563 | 0.012 |
| Proliferative phase | 0.472 | 0.041 |
| Secretory phase | 0.56 | 0.013 |

partial correlation coefficient: adjusted for the age and BMI

**Table 1** Age, anthropometric variables, and SDS scores in the Normal and Depressive Groups

| | | Normal (n = 47) | | | Depressive (n = 10) | | | |
|---|---|---|---|---|---|---|---|---|
| | | Mean | SD | | Mean | SD | | *p* |
| Age (years) | | 21.5 | 0.7 | | 22.0 | 0.9 | | 0.050 |
| Height (cm) | | 158.6 | 4.6 | | 160.1 | 5.1 | | 0.361 |
| Body weight (kg) | | 51.2 | 6.3 | | 49.1 | 4.8 | | 0.325 |
| BMI (kg/cm$^2$) | | 20.3 | 2.3 | | 19.1 | 1.3 | | 0.118 |
| SDS | Menstrual phase | 42.2 | 6.2 | | 55.1 | 4.2 | | < 0.001 |
| | Proliferative phase | 41.9 | 5.7 | † | 52.3 | 9.2 | ‡ | < 0.001 |
| | Secretory phase | 41.4 | 5.7 | | 52.9 | 7.8 | | < 0.001 |

One-factor repeated measures analysis of variance: † $p = 0.865$, ‡ $p = 0.361$
SD, standard deviation; BMI, body mass index; SDS, self-rating depression scale.

The AUC was 0.81 ($p = 0.005$) during the menstrual, 0.73 ($p = 0.038$) during the proliferative, and 0.80 ($p = 0.006$) during the secretory phases (Fig. 2).

## Discussion

Urinary 8-OHdG levels were significantly higher in the depressive group independent of the menstrual cycle, and partial correlation analysis between urinary 8-OHdG levels and the SDS scores revealed a significant positive correlation in each menstrual phase; these findings were consistent with the previous studies [1, 3]. This is, to our knowledge, the first study on the relation between urinary 8-OhdG and depression to take the menstrual cycle into account. An increased reactive oxygen level due to psychological stress [13, 14] might have led to an increase in the urinary 8-OHdG level [15]. Indeed, reactive oxygen species (ROS) are produced in response to the secretion and decomposition of stress hormones, and specifically of adrenocortical hormones [16]. In addition, large amounts of vitamin C are consumed under stress as antioxidants [17], and this process is generally thought to lead to a further increase of ROS. Further, reperfusion to tissues exposed to reduced blood flow due to vasoconstriction caused by sympathetic nervous activation has been associated with increased production of ROS [18–20]. Subjects with depressive symptoms have been reported to exhibit high susceptibility to sympathetic nervous [21–25] and high sympathetic nervous activity [26–28] in response to stress. The increase in ROS by the activated sympathetic nervous system might cause oxidative damage to the DNA of the person experiencing depressive symptoms. Alternatively, slower recovery of lymphocyte DNA from X-ray-induced damage has been reported in depressive individuals compared to the healthy ones [29]. Although the present study was not carried out to identify mechanisms explaining the association between depression and urinary 8-OHdG levels, it did reveal that those with depressive symptoms often have increased urinary 8-OHdG levels, possibly due to increased ROS levels or slower recovery of DNA damage. Forlenza *et al.* examined the association between depression and serum 8-OHdG in 169 young persons (males/females) using covariance analysis and a trend test after dividing them into depression and comparison groups and adjusting the results by gender and age. They reported that the mean serum 8-OHdG level was significantly higher in the depression group

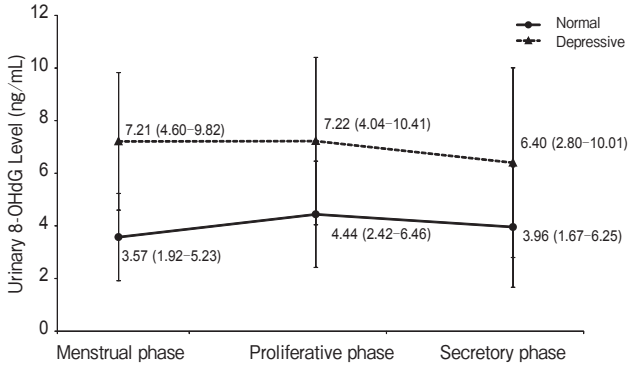


**Fig. 1** Comparison of Urinary 8-OHdG Levels between the Normal and Depressive Symptoms by Menstrual Phases. The solid line indicates the results for normal individuals, the dotted line the results for individuals with depressive symptoms. Two-way factorial analysis of variance with the presence/absence of depression and menstrual cycle as factors (adjusted for the age and BMI).
Depression: $p = 0.040$; menstrual cycle: $p = 0.529$; interaction: $p = 0.863$
Adjusted mean: depressive: 7.01 ng/mL; normal: 3.98 ng/mL.

