response on BPRS during the study, responders were defined as patients showing a reduction of greater than 20% from baseline. Extrapyramidal symptoms (EPS) were evaluated using the Extrapyramidal Symptom Rating Scale (ESRS: 0–257 point that is summed from all of the factors including the last four sections of clinical impressions: Chouinard and Margolese, 2005). Compliance with treatment medication was monitored using by both a self-rating visual analog scale for patient and objective observation by their respective physicians, which rated medication administration from 0 to 100% (Garfield et al., 2011). If these measurements differed from each other by no more than 25%, the mean of both values was used as the patient's adherence rate. To reliably evaluate with these measurements, physicians on the study underwent several rounds of assessment training.

2.4. Statistical analysis

All analyses were conducted using SPSS, version 19.0 (IBM, NY, US). Data analyses were conducted on an intent-to-treat basis including all dropout cases (Fig. 1). Analyses for the primary efficacy measure were performed using a mixed-effects model repeated-measures analysis (Gueorguieva and Krystal, 2004). Treatment group, time and each time-by-group interaction were included as fixed effects, while baseline scale scores and age were included as covariates. The within-subject factor was considered as a random effect. Compound symmetry was used.

Logistic regression analyses was also performed to look at the effect of treatment group on the outcome measure of treatment response or nonresponse at T4, with age, sex, duration of illness, baseline BPRS and ESRS scores, treatment adherence and the presence or absence of DSP included as items. Continuous and categorical variables were compared by independent t test and chi-square test, respectively. A P value of .05 was set as the threshold of significance.

3. Results

3.1. Patient characteristics and analysis of drop-out cases

Of the 115 patients screened, 21 patients were excluded due to meeting exclusion criteria, being lost to follow-up or refusing to participate before the evaluation for DSP, yielding a final analytic sample of 94 patients (Fig. 1: DSP group: N = 61, NonDSP group: N = 33).

Baseline demographics and clinical characteristics were similar between the two groups (Table 1). The BPRS positive symptoms score showed no difference between the two groups, whereas the BPRS negative symptoms score and ESRS score in the DSP group were significantly higher than those of the NonDSP group ($P < .001$). A total of 75 patients (79.8%) completed the 12-month RLAI treatment. There was no significant difference in the dropout rates between the two groups: 14.8% (N = 9) in the DSP group and 30.3% (N = 10) in the NonDSP group ($P > .05$). Seven DSP and 7 NonDSP patients left the study due to an exacerbation of psychotic symptoms. Two DSP patients discontinued due to dystonia and akathisia, and 3 NonDSP patients discontinued due to constipation, hyperglycemia and dystonia.

3.2. Treatment with RLAI and other oral antipsychotics, medication adherence

The mean daily total CPZeq-dose of oral antipsychotics at baseline was about 1000 mg in both groups (Table 1). The subjects received quite variable types and combinations of antipsychotics with variable dose ranges. The primary types of antipsychotics used in the present patients were risperidone (1–18 mg), olanzapine (4–40 mg) and quetiapine (200–825 mg). Percent rate of RLAI patients receiving dose of 25 mg, 37.5 mg and 50 mg at T4 was 13.5%, 19.2% and 67.3% respectively in the DSP group, and 13.0%, 21.7% and 65.2% respectively in the NonDSP group. For daily oral antipsychotics dosing (CPZeq-dose), the mean (±SD) doses at T4 were 605 (791) mg/day and 471 (421) mg/day in the DSP group and the NonDSP group, respectively. There was a significant main effect for Time ($F = 9.70$, $P < .001$), but no main effect for Group ($F = 0.37$, $P > .05$) or for an interaction of Time × Group ($F = 0.07$, $P > .05$). There was no significant difference in the total amount of daily oral antipsychotics and RLAI dose (CPZeq), at T4, between the two groups nor were there significant time effects during the treatment between the groups (Table 2).

Mood stabilizers were prescribed for 19 of 61 DSP patients and 14 of 33 NonDSP patients. Among them, 13 DSP patients and 9 NonDSP patients took sodium valproate at T0: their distributions and their mean doses did not differ between the two groups. These doses tended to be lower during the study, though not significantly, in both groups (data not shown). Regarding benzodiazepine and antiparkinsonism agents, none of the groups showed any significant differences either in baseline doses or in dose changes between T0 and T4.

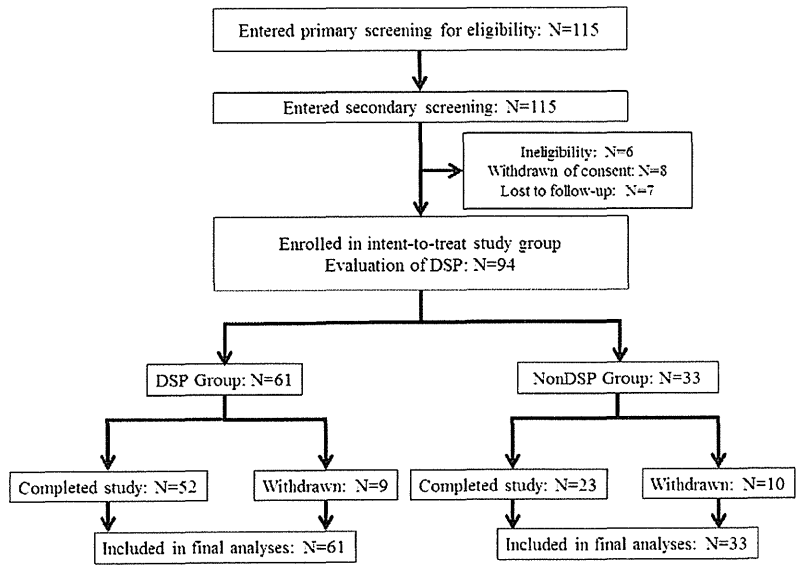

Fig. 1. Overview of participant flow. Initially, 115 patients were screened. Of these, 21 were lost to the study due to meeting the exclusion criteria, being lost to follow-up, or a withdrawal of consent before evaluation of DSP status, yielding a final analytic sample of 94 patients (DSP group: N = 61, NonDSP group: N = 33).

