logistic model. The calibration curve generated for each cytokine was used to determine the cytokine concentrations in the samples. The lower limits of detection for IL-6, TNF-α, IFN-γ, IL-2, IL-4, and IL-10 were 2.5 pg/mL, 2.8 pg/mL, 7.1 pg/mL, 2.6 pg/mL, 2.6 pg/mL and 2.8 pg/mL, respectively.

The concentrations of sTNFR1 in serum were determined with an sTNFR1 ELISA kit (Bender Medsystems, Vienna, Austria), with a detection limit of 0.05 ng/mL. The concentrations of sE-selectin in serum were determined with sandwich-type ELISA kits (R&D Systems, Minneapolis, MN). The detection limits were 0.1 ng/mL.

The serum concentrations of MMP-9 and TIMP-1 were determined with sandwich-type ELISA kits (Amersham, Buckinghamshire, UK). Assays were performed according to the manufacturer's instructions. The detection limits were 2.5 ng/mL for MMP-9 and 2.4 ng/mL for TIMP-1.

2.4. Clinical data

The relationships between HUS with or without encephalopathy and clinical data including hemoglobin, platelet counts, leukocyte counts, aspartate aminotransferase (AST), lactate dehydrogenase (LDH), blood urea nitrogen (BUN), creatinine, or C-reactive protein (CRP) at the time of specimen collection were investigated.

2.5. Statistical analysis

The differences between groups were analyzed using the Mann–Whitney *U* test. *P*-values less than 0.05 were considered significant. Analyses and calculations were performed using SPSS-12.0 (SPSS, Inc., Chicago, IL, USA).

3. Results

Serum concentrations of IL-6 ($P<0.001$), IL-10 ($P=0.041$), sTNFR1 ($P<0.001$), sE-selectin ($P<0.001$), MMP-9 ($P<0.001$) and TIMP-1 ($P<0.001$), but not TNF-α, IFN-γ, IL-2 or IL-4, were significantly higher in patients with HUS with encephalopathy compared with controls (Fig. 1). Serum IL-6, sTNFR1 and TIMP-1 concentrations were significantly higher in patients with HUS with encephalopathy compared with those with HUS without encephalopathy ($P=0.031$, $P=0.005$ and $P=0.007$, respectively) (Fig. 1 and Table 2). There were no significant differences in the serum concentrations of IL-10, sE-selectin, MMP-9, hemoglobin, platelet counts, leukocyte counts, AST, LDH, BUN, creatinine or CRP between the HUS patients with and without encephalopathy (Table 2). Serum IL-6, IL-10, sTNFR1, MMP-9 and TIMP-1 concentrations were significantly higher in patients with HUS with encephalopathy compared with those with acute colitis without HUS ($P=0.011$, $P=0.041$, $P<0.001$, $P=0.042$ and $P=0.005$, respectively) (Fig. 1). Serum sTNFR1 concentrations were significantly higher in patients with HUS without encephalopathy compared with those with acute colitis without HUS ($P<0.001$). Serum concentrations of IL-6 ($P=0.037$), sTNFR1 ($P<0.001$), sE-selectin ($P<0.001$), MMP-9 ($P=0.008$) and TIMP-1 ($P=0.001$), but not TNF-α, IFN-γ, IL-2, IL-4 or IL-10, were significantly higher in patients with HUS without encephalopathy compared with controls (Fig. 1). Serum concentrations of IL-6 ($P=0.026$), sTNFR1 ($P<0.001$), sE-selectin ($P=0.004$), MMP-9 ($P=0.001$) and TIMP-1 ($P=0.002$), but not TNF-α, IFN-γ, IL-2, IL-4 or IL-10, were significantly higher in patients with acute colitis without HUS compared with controls (Fig. 1).