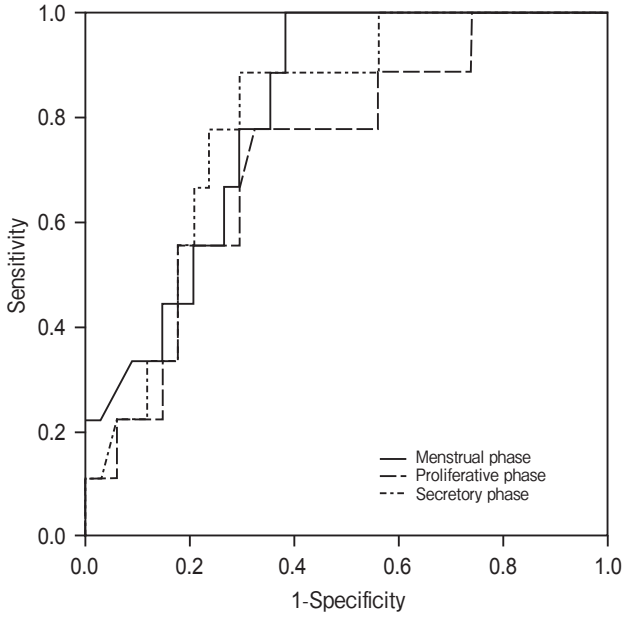


**Fig. 2** ROC Curve during Each Menstrual Phase. The solid line shows the results for the menstrual phase, the dashed line those for the proliferative phase, and the dotted line those for the secretory phase. The AUCs (SDs) and *p*-values in each phase were 0.81 (0.07), 0.005 (menstrual); 0.73 (0.90), 0.038 (proliferative); and 0.80 (0.72), 0.006 (secretory), respectively.

[3]. Although our present results were limited to young women, our findings were similar to those of the previous studies [3, 7], and thus further clarify the association between depression and urinary 8-OHdG. In addition, we found that these factors were not influenced by the menstrual cycle. Therefore, the results suggest that urinary 8-OHdG is useful for the early detection of depression in young women, and that this parameter is not influenced by the menstrual cycle. Based on previous studies [3, 7], 8-OHdG may be a depression-associated marker. In addition, this tendency may be present not only in young females but also in those of other ages and males. Based on the results of this study, the use of urinary 8-OHdG for objective assessment along with SDS and other questionnaires for subjective assessment may contribute to the early detection of depression in young women and the arrangement of the lifestyle/environment/support system to assist persons with depressive symptoms in schools and workplaces.