Table 1
Characteristics of eligible participants.

	DSP group N = 61	NonDSP group N = 33	All patients N = 94	Statistical value[c]
Age (years)	43.6 (14.7)	48.5 (11.1)	45.4 (13.7)	N.S.
[Age range]	[18–69]	[26–69]	[18–69]	
Sex (male/female)	30/31	17/16	47/47	N.S.
Duration of illness (years)	20.4 (12.5)	21.2 (11.9)	20.7 (12.3)	N.S.
Inpatient/outpatient	32/29	14/19	46/48	N.S.
Non-responder/intolerance to antipsychotics	57/4	33/0	90/4	N.S.
Diagnosis				
Schizophrenia	58	29	87	
Schizoaffective disorder	3	4	7	
DSP type				
Withdrawal psychosis	41	-	41	-
Tolerant to antipsychotics	35	-	35	-
Relapse with great severity	27	-	27	-
Tardive dyskinesia	24	-	24	-
Antipsychotics dose (CPZeq: mg)	1084.6 (741.4)	960.1(444.1)	1040.4 (651.7)	N.S.
[Dose range]	[0–4512.5]	[200–2050.0]	[0–4512.5]	
BPRS				
Total score	63.0 (18.6)	58.5 (15.7)	61.4 (17.7)	N.S.
Positive symptom score[a]	17.0 (5.5)	16.7 (5.6)	16.9 (5.5)	N.S.
Negative symptom score[b]	13.0 (3.8)	10.8 (3.1)	12.2 (3.7)	P = .004
CGI-S	5.5 (1.1)	5.3 (1.0)	5.4 (1.0)	N.S.
GAF	30.9 (13.1)	32.7 (11.4)	31.5 (12.5)	N.S.
ESRS	34.2 (32.4)	17.8 (17.5)	28.5 (29.1)	P = .001
Adherence	89.2	80.6	86.3	N.S.

Data are mean (SD) [absolute range]. Unless otherwise noted, differences between the DSP and NonDSP groups were not statistically significant (P > .05).
Abbreviations: DSP = dopamine supersensitivity psychosis, CPZeq = chlorpromazine equivalent, BPRS = Brief Psychiatric Rating Scale, CGI-S = Clinical Global Impression Severity, GAF = Global Assessment of Functioning, ESRS = Extrapyramidal Symptom Rating Scale.
[a] The summed scores for conceptual disorganization (#4), suspiciousness (#11), hallucination (#12), and unusual thoughts (#15).
[b] The summed scores for emotional withdrawal (#3), motor retardation (#13), and blunted affect (#16).
[c] Statistical result of each comparison between the DSP and NonDSP groups. Student's t test is applied for continuous variables and the chi-square test is applied for categorical variables.

Adherence to treatment medication, which was measured by a self-administered visual analog scale at T0, T2 and T4, was 89.2%, 92.2% and 90.0% in the DSP group and 80.6%, 86.8% and 88.4% in the NonDSP group, respectively (Table 1). The difference between the self-administered visual analog scale by each patient and assessment of medication adherence rate by his/her physician was within 25% in all patients. Throughout the study period, all patients received RLAI procedures at over 90% of the scheduled visits (once every two weeks).

3.3. Primary outcome measures

Mixed-model analysis of the percentage change in BPRS total scores from baseline to 12 months showed significant improvement in DSP relative to NonDSP patients. This difference was observed from T1 to T4 at each time point analysis (Fig. 2A and Table 2). Average BPRS total scores in both groups were also significantly decreased after the 12-month treatment period (P < .05). Based on percentage changes in BPRS positive and negative symptom scores, DSP patients showed significantly greater improvements compared with NonDSP patients (Fig. 2B, C and Table 2).

Furthermore, we analyzed the percentage BPRS changes only among inpatients with DSP (N = 32) whose adherence was approximately 100%, because they took their medication under staff observation. The results revealed that BPRS scores at T0 and T4 were 68.1 ± 20.3 and 53.6 ± 25.2, respectively, indicating change of more than 20%, suggesting that amelioration in the DSP group was not caused simply by improvement of medication adherence.

3.4. Secondary outcome measures

The mean CGI and GAF scores significantly improved in both groups. The CGI and GAF scores significantly decreased and increased respectively, in each DSP and NonDSP group (P < .05). The improvements during treatment were significantly more pronounced in the DSP

Table 2
Follow-up assessment outcomes over all time points up to 12 months.