Table 2
The serum concentrations of IL6, IL-10, sTNFR1, sE-selectin, MMP-9, TIMP-1, and laboratory parameters in HUS patients with/without encephalopathy

	HUS with encephalopathy	HUS without encephalopathy	*P*-value
	Median, range	Median, range	
IL-6	28.2, 14.0–1352	8.7, <2.5–44.9	0.031
IL-10	22.1, <2.8–263.5	3.8, <2.8–277.2	N. S.
sTNFR1	19.85, 13.34–31.18	6.52, 2.94–19.74	0.005
sE-selectin	155.7, 88.2–373.6	198.2, 38.4–352.4	N. S.
MMP-9	183.6, 133.6–301.2	151.1, 55.1–189.6	N. S.
TIMP-1	719.0, 319.2–1175	274.8, 85.2–716.5	0.007
Hemoglobin	9.5, 8.2–12.1	7.6, 5.8–12.4	N. S.
Platelet	4.6, 2.7–11.7	2.6, 1.5–20.9	N. S.
Leukocyte	21,520, 10,020–62,260	12,300, 7500–19,600	N. S.
AST	128, 30–260	58, 25–230	N. S.
LDH	3503, 1529–4403	2189, 1682–3650	N. S.
BUN	74, 27–101	65, 26–161	N. S.
Creatinine	2.88, 1.20–4.30	1.57, 0.43–9.74	N. S.
CRP	1.67, 0.15–13.60	1.58, 0.14–3.23	N. S.

N. S., not significant.

4. Discussion

Previous reports have demonstrated that proinflammatory cytokines are related to the pathogenesis of HUS by EHEC infection, while we could not find any previous reports regarding the serum cytokine levels of acute colitis without HUS by EHEC infection (Inward et al., 1997; Karpman et al., 1995; López et al., 1995; Murata et al., 1998; van de Kar et al., 1995). Previous investigations have revealed that IL-6, TNF-α, sTNFR1, and sTNFR2 were elevated in the peripheral blood of patients with HUS (Karpman et al., 1995; López et al., 1995; van de Kar et al., 1995), and plasma IL-6 levels of 4 HUS patients with encephalopathy were higher than those without encephalopathy (van de Kar et al., 1995). Our present data were in agreement with the previous data, except for TNF-α. It has been reported that sTNFR is the natural homeostatic regulator of the action of TNF-α, and that its level, rather than that of TNF-α, reflects the true biological activity of TNF-α (Duncombe and Brenner, 1988; Engelmann et al., 1990; Seckinger et al., 1988). Our present data indicating that serum sTNFR1 levels were elevated did not contradict the previous results of TNF-α in patients with HUS.

Hemoglobin and platelet counts are decreased, and serum levels of AST, LDH, BUN, and creatinine are increased in HUS. Therefore, these parameters could be indicators of the onset of HUS in acute colitis by EHEC infection. However, there are no significant differences of these parameters between HUS with and without CNS involvement. We also investigated IFN-γ, IL-

2, IL-4, sE-selectin, MMP-9, and TIMP-1 in patients with EHEC infection. Moreover, we divided the patients with EHEC infection into three groups, including HUS with encephalopathy, HUS without encephalopathy, and acute colitis without HUS, and compared various markers among the three groups.

We focused on the pathogenesis of HUS with neurologic involvement, and investigated whether or not any biomarkers could distinguish HUS with encephalopathy from that without encephalopathy. Serum IL-6 levels were significantly higher in the HUS patients with encephalopathy compared with those without encephalopathy, as reported previously (van de Kar et al., 1995). We found that serum sTNFR1 levels for HUS patients with encephalopathy were higher than those for HUS without encephalopathy, which were higher than those for acute colitis. TNF-α injures vascular endothelial cells and damages the BBB (Sato et al., 1986). It is hypothesized that the BBB is damaged in HUS with encephalopathy because TNF-α injures the vascular endothelial cells. The patients with EHEC infection including HUS with encephalopathy, HUS without encephalopathy, and acute colitis without HUS, exhibited elevated sE-selectin levels in the serum. Elevated sE-selectin in the serum indicates vascular endothelial injury (Boehme et al., 1996). It is likely that EHEC infection easily promotes vascular endothelial damage.