There were several limitations in the present study. First, our sample was limited to female students of a single university, and thus it would be inappropriate to generalize the findings, although the participants' height and weight were similar to those of a Japanese national survey [30]. In addition, the students' lifestyles were likely to be uniform, unlike lifestyles in the general population, and while this homogeneity was likely to have contributed to the internal validity of the results, it also make our findings less generalizable. Nonetheless, the present findings clearly revealed a potential association between depression and premenstrual syndrome in the secretory phase. Second, our participants' smoking habits were self-reported. Although the participants were informed of the strict confidentially of this study, it is possible that some of them misreported. In future studies, it would be preferable to obtain urinary or salivary cotinine levels. Third, the collected urinary samples were extracted between 12 : 00 and 13 : 00 before lunch in consideration of daily fluctuations in the levels of the parameters measured. Since these samples were collected as spot urine samples, in future it might be useful to add creatinine correction or generation speed correction. Fourth, we evaluated the presence of depressive symptom based only on SDS scores. As there were no confirmatory diagnoses from psychiatrists, false-positive or -negative depression might have been present, which could have distorted the association. In an attempt to avoid systematic over- or under-diagnosis, we performed sensitivity analyses by changing the cut-off point of the SDS score. In comparison of the normal and the depressive which considered only in 1 time of a menstrual cycle as cutoff of 53 (Student's *t*-test), the menstrual phases 3 periods was that the depression was a high value (menstrual phase: normal, 4.1 ng/mL; depressive, 7.3 ng/mL ($p = 0.006$); proliferative phase: normal, 4.4 ng/mL; depressive, 7.9 ng/mL ($p = 0.003$); secretory phase: normal, 3.8 ng/mL; depressive, 6.8 ng/mL ($p = 0.016$)). Even when adopting a lower score of 40 [31], the depressive-group participants had higher urinary 8-OHdG levels (6.6 ng/mL) than in the normal group (3.2 ng/mL). ROC curve analysis yielded similar results: the AUCs during the menstrual, proliferative, and secretory phases were 0.73, 0.77, and 0.67, respectively. In future studies, however, it will be necessary to have depression evaluated by psychiatrists. In conclusion, the results of this study confirmed that SDS scores are associated with urinary 8-OHdG levels independent of the menstrual cycle. Although this study was cross sectional, it would appear to suggest that young females with depressive symptoms are in a state of increased oxidative stress.

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## References

1. Lopresti AL, Maker GL, Hood SD and Drummond PD: A review of peripheral biomarkers in major depression: the potential of inflammatory and oxidative stress biomarkers. Prog Neuropsychopharmacol Biol Psychiatry (2014) 48: 102–111.
2. Ishihara I, Nakano M, Ikushima M, Hara Y, Yoshimine T, Haraga M, Nakatani J, Kawamoto R and Kasai H: Effect of work conditions and work environments on the formation of 8-OH-dG in nurses and non-nurse female workers. J UOEH (2008) 30: 293–308.
3. Forlenza MJ and Miller GE: Increased serum levels of 8-hydroxy-2'-deoxyguanosine in clinical depression. Psychosom Med (2006) 68: 1–7.
4. Yi S, Nanri A, Matsushita Y, Kasai H, Kawai K and Mizoue T: Depressive symptoms and oxidative DNA damage in Japanese municipal employees. Psychiatry Res (2012) 200: 318–322.
5. Matsumoto Y, Ogawa Y, Yoshida R, Shimamori A, Kasai H and Ohta H: The stability of the oxidative stress marker, urinary 8-hydroxy-2'-deoxyguanosine (8-OHdG), when stored at room temperature. J Occup Health (2008) 50: 366–372.
6. Chikamura C, Iida T, Ishizaki F, Aoi S, Kobayashi T and Kataoka