BPRS total score	DSP group		NonDSP group		
	Score at T4	Percentage change in score	Score at T4	Percentage change in score	P value[a]
BPRS total score	42.1 (18.0)[b]	33.0 (19.9)	44.3 (16.5)[b]	17.0 (20.5)	<.01
Positive symptom score	11.3 (5.5)[b]	33.3 (22.9)	12.1 (5.2)	16.7 (27.7)	<.01
Negative symptom score	8.8 (3.9)[b]	31.7 (24.0)	8.6 (2.7)[b]	16.6 (22.2)	<.01
CGI-S	3.8 (1.4)[b]		4.3 (1.3)[b]		<.01
GAF	49.2 (16.9)[b]		42.5 (14.9)[b]		<.01
ESRS	19.2 (23.6)[b]		18.1 (16.7)		N.S.
Antipsychotics dose (CPZeq: mg)	1034.7 (823.4)		870.5 (466.9)		N.S.
Adherence (%)	90.0		88.4		N.S.

Data are mean (SD). T4 indicate time points at 12 months. The numbers of patients at T4 were 52 in the DSP group and 23 in the NonDSP group.
Abbreviations: N.S. = not significant.
[a] P values for the comparison in % change score or each measurement score between the DSP and NonDSP groups. The treatment comparison was a liner contrast based on a mixed-effects model with three fixed effects (time, treatment group, and time-treatment group interaction). The within-subject factor was considered as a random effect.
[b] P < .01 comparisons in each score between baseline (T0) and T4 within the group.

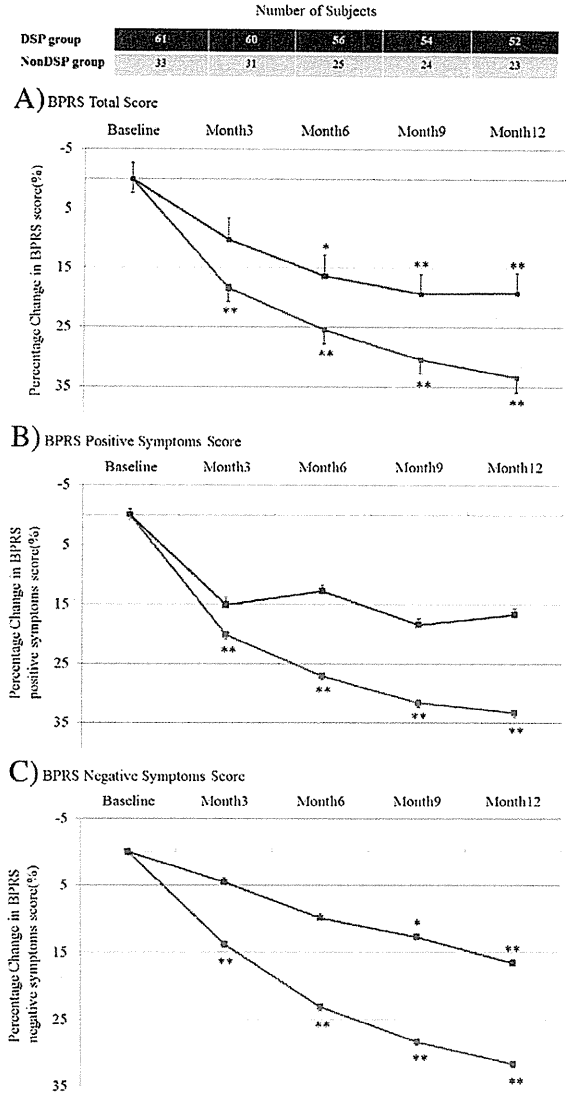

Fig. 2. Percentage change in BPRS total, positive and negative symptom scores over time. The red and blue lines indicate changes in the DSP and the NonDSP group, respectively. Error bars indicate standard error of the mean. Percentage changes in BPRS total, positive and negative symptom scores were analyzed using mixed effects model repeated-measures analysis. There were significant differences in A) total, B) positive and C) negative symptom scores between the DSP and NonDSP group ($P < .01$). $^{*}P < .05$ and $^{**}P < .01$ represent significant improvement in each group and the percentage change in BPRS score from baseline respectively.

group relative to the NonDSP group. Mean ESRS scores showed no significant difference between the two groups at the end of the study (Table 2). However, there were significant reductions in this value from T0 to each subsequent time point in the DSP group, whereas there was no change in the NonDSP group. Furthermore, the TD score of ESRS was significantly lower in the completers of the DSP group. On the other hand, no patients in the NonDSP group exhibited new TD during the study period.

Responder rates were 62.3% (N = 38) in the DSP group and 21.2% (N = 7) in the NonDSP group, indicating a significant difference ($\chi 2 = 14.5$, $P < .001$) between the two groups.

Logistic regression analysis revealed DSP as the only factor significantly related to RLAI response (odds ratio = 6.90, $P < .01$: Table 3).