Moreover, serum TIMP-1 levels were significantly higher in the HUS patients with encephalopathy compared with those without encephalopathy. TIMP-1 is thought to be important in the function of the BBB and can protect BBB function (Sellner and Leib, 2006; Yamamoto and Nguyen, 2006). We have previously investigated serum MMP-9 and TIMP-1 levels in neurologic diseases, including acute disseminated encephalomyelitis, subacute sclerosing panencephalitis, influenza-associated encephalopathy, and acute encephalopathy following prolonged febrile seizures (Ichiyama et al., 2006, 2007a,b; Suenaga et al., 2008). It is likely that high levels of TIMP-1 in HUS with encephalopathy protect the damaged BBB in response to TNF-α and MMP-9. Therefore, serum TIMP-1 levels may reflect the severity of the BBB dysfunction in HUS. However, further studies are needed to clarify the relationship between serum TIMP-1 levels and the function of the BBB because TIMP-1 in the serum is part of the regulatory system of the BBB function. It is interesting that sTNFR1 and TIMP-1 levels were increased in HUS with encephalopathy because TNF-α and TIMP-1 are responsible for BBB function. Furthermore, the serum samples were obtained from the HUS patients before the onset of encephalopathy. The size of our present study was small. Further large-scale studies are necessary to confirm our results.

In conclusion, we suggest that IL-6, sTNFR1 and TIMP-1, especially sTNFR1 and TIMP-1, are predictive indicators of CNS involvement in patients with HUS by EHEC infection.

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Correlation between depressive symptoms and nocturnal disturbances in Japanese patients with Parkinson's disease

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Abstract

Depression and nocturnal disturbances are frequent in patients with Parkinson's disease (PD). The aim of this study was to determine the correlation between depressive symptoms and nocturnal disturbances in patients with PD in Japan. The subjects of this multi-center cross-sectional study were 188 patients with PD and 144 age-matched controls who were assessed for nocturnal disturbances by the Parkinson's disease sleep scale (PDSS) and for depressive symptoms by Zung Self-Rating Depression Scale (SDS). Depressive symptoms (SDS score of $\geq$40) were identified in 122 patients (64.9%). The SDS was significantly higher in PD patients than control subjects. The stepwise regression model identified PDSS ($p < 0.001$) and Unified Parkinson's Disease Rating Scale I (mental state) ($p = 0.002$) as significant determinants of SDS. Stepwise regression analysis identified item 15 (daytime sleepiness) ($p = 0.002$), item 13 (early morning tremor) ($p = 0.008$), item 12 (nocturnal dystonia) ($p = 0.015$), and item 3 (sleep maintenance insomnia) ($p = 0.026$) as significant predictors of SDS. Our results indicated that depressive symptoms in PD correlate significantly with nocturnal disturbances, and that daytime sleepiness, dystonia, tremor and sleep fragmentation are the most common nocturnal disturbances in depressed patients with PD.

Keywords: Parkinson's disease; Depression; Zung Self-Rating Depression Scale (SDS); Parkinson's disease sleep scale (PDSS); Nocturnal disturbances

1. Introduction

Non-motor symptoms such as cognitive dysfunction, psychiatric symptoms, and sleep disorders have attracted attention recently, in addition to motor symptoms, in Parkinson's disease (PD). In a community-based study, two-thirds of the patients with PD reported sleep disorders [1]; however, the etiology is still controversial. About 40% of PD patients have depression [2], which also involves sleep disorders [3] and is associated with impairment of activities of daily living [4]. Chaudhuri and Martinez-Martin [5] found a significant correlation between sleep disturbances and depression using the Parkinson's disease sleep scale (PDSS) [6], useful for multifactorial nocturnal problems. Using PDSS, other studies reported that sleep disturbances were associated with disease severity, daytime sleepiness [7], and impairment of activities of daily living [8]. Dhawan et al. [9] reported that untreated