T: The relationship between stress levels and biological responses in a clinical nursing practicum. Hiroshima J Med Sci (2008) 57: 93–98.
7. Iida T, Chikamura C, Inoue K, Ito Y, Ishikawa H, Teradaira R and Ono Y: Association of STAI and SDS scores with 8-hydroxydeoxyguanosine and serotonin levels in young women with depressive symptoms. J Neuropsychiatry Clin Neurosci (2011) 23: E10.
8. Johnson SR: The epidemiology and social impact of premenstrual symptoms. Clin Obstet Gynecol (1987) 30: 367–376.
9. Michell D: Reproductive endocrinology; in Comprehensive Gynecology, Herbst AL, Mixhell DR, Stenchever MA and droegemueller W, 2nd Ed, Mosby Year Book Inc, St Louis (1992) pp121.
10. Taylor JR, Combs-Orme T, Anderson D, Taylor DA and Koppenol C: Alcohol, hypertension, and stroke. Alcohol Clin Exp Res (1984) 8: 283–286.
11. Fukuda K KS: The study of the Zung Self-rating Depressive Scale. Seishin Shinkeigaku Zasshi (1973) 75: 673–679 (in Japanese).
12. American Psychiatric Association: Diagnostic and statistical manual of mental disorders; DSM-IV 4th Ed, American Psychiatric Association, Washington (DC) (1994) pp866.
13. Atanackovic D, Brunner-Weinzierl MC, Kroger H, Serke S and Deter HC: Acute psychological stress simultaneously alters hormone levels, recruitment of lymphocyte subsets, and production of reactive oxygen species. Immunol Invest (2002) 31: 73–91.
14. Miyaoka T, Yasukawa R, Yasuda H, Shimizu M, Mizuno S, Sukegawa T, Inagaki T and Horiguchi J: Urinary excretion of biopyrrins, oxidative metabolites of bilirubin, increases in patients with psychiatric disorders. Eur Neuropsychopharmacol (2005) 15: 249–252.
15. Zhang Y, Jiang L, Jiang L, Geng C, Li L, Shao J and Zhong L: Possible involvement of oxidative stress in potassium bromate-induced genotoxicity in human HepG2 cells. Chem Biol Interact (2011) 189: 186–191.
16. Shiga N: Changes of plasma cortisol and inflammatory cytokine (IL-1 $\beta$ and TNF-$\alpha$) in depressive women. Nihon Jyosei Shinshinigaku Zasshi (2011) 16: 89–94 (in Japanese).
17. Hiraoka A: A Biochemical Study of the Mechanisms of Oxidative Damage Caused by Mental Stress. Current biofeedback research in Japan (2003) 29: 29–34.
18. Clempus RE and Griendling KK: Reactive oxygen species signaling in vascular smooth muscle cells. Cardiovasc Res (2006) 71: 216–225.
19. Mehta PK and Griendling KK: Angiotensin Ⅱ cell signaling: physiological and pathological effects in the cardiovascular system. Am J Physiol Cell Physiol (2007) 292: C82–97.
20. Taniyama Y and Griendling KK: Reactive oxygen species in the vasculature: molecular and cellular mechanisms. Hypertension (2003) 42: 1075–1081.
21. Habets P, Collip D, Myin-Germeys I, Gronenschild E, van Bronswijk S, Hofman P, Lataster T, Lardinois M, Nicolson NA, van Os J and Marcelis M: Pituitary volume, stress reactivity and genetic risk for psychotic disorder. Psychol Med (2012) 42: 1523–1533.
22. Hankin BL, Wetter EK and Flory K: Appetitive motivation and negative emotion reactivity among remitted depressed youth. J Clin Child Adolesc Psychol (2012) 41: 611–620.
23. Morris MC, Rao U and Garber J: Cortisol responses to psychosocial stress predict depression trajectories: social-evaluative threat and prior depressive episodes as moderators. J Affect Disord (2012) 143: 223–230.
24. Morris MC, Rao U, Wang L and Garber J: Cortisol Reactivity to Experimentally Manipulated Psychosocial Stress in Young Adults at Varied Risk for Depression. Depress Anxiety (2014) 31: 44–52.
25. Rao U, Hammen C, Ortiz LR, Chen LA and Poland RE: Effects of early and recent adverse experiences on adrenal response to psychosocial stress in depressed adolescents. Biol Psychiatry (2008) 64: 521–526.
26. Barton DA, Dawood T, Lambert EA, Esler MD, Haikerwal D, Brenchley C, Socratous F, Kaye DM, Schlaich MP, Hickie I and Lambert GW: Sympathetic activity in major depressive disorder: identifying those at increased cardiac risk? J Hypertens (2007) 25: 2117–2124.
27. Sanchez-Gonzalez MA, May RW, Koutnik AP, Kabbaj M and Fincham FD: Sympathetic Vasomotor Tone Is Associated With Depressive Symptoms in Young Females: A Potential Link Between Depression and Cardiovascular Disease. Am J Hypertens (2013) 26: 1389–1397.
28. Veith RC, Lewis N, Linares OA, Barnes RF, Raskind MA, Villacres EC, Murburg MM, Ashleigh EA, Castillo S, Peskind ER, Pascualy M and Halter JB: Sympathetic nervous system activity in major depression. Basal and desipramine-induced alterations in plasma norepinephrine kinetics. Arch Gen Psychiatry (1994) 51: 411–22.
29. Kiecolt-Glaser JK, Stephens RE, Lipetz PD, Speicher CE and Glaser R: Distress and DNA repair in human lymphocytes. J Behav Med (1985) 8: 311–320.
30. Health and Welfare Statistics Association: Journal of Health and Welfare Statistics Kosaido Co., Ltd, Tokyo (2009) 56: 454 (in Japanese).
31. Zung WW: A Self-Rating Depression Scale. Arch Gen Psychiatry (1965) 12: 63–70.