4. Discussion

To our knowledge, this is the first study to investigate the efficacy of a 12-month RLAI treatment regime in patients with TRS and DSP. The treatment yielded significantly greater improvement in psychiatric symptoms and global functioning in DSP patients compared with DSP-free patients. DSP patients also showed a higher response rate (62%) relative to those without DSP (21%). Multiple logistic regression analyses revealed that the presence of DSP greatly contributed to clinical improvements in this study. Furthermore, at the end of the study, patients who received high antipsychotic doses (both oral antipsychotics and RLAI), took comparable daily oral antipsychotic doses at baseline prior to RLAI initiation. These results imply that adjunctive RLAI treatment with a gradual reduction of oral antipsychotics can help to promote a remarkable improvement in DSP patients. Unsurprisingly, DSP patients showed severer EPS at baseline, including TD, a neurological DRD2 supersensitivity (Sasaki et al., 1995a, 1995b) and an important criteria in the diagnosis of DSP (Chouinard, 1991; Fallon and Dursun, 2011). In the DSP group, the possibility that RLAI treatment lessens severe EPS was observed. Taken together, our findings suggest that achieving and maintaining stable therapeutic blood levels of antipsychotics could improve symptoms in patients with severe and treatment-resistant DSP, supporting our original hypothesis (Iyo et al., 2013). In addition, the development of other long acting injectable antipsychotics, such as other classes of atypical antipsychotics or longer-acting forms, may be desirable for the treatment of DSP.

The ESRS score and the TD score were lower overall in the DSP group, whereas no change was observed in the NonDSP group. When we consider that the mean of the total chlorpromazine equivalent doses was not different between the entry (T0) and the end (T4) of this study, we can infer that the reduced fluctuation of plasma antipsychotic levels contributes not only to the stabilization of psychosis but also to the reduction in antipsychotic-induced EPS and TD, which can be considered neurological manifestations of dopamine supersensitivity.

In this study, DSP patients exhibited significant negative symptoms at baseline, which improved remarkably during treatment. Antipsychotics are capable of improving negative and depressive symptoms, depending on the extent to which positive symptoms and EPS are reduced (Tandon, 2011). In DSP patients, the dramatic improvement in positive symptoms and EPS plays a contributory role in the improved negative symptoms and general functioning.

Table 3
Multiple logistic regression model of factors associated with responders.

	Partial regression coefficient	P value	Odds ratio	95% confidence intervals
Presence of DSP	1.93	<.01	6.90	2.19–21.80
BPRS at baseline				
Total score	−0.02	.45	0.98	0.92–1.04
Positive symptom score	0.01	.87	1.01	0.86–1.19
Negative symptom score	0.07	.46	1.07	0.90–1.28
ESRS	<−0.01	.79	1.00	0.98–1.02
Sex	−0.23	.63	0.95	0.31–2.05
Age	−0.02	.58	0.99	0.94–1.04
Duration of illness	<0.01	.94	1.00	0.94–1.06
Adherence	0.19	.38	1.20	0.80–1.82

One part of DSP patients didn't respond to the treatment. One possible reason may be sub-optimal dosing, with the combined RLAI and oral antipsychotic treatment. If the total dosages were too low to achieve optimal receptor occupancy, or if the elimination half-life of the oral drugs was too short to maintain optimal occupancy, RLAI therapy may not be sufficient to control disease symptoms. In Japan, the maximum dose of RLAI is limited to 50 mg/2-week, which is estimated to produce an occupancy range of 65.4 to 74.4% (Remington et al., 2006), corresponding to the optimal range for patients with a first schizophrenic episode (Kapur et al., 2000). Further studies are needed to clarify the accuracy of this data and its validity for subsequent episodes.

The study treatment provided only limited efficacy for NonDSP patients. In this group, positive symptoms failed to show significant improvement, while the negative symptoms showed only slight significant improvement. Reports highlight that patients with deficit syndrome (Galderisi and Maj, 2009) respond poorly to antipsychotic treatment and show profound continued negative symptoms. It is possible that there were a significant number of patients with deficit syndrome within our NonDSP cohort. That said, there may be patients with other types of confounding factors, as schizophrenia is known to be a heterogeneous disease (Tandon et al., 2009; Insel, 2010; Kanahara et al., 2013). Clozapine is known to improve symptoms in deficit syndrome (Rosenheck et al., 1999; Kelly et al., 2010). It is highly possible that in these patients, the mechanistic action is not via blockade of DRD2, but by modulation of other sites, such as the N-methyl-D-aspartate receptor, a candidate target of clozapine in the treatment of schizophrenia (Hashimoto, 2011; Miyamoto et al., 2012). However, further studies are needed to fully explore this point.

To date, there are two previous reports on clinical trials using RLAI in TRS (Procyshyn et al., 2010; Volonteri et al., 2010), although in these studies, patients were switched from other antipsychotics to RLAI. This differs from our study where RLAI was used adjunctively. In one study, a 6-month RLAI treatment achieved a 60% response rate in treatment-resistant patients with severe symptoms (Volonteri et al., 2010). The other study failed to show an advantage for RLAI (Procyshyn et al., 2010). Neither of these studies made special reference to DSP, nor did they report on the dosages of antipsychotics in use before patients entered the study. Therefore, it is unknown what percentage, if any of their study participants suffered from DSP and whether the doses of RLAI were high enough to improve symptoms in these studies.

As with all reports of this nature, there are some limitations to this study. First, this was a relatively short term observational study, because our aim was to maximize efficacy of the RLAI regime to effect improved conditions for TRS patients. A randomized, controlled study with a longer follow-up duration is needed to confirm our observation. Second, we didn't directly measure D2 receptor occupancy or the fluctuation of plasma levels of antipsychotics. Therefore, further studies, including direct measurements of these parameters, are needed to confirm our hypothesis on the mechanisms underlying DSP and treatment of patients with DSP. Third, the medication adherence level may affect the results to some extent in this study, since it has been suggested that most patients actually are under partial adherence (Oehl et al., 2000), especially patients with TRS, like our participants. Therefore, we evaluated our patients' adherence using self-reported data and the observations of their physicians. The results confirmed no differences between these two reports, although we didn't use pill-count methods. Furthermore, we analyzed BPRS scores and their changes only among the inpatients with DSP, whose adherence rates could be considered almost 100%, and the results were similar to those obtained by the analysis of all patients with DSP. In this light, we consider that the present results on the improvement of symptoms were not likely attained simply by improvements in medication adherence alone.