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PD had many nocturnal problems, such as nocturia, nighttime cramps, dystonia, tremor, and daytime sleepiness in PDSS sub-items, suggesting that sleep disorders in PD are more likely to be related to the underlying dopaminergic deficit rather than the effect of dopaminergic treatment. On the other hand, severer changes in monoamines, such as dopamine, serotonin and noradrenalin are reported in the brain of depressed patients than non-depressed patients in PD [2, 10], and it has been reported that depression develops prior to the motor symptoms in PD patients [11]. Therefore, sleep disruption caused by depression may reflect the pathological course of the disease itself in PD.

Although we reported previously that the depressive state is a significant determinant of sleep disorders in PD [12], the specific nocturnal disturbances related to depressive symptoms remain elusive.

To determine the true status of nocturnal disturbances associated with depressive symptoms and the frequency of depressive state in Japanese patients with PD, we conducted the present survey using the PDSS [6] and Zung Self-Rating Depression Scale (SDS) [13] at multiple facilities. This study was a part of an epidemiological study on non-motor symptoms in PD [12].

2. Subjects and methods

A consecutive series of 251 patients with idiopathic PD consulted the participating eight medical university hospitals in the Kanto area of Japan during the period between April and December 2005. The current population of Kanto area is, approximately, 43 million (34.3% of all Japan). The area is called the metropolitan area and includes the city of Tokyo. Semi-structured, questionnaire-based interviews were conducted among these 251 patients. Of the 251 patients, 188 (85 men and 103 women) were assessed for sleep problems and depressed symptoms. Thirty-six gave incomplete answers in the questionnaire, 1 was bedridden, 1 was less than 40 years of age and had juvenile PD, and 25 had dementia. The cognitive function and dementia were evaluated by the Mini Mental State Examination and a score of less than 24 points was regarded as indicative of dementia. The mean age of patients was 66.4 ± 8.7 years ($\pm$ standard deviation) and the disease duration was 6.9 ± 5.3 years. For comparison, we studied 144 age-matched healthy control subjects (65.1 ± 6.8 years, 64 men and 80 women) in the Kanto area of Japan. The control subjects had no history of ischemic heart disease, painful joint disease, neurologic disease (include stroke), chronic obstructive airway disease and psychiatric disease and taking no hypnotic drugs and antidepressants.

The diagnosis of PD was based on the UK Parkinson's Disease Society Brain Bank clinical diagnostic criteria [14]. In other words, PD patients were defined as having bradykinesia and at least one of the following three symptoms: resting tremor, muscular rigidity, and/or postural instability. Parkinsonism, such as that induced by chemical or vascular insults, was excluded from disease history and imaging diagnosis. Furthermore, all patients were assessed by a neurologist and confirmed to be free of progressive supranuclear palsy, multiple system atrophy, corticobasal degeneration and other forms of atypical parkinsonism.

PD patients were evaluated using the Unified Parkinson's Disease Rating Scale (UPDRS), Hoehn and Yahr (H&Y) stage for evaluation of disease severity. The mean H&Y stage and UPDRS of all PD patients were 2.5 ± 0.8 and 32.9 ± 18.1, respectively.

With regard to medications, 148 patients had taken levodopa with decarboxylase inhibitor (levodopa/DCI), with a mean dosage of 366.9 ± 157.7 mg/day, while 130 patients had taken dopamine agonists (DA), with a mean equivalent levodopa dose of 240.5 ± 161.1 mg/day [15].

Symptoms of depression were assessed using the SDS [13]. The SDS Japanese version has been well validated [16]. The SDS has been used widely in various patient groups and in healthy subjects, providing considerable validation data as well as a large number of comparison groups [17]. The SDS scale has 20 items selected to represent the various symptoms of clinically significant depression. Each item is rated 1–4, with higher scores representing greater symptom severity. The presence of depressive symptom was defined as SDS raw score of $\geq$40, since this cut-off point was recommended to distinguish depressed patients from controls [18].

All patients were evaluated for sleep disturbances using the PDSS-Japanese version [19]. The PDSS, which is a visual analog scale type questionnaire, consists of 15 items on sleep disorders and nocturnal problems associated with PD (see Appendix 1) [6]. Sub-items of PDSS address the following: overall quality of night's sleep (item 1); sleep onset and maintenance insomnia (items 2 and 3); nocturnal restlessness (items 4 and 5); nocturnal psychosis (items 6 and 7); nocturia (items 8 and 9); nocturnal motor symptoms (items 10–13); sleep refreshment (item 14); daytime dozing (item 15). Scores for a given individual item range from 0 to 10. Ten represents the best, 0 represents the worst score. The maximum total score for PDSS is 150 (patient is free of symptoms associated sleep disorders).

The study was approved by the institutional review boards appropriate for each investigator and all study participants gave written informed consent.

3. Statistical analysis

The Mann–Whitney U test was used for continuous data and the chi-square test was used for categorical variables. Kruskal Wallis test was used to test differences in sub-items of PDSS and PDSS total score between PD (depressed and non-depressed patients) and controls. The Spearman's correlation was calculated to compare the SDS and the PDSS total score in PD patients. A stepwise regression model was used to identify the determinants of SDS in PD patients that included age, gender, disease duration, PDSS, H&Y stage, UPDRS I (mental state), UPDRS II (activities of daily living), UPDRS III (motor performance), UPDRS IV (complications of treatment), use of any DA, and use of levodopa/DCI. Subsequently, stepwise regression analysis was applied to determine the important PDSS sub-items for SDS. Significance of differences was defined as two-tailed $p < 0.05$. SPSS II Windows Ver 11.0 (SPSS Japan Inc.) was used for statistical analyses. All data are expressed as mean $\pm$ standard deviation.

4. Results

The SDS score was higher in PD patients (43.4 ± 9.6) than in controls (35.4 ± 8.2) ($p < 0.001$). Of the 188 PD patients, 122 (64.9%) had depressive symptoms. Table 1 shows the clinical characteristics of depressed and non-depressed patients with PD. There were no differences in age, gender differences, and disease duration. Depressed patients had significantly higher scores of H&Y stages and UPDRS I–IV, and higher dose of levodopa/DCI compared with non-depressed patients. Significant differences in PDSS total scores and PDSS sub-items except PDSS items 2 and 11 were seen among depressed patients, non-depressed patients, and controls (Table 2, Fig. 1). However, there was no difference in PDSS total scores and PDSS sub-items except PDSS item 13 between controls and non-depressed patients.

The PDSS total score correlated significantly with the SDS in PD patients ($r = -0.41$, $p < 0.001$, Fig. 2). In PD patients, stepwise regression model with SDS as the dependent variable

Table 1
Clinical characteristics of depressed PD patients, non-depressed PD patients and control subjects

	Non-depressed PD	Depressed PD	Total PD	*p* Value
Number	66	122	188	
Men/women	36/30	49/73	85/103	0.059[a]
Age	66.8 ± 9.7	66.2 ± 8.2	66.4 ± 8.7	0.473
Disease duration	6.0 ± 5.1	7.4 ± 5.3	6.9 ± 5.3	0.056
H&Y stage	2.3 ± 0.7	2.6 ± 0.8	2.5 ± 0.8	0.014
UPDRS total score	27.0 ± 14.7	36.0 ± 19.1	32.9 ± 18.1	0.001
UPDRS I	0.6 ± 0.9	1.4 ± 1.9	1.1 ± 1.7	<0.001
UPDRS II	7.8 ± 5.3	10.3 ± 6.6	9.4 ± 6.3	0.010
UPDRS III	17.3 ± 9.7	21.8 ± 11.7	20.2 ± 11.2	0.010
UPDRS IV	1.3 ± 1.6	2.5 ± 3.4	2.1 ± 3.0	0.045
Levodopa/DCI (mg/day)	240.9 ± 193.5	314.8 ± 207.9	366.9 ± 157.7	0.019
DA (mg/day)	224.6 ± 140.7	248.8 ± 171.0	240.5 ± 161.1	0.595