In conclusion, our study demonstrated that adjunctive RLAI treatment significantly improved psychotic symptoms and global functioning in TRS patients with DSP. While clozapine is considered the standard antipsychotic drug of choice for TRS (Kane et al., 1988), it is associated with serious adverse events, such as agranulocytosis and diabetes mellitus (Fakra and Azorin, 2012). This study suggests that therapeutic regimes using antipsychotics with long elimination half-lives may prove suitable alternatives to clozapine for this cohort of patients.

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Conflict of interest

Dr. Kimura reported honoraria from Janssen, Meiji Seika and Otsuka. Dr. Kanahara received grant funding from Grant-in-Aid for Young Scientists (B) (grant number is 25860989) from Japan Society for the Promotion of Science (JSPS) and grant of Heisei-24 Schizophrenia research field from SENSHIN Medical Research Foundation and reported honoraria from Eli Lilly, Otsuka and Janssen. Dr. N. Komatsu reported honoraria from Eli Lilly, Otsuka, Janssen, Yoshitomi and Ono. Dr. Ishige reported honoraria from Janssen, Eli Lilly, Otsuka and Astellas. Dr. Yamanaka reported honoraria from Otsuka, Dainippon Sumitomo, Janssen and Eli Lilly. Dr. Sasaki reported honoraria from Otsuka, Dainippon Sumitomo, Pfizer, Eli Lilly and Mochida. Dr. T. Hashimoto received grant funding from Ministry of Health, Labour and Welfare and reported honoraria from Mochida and Meiji Seika. Dr. Hasegawa reported honoraria from Eli Lilly, Astellas, Otsuka, Dainippon Sumitomo, GlaxoSmithKline and Shionogi. Dr. Shiina reported grant funding from Ministry of Health, Labour and Welfare. Dr. Sekine reported honoraria from Eli Lilly, Otsuka and Janssen. Dr. Watanabe reported honoraria from Eli Lilly and Dainippon Sumitomo. Dr. Shimizu reported honoraria from Meiji Seika, Mochida, Eli Lilly, Janssen and Yoshitomi. Dr. K. Hashimoto received the research grant or consultant fee from Abbott, Astellas, Otsuka and Taisho. Dr. Iyo received consultant fee from Eli Lilly, Dainippon Sumitomo, Pfizer and Abbott and reported honoraria from Janssen, Eli Lilly, Otsuka, Meiji Seika, Astellas, Dainippon Sumitomo, Ono, GlaxoSmithKline, Takeda, Mochida, Kyowa Hakko, MSD, Eisai, Daiichi-Sankyo, Novartis, Teijin, Shionogi, Hisamitsu and Asahi Kasei. Dr. Muneoka, Dr. Yoshimura, Dr. Suzuki, Dr. H. Komatsu, Dr. Ishikawa and Dr. Shiraishi reported no conflict of interest.

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Case Report

The Melatonin Receptor Agonist Ramelteon Effectively Treats Insomnia and Behavioral Symptoms in Autistic Disorder

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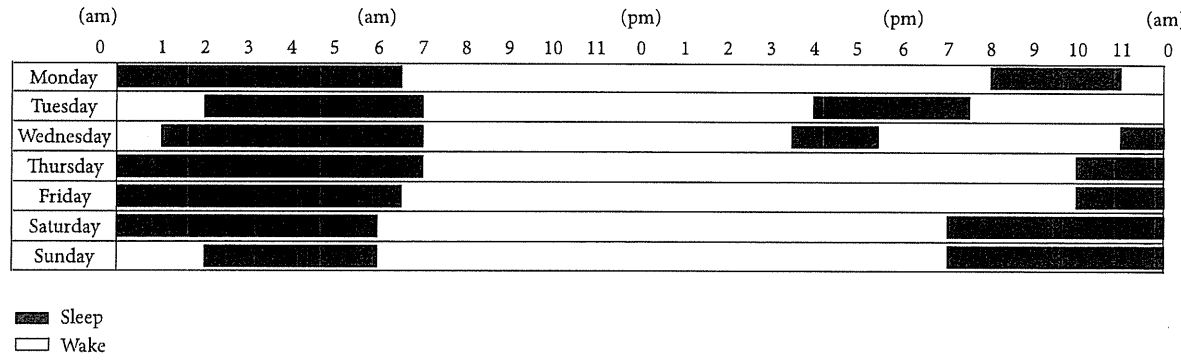
Children with autism spectrum disorders (ASD), including autistic disorder, frequently suffer from comorbid sleep problems. An altered melatonin rhythm is considered to underlie the impairment in sleep onset and maintenance in ASD. We report three cases with autistic disorder in whom nocturnal symptoms improved with ramelteon, a selective melatonin receptor agonist. Insomnia and behavior, assessed using the Clinical Global Impression-Improvement Scale, improved in two cases with 2 mg ramelteon and in the third case with 8 mg ramelteon. Our findings demonstrate that ramelteon is effective not only for insomnia, but for behavioral problems as well, in patients with autistic disorder.