Depressed patients with PD were defined as SDS ≥ 40. Data are mean ± SD.
PD: Parkinson's disease, H&Y stage: Hoehn and Yahr stage, UPDRS: Unified Parkinson's Disease Rating Scale, PDSS: Parkinson's disease sleep scale, Levodopa/DCI: levodopa with decarboxylase inhibitor, DA: dopamine agonists, SDS: Zung Self-Rating Depression Scale.
[a] Chi-square test.

and age, gender, disease duration, PDSS, H&Y stage, UPDRS I, UPDRS II, UPDRS III, UPDRS IV, use of any DA, and use of levodopa/DCI as the independent variables identified PDSS ($p < 0.001$) and UPDRS I ($p = 0.002$) as significant determinants of SDS (Table 3). Another model, in which the SDS was used as the dependent variable, while sub-items of PDSS (items 1–15), age, gender, disease duration, H&Y stage, use of any DA, and use of levodopa/DCI were the independent variables, identified item 15 (daytime sleepiness) ($p = 0.002$), item 13 (early morning tremor) ($p = 0.008$), item 12 (nocturnal dystonia) ($p = 0.015$), and item 3 (sleep maintenance insomnia) ($p = 0.026$) as significant determinants of the SDS in PD patients (Table 4).

5. Discussion

In this study, depressive symptoms were observed in 64.9% of patients with PD. The higher prevalence rates for depressive symptoms compared with other study may be attributed to screening instrument or patient population. Although a number of studies have used various evaluation methods such as SDS, the Beck Depression Inventory, and the diagnostic criteria of Diagnostic and Statistical Manual of Mental Disorders-IV, the PD-specific evaluation method for depression is still lacking [20]. In SDS, sub-items such as fatigue, sleep disorders, and constipation may be based on the symptoms of PD itself, while items related to daily life and work may be affected by impairments of motor function. In addition, because the SDS is an evaluation tool that relies on the subject's own self-assessment, there is a tendency for overestimating depression in PD.

In neurobiological findings, postmortem examination of the brain of PD patients demonstrated 50–85% cell loss in the substantia nigra and locus ceruleus, 0–43% in the dorsal raphe nucleus and 32–87% cell loss in the basal nucleus of Meynert [21]. Thus, the depletion of endogenous neurotransmitters in the brain varies among patients with PD, suggesting that this variation may have an effect on varied prevalence rates for

Table 2
Total scores and sub-items of PDSS in Parkinson's disease and controls

	Controls	Non-depressed PD	Depressed PD	Total PD	*p* Value
Total	126.6 ± 17.8	123.2 ± 17.9	107.2 ± 27.1	112.8 ± 25.4	<0.001
Item 1	7.4 ± 3.1	7.2 ± 3.1	6.0 ± 3.5	6.4 ± 3.4	0.001
Item 2	7.9 ± 2.8	7.6 ± 3.4	7.2 ± 3.5	7.4 ± 3.4	0.316
Item 3	6.5 ± 3.2	6.5 ± 3.4	5.2 ± 3.8	5.7 ± 3.7	0.011
Item 4	9.2 ± 1.8	9.2 ± 1.9	8.2 ± 2.8	8.6 ± 2.6	<0.001
Item 5	9.1 ± 1.8	9.0 ± 2.1	8.1 ± 2.9	8.4 ± 2.7	<0.001
Item 6	9.0 ± 1.9	8.7 ± 2.1	7.4 ± 3.2	7.9 ± 2.9	<0.001
Item 7	9.6 ± 1.3	9.2 ± 2.1	8.1 ± 3.0	8.5 ± 2.8	<0.001
Item 8	5.3 ± 3.8	4.4 ± 3.9	4.1 ± 3.7	4.2 ± 3.8	0.045
Item 9	9.7 ± 1.4	9.1 ± 2.1	8.5 ± 2.6	8.7 ± 2.4	<0.001
Item 10	9.5 ± 1.6	9.4 ± 1.5	7.8 ± 3.3	8.4 ± 2.9	<0.001
Item 11	8.5 ± 2.3	9.0 ± 2.0	8.3 ± 2.5	8.5 ± 2.4	0.131
Item 12	9.5 ± 1.3	9.4 ± 1.3	8.0 ± 3.1	8.5 ± 2.7	<0.001
Item 13	9.8 ± 1.1	8.4 ± 2.7	6.9 ± 3.6	7.4 ± 3.4	<0.001
Item 14	7.6 ± 2.9	7.8 ± 3.0	6.6 ± 3.4	7.0 ± 3.3	0.012
Item 15	8.0 ± 2.6	8.3 ± 2.6	6.6 ± 3.4	7.2 ± 3.3	0.001