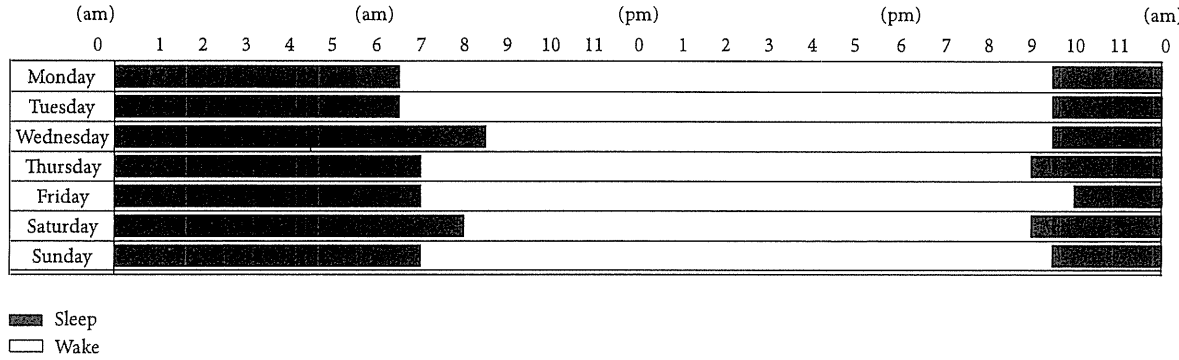
1. Introduction

Autistic disorder is characterized by persistent deficits in social communication and social interaction and communication abilities and by the presence of restricted, repetitive patterns of behavior [1]. The category of autistic disorder is combined into autism spectrum disorders (ASD) in the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) [2]. A systematic literature review found that autistic disorder in the DSM, Fourth Edition, Text Revision (DSM-IV-TR), is a fairly stable diagnosis supporting the more stringent DSM-5 criteria [3]. Many individuals with ASD present not only with diagnostic features but also with associated features, including intellectual impairment, motor deficits, and sleep problems. Sleep difficulties, particularly insomnia, occur in 50–80% of children with ASD [4, 5] and are often accompanied by child and family distress [6]. Sleep disturbance also exacerbates core and related symptoms of autism, including social interaction deficits, repetitive behaviors, affective problems, and hyperactivity/inattention [5, 7]. Therefore, interventions targeting sleep not only alleviate sleep difficulties, but also ameliorate core and related ASD symptoms and reduce familial distress.

Various neurobiological factors are known to modulate the sleep-wake cycle, and sleep problems are common in ASD. Neurotransmitter systems, such as gamma-aminobutyric acid (GABA), serotonin, and melatonin, are reported to be disturbed in ASD [8]. Melatonin is involved in promoting sleep onset and establishing a regular sleep-wake cycle. An altered melatonin rhythm seems to be responsible for the sleep onset and maintenance problems in ASD. Earlier studies showed that children with ASD have significantly lower mean concentrations of melatonin, mainly during the dark phase of the day, compared with controls [9]. Another study showed that melatonin may be helpful in treating sleep problems in children with ASD. In a randomized, placebo-controlled, double-blind, crossover study of 11 children with ASD, melatonin treatment effectively alleviated sleep difficulties [10]. In an open-label study of 107 children with ASD, 60% of the subjects treated with melatonin had an improvement in insomnia [11]. Furthermore, in an open-label dose-escalation study of 24 children with ASD conducted over



(a) Before starting ramelteon



(b) 12 weeks after starting ramelteon

FIGURE 1: Sleep diaries of Case 1.

a 14-week treatment period, melatonin improved symptoms after 1 week of supplementation, and the beneficial effects were maintained over several months. Melatonin effectively alleviated sleep and behavioral problems, reduced parenting stress, and was safe and well tolerated by the subjects [12].

Although melatonin is presently unavailable in many countries, including Japan, ramelteon, a melatonin agonist, is used for the treatment of insomnia in the United States and Asia. Ramelteon is a relatively new drug with high selectivity for the melatonin MT1 and MT2 receptors, which are located in the suprachiasmatic nucleus and have been implicated in the regulation of the sleep-wake cycle. Ramelteon has rapid oral absorption and a short elimination half-life [13] and has negligible affinity for a wide range of other binding sites in the central nervous system (including GABA, benzodiazepine, opioid, muscarinic, histamine, serotonin, and dopamine receptors) [14]. Here, we present three case reports of children with ASD treated with ramelteon.

2. Case Presentation

2.1. Case 1. A 9-year-old boy was treated and followed since being diagnosed at the age of 4 years. He had a significant impairment in social skills, including reduced eye contact, lack of imaginative play, and absence of joint attention. He also had autistic regression. He had apparently normal language development when he aged 1 year; however, he lost acquired language abilities at the age of 2.5 years. He had a heavy acoustic hypersensitivity from childhood. He let out a strange noise and would panic, particularly on rainy days. He was diagnosed with autistic disorder according to the criteria in the DSM-IV-TR [2]. Background and clinical data are given in Table 1. At the age of 7 years, he had interfering behaviors consisting of hyperactivity, restless activities, and significant insomnia, including a delay in sleep onset of 2-3 h. Risperidone (0.5 mg/day) was started for sedating hyperactivity and restless activities. It was effective for approximately 1 year; however, restless activities and insomnia gradually relapsed. In addition, self-injury, such as knocking his head, appeared. Sodium valproate (200 mg/day) was added; however, he continued to exhibit self-injury. He could not keep regular sleep hours nor maintain a regular sleep-wake rhythm. As a result, he was not able to attend school. Ramelteon (2 mg/day) was started orally at 9:00 pm to advance the sleep phase, and other drugs were discontinued. Four weeks after starting ramelteon, he began to sleep before 11:00 pm and was able to maintain sleep throughout the night (Table 2). Adverse effects were not observed. He could wake up in a better mood in the morning than before. As he acquired the ability to maintain a regular sleep-wake rhythm, he was able to go outside and start attending school (Figure 1). His acoustic hypersensitivity became mildly improved and the frequency of panic attacks had been reduced two to three times weekly.

Table 1: Patient background.

	Sex/age	Age diagnosed as ASD	CGI-S	School	Associated psychotropic medication (mg/day)
Case 1	M/9	4	6	Special school	Risperidone 0.5
Case 2	M/11	3	4	Special classroom	No drug
Case 3	F/12	6	6	Special school	Risperidone 0.5 Brotizolam 0.25 Flunitrazepam 2 Carbamazepine 100 Lamotrigine 50

The Clinical Global Impression-Severity (CGI-S) Scale is a 7-point scale used to assess symptom severity (1: normal, not ill; 2: minimally ill; 3: mildly ill; 4: moderately ill; 5: markedly ill; 6: severely ill; and 7: extremely ill) [24].

Table 2: Effectiveness of ramelteon.

	Ramelteon	Ramelteon (mg/day)			Effectiveness
	Start age	Initial dose	Maintenance dose	CGI-I	Improvement
Case 1	9	2	2	2	Reduced LPS Reduced acoustic hypersensitivity Reduced panic attacks
Case 2	11	2	2	3	Reduced LPS Maintained sleep-wake rhythm Reduced hyperactivity
Case 3	12	4	8	2	Reduced LPS Increased TST Reduced overeating

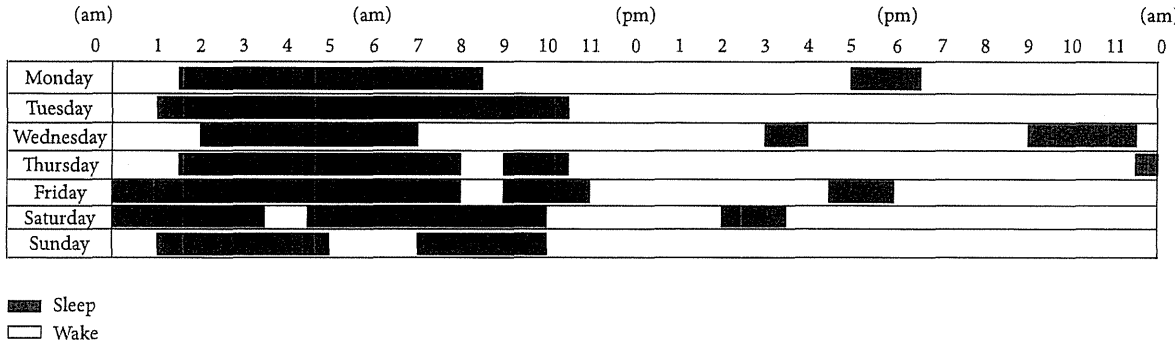
The Clinical Global Impression-Improvement (CGI-I) Scale is used to assess the degree of symptom improvement or worsening (1: very much improved; 2: much improved; 3: moderately improved; 4: minimally improved; 5: no change; 6: minimally worse; 7: moderately worse; 8: much worse; and 9: very much worse). LPS: latency to persistent sleep; TST: total sleep time.

2.2. Case 2. An 11-year-old-boy was diagnosed with pervasive developmental disorders in another hospital at the age of 3 years. He was absent from most morning classes because he slept late at night and was unable to wake up until noon. His full intelligence quotient was 51, measured with the Wechsler Intelligence Scale for Children, Fourth Edition. He was diagnosed with autistic disorder and mental retardation according to criteria in the DSM-IV-TR. He was also observed to be hyperactive and impulsive. To treat the sleep-wake disturbance, ramelteon (2 mg/day) was administered at 10:00 pm. After 1 week, his sleep-wake phase had advanced and he was able to sleep from 12:00 pm to 9:00 am. He became able to keep regular sleep-wake rhythm both on weekdays and got up more comfortably. He started to go to school in the midmorning. In addition the symptom of hyperactivity at school and home was reduced. This beneficial effect of ramelteon has been maintained for over 50 weeks. No adverse effects, such as residual daytime sleepiness, were present during treatment with ramelteon.