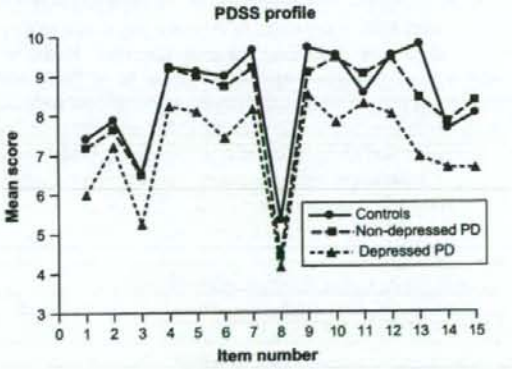

Fig. 1. Profiles of mean PDSS scores of sub-items in depressed PD, non-depressed PD and controls. Significant differences in PDSS sub-items except items 2 and 11 were seen among depressed patients, non-depressed patients, and controls.

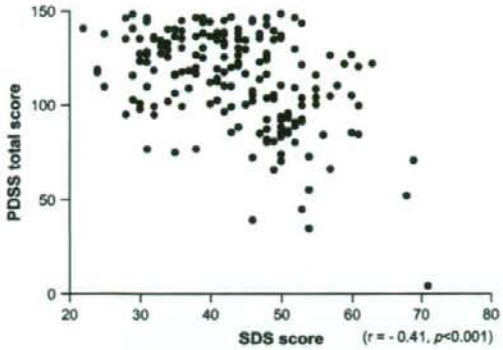

Fig. 2. Spearman's correlation analysis of SDS and PDSS in PD patients. Correlation coefficient = −0.41, $p < 0.001$.

depression in PD. As for the relationship between excessive daytime sleepiness, it has been demonstrated that bilateral destruction of ventral tegmental area (VTA) causes excessive daytime sleepiness in animal experiments [22] and PD patients with depression show greater dopamine loss in the mesolimbic dopaminergic pathway from VTA, as well as serotonin depletion in the dorsal raphe nucleus, noradrenalin depletion in the locus ceruleus, and acetylcholine depletion in the pedunculopontine nucleus and nucleus basalis of Meynert compared with patients without depression [23]. Moreover, it is believed that degeneration of cholinergic neurons and depletion of noradrenalin can cause disorders of rapid eye movement sleep, and serotonin depletion can reduce the amount of slow-wave sleep [24]. Based on these findings, PD patients with depression may show exacerbation of daytime sleepiness due to change in neurotransmitters.

In our study, Spearman's correlation analysis showed a significant correlation between depressive symptoms and nocturnal disturbances. Although depressed PD showed higher disease severity and severer motor dysfunction compared to non-depressed PD, motor function (UPDRS III) did not enter the final models as a significant variable in stepwise regression analysis with SDS. The results of stepwise regression analysis with SDS as the dependent variable identified PDSS and UPDRS I as significant determinants of the SDS. These findings support the previous reports that sleep disorders are exacerbated by depression in PD patients [3,25] and that cognitive dysfunction often complicates major depression [26]. We believe that depression, sleep disorders, and cognitive dysfunction interact with one another.