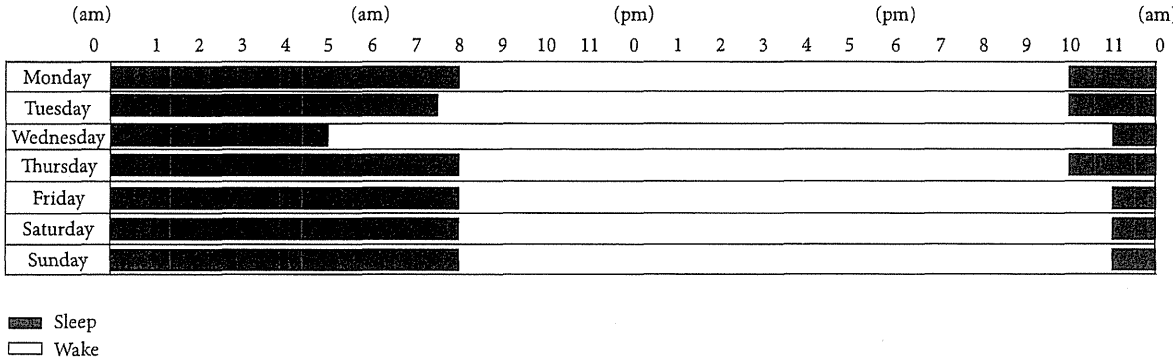
2.3. Case 3. A 12-year-old-girl was followed since the age of 6 years. At the initial visit, she exhibited a delay in language development, hyperactivity, stereotyped movements, and perseverative behavior. She had autistic regression. She had apparently normal language development until she was aged 1 year but lost acquired language abilities at the age of 1.5 years. Her developmental quotient was 30. She was diagnosed with autistic disorder according to criteria in the DSM-IV-TR. When she was 9 years old, she suffered from an irregular sleep-wake pattern and midnight awakening with beating and kicking her mother. For sedation, risperidone (0.5 mg/day) was administered. Although her agitated mood improved, difficulty in initiating and maintaining sleep remained. Flunitrazepam (2 mg/day) and brotizolam (0.25 mg/day) were administered. However, her sleep disturbance did not improve. She suffered from partial convulsive seizures at the ages of 5, 10, and 13 years. Carbamazepine (100 mg/day) and lamotrigine (50 mg/day) were administered at the age of 12 years. She began to wake up at midnight, associated with repeated overeating, which caused a rapid weight gain. As sleep duration shortened to 6 h, from 10:00 pm to 4:00 am, ramelteon (4 mg/day) was administered orally at 9:00 pm. After 2 weeks, the dose was titrated to 8 mg/day. One week later, she was able to sleep from 10:00 pm to 6:00 am (Figure 2). The intermittent awakenings were reduced, and overeating at midnight disappeared although overeating before going to bed still continued. Ramelteon was effective for bedtime resistance for more than 1 year, and risperidone was discontinued.

3. Discussion

The three cases described here illustrate our clinical experience with ramelteon for children with autistic disorder. There



(a) Before starting ramelteon



(b) 12 weeks after starting ramelteon

FIGURE 2: Sleep diaries of Case 3.

are only few case reports on the use of ramelteon. Stigler et al. reported the potential effectiveness and tolerability of ramelteon for sleep disturbances in two males aged 7 and 18 with autistic disorder [15]. In our study, we presented not only male youth, but also female youth with autistic disorder, with sleep diaries for the evaluation of sleep-wake schedule.

Cases 1 and 2 demonstrate the effectiveness of 2 mg/day ramelteon. Case 3 required a relatively higher dose of the drug (8 mg/day). Ramelteon (4–32 mg/day) has been shown to work without dose dependency in both efficacy and adverse effects in randomized controlled trials [14, 16]. Our cases show that lower doses of ramelteon are effective for insomnia and behavioral symptoms in autistic disorder.

Cases 1 and 3 had a history of autistic regression. Giannotti et al. reported that a history of autistic regression is strongly associated with disrupted sleep [17]. The overall prevalence rate for autistic regression is 32.1% and occurs at a mean age of 1.78 years [18]. Over 50% of children with autism had at least one sleep problem during the second year of life, coinciding with the period during which autistic regression most frequently occurs. Several neurotransmitter systems, including GABA, serotonin, and melatonin, which have been implicated in promoting sleep and establishing a regular sleep-wake cycle, are affected in autism and may contribute to the sleep disruptions [19]. Abnormal melatonin regulation has been found in ASD, including elevated daytime melatonin and significantly decreased nocturnal melatonin. An abnormal circadian pattern may be due to dysfunction of the pineal gland in ASD [20], and perturbed synthesis and secretion of melatonin may increase the severity of ASD [21]. Furthermore, melatonin deficit is associated with social communication impairments [22]. Only a few clinical trials have examined the effects of melatonin on parameters other than sleep, such as autistic behavioral impairment. Improvement in communication [23], social withdrawal, and stereotyped behaviors [12] have been reported in children with ASD given melatonin treatment. In the present study, we found that ramelteon alleviates not only the sleep disturbance, but autistic behaviors as well.

There are several limitations to our report. We did not use instruments specialized for sleep evaluation, such as actigraphy or polysomnography. Consequently, the improvements in sleep are not definitive. In addition, we used the Clinical Global Impression-Improvement Scale for sleep and behavioral assessment. A more detailed evaluation is required to validate our behavioral findings. Furthermore, a larger sample size is needed to examine the efficacy of ramelteon in children with different autism subtypes.

In summary, ramelteon appears to be an effective treatment for sleep disorders as well as behavioral symptoms in

autistic disorder during childhood. Further clinical studies are required to more thoroughly evaluate the effectiveness of ramelteon in children with ASD.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Re: [MS#14-42] 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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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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running title: 8-OHdG levels in females with and without Depressive Symptoms

Abstract

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

Introduction

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 urinary 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 three 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), 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 two 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) ($p=0.040$).

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 [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.

Acknowledgments

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Conflict of interest

The authors declare that they have no conflicts of interest to report.

References