Table 3
Stepwise regression analysis of SDS in Parkinson's disease

Parameter	Clinical variable	R^2
SDS	PDSS	0.200
	PDSS + UPDRS I	0.301

SDS was used as the dependent variable, while age, gender, disease duration, PDSS, H&Y stage, UPDRS I, UPDRS II, UPDRS III, UPDRS IV, use of levodopa/DCI, and use of any DA were independent variables.
For abbreviations, see Table 1.

Table 4
Stepwise regression analysis of SDS in Parkinson's disease (including subitems of PDSS)

Parameter	Clinical variable	R^2
SDS	Item 15	0.108
	Item 15 + item 13	0.168
	Item 15 + item 13 + item 12	0.196
	Item 15 + item 13 + item 12 + item 3	0.218

SDS was used as the dependent variable, while age, gender, disease duration, PDSS (items 1–15), H&Y stage, use of any DA, and use of levodopa/DCI were independent variables.
Item 15: daytime sleepiness, item 13: early morning tremor, item 12: nocturnal dystonia, item 3: sleep maintenance insomnia.

Happe et al. [3] reported significant correlations between SDS and the narcolepsy score and periodic limb movement disorder score (including items regarding restless legs syndrome) in their study of 56 patients with PD and 59 control subjects. On the other hand, in our study, daytime sleepiness and sleep maintenance insomnia and the causes thereof, including early morning tremors and nocturnal paroxysmal dystonia, correlated with the SDS (depressive symptoms) in PD. It is widely believed that sleep maintenance insomnia is more common than difficulty in falling asleep in patients with PD [1]. Because sleep maintenance insomnia was closely associated with depressive symptoms in this study, we consider depression to be associated with nocturnal symptoms, or it may potentially be due to abnormality of the structures involved in sleep regulation caused by the disease process itself.

Dhawan et al. [9] compared nocturnal disturbances among 59 cases of PD (25 cases were untreated and 34 cases were treated) and 131 control subjects using PDSS. The results showed that nocturia, nighttime cramps, dystonia, tremors, and daytime somnolence were important factors for untreated PD. However, these symptoms were similar to the nocturnal symptoms that are important to PD with severe depressive symptoms in our study, they did not include a description of depressive symptoms. It has been reported that dystonia and tremors in PD increase in the "off" state, and depression exacerbates nocturnal off-period-related motor symptoms [2]. Furthermore, depression is reported to trigger the "wearing off", and a hypodopaminergic state and a psychological "off" have been considered as causes for this phenomenon [27].

With regard to the relationship between dystonia and nocturnal symptoms, Lees et al. [28] found dystonia in 34% of 215 cases of PD, and Starkstein et al. [25] reported that depression was the most important factor associated with sleep disorders and pain. Goetz et al. [29] discussed the possibility that depression changes the interpretation of pain and the possibility that depression exacerbates pain, because pain was frequently observed in patients with severe depression among 95 cases of PD. Animal experiments have shown that tremors are due to depletion of the neostriatal content of serotonin and dopamine [30], while clinical studies indicated that bradykinesia and rigidity are more severe than tremors as characteristics of motor function in depressed PD [2]. Conversely, early morning tremors were closely associated with the depressive state in

our study, and this is believed to be due to nocturnal off-period-related symptoms rather than daytime motor function.

In conclusion, there was a significant correlation between patients with depressive symptoms and nocturnal disturbances. We demonstrate that nocturnal disturbances and cognitive dysfunction were significant determinants of depressive symptoms and that daytime sleepiness, dystonia, tremor and sleep fragmentation seem to be the important nocturnal disturbances in depressed patients with PD.

Appendix 1. The Parkinson's disease sleep scale (from Ref. [6])

Item 1 The overall quality of your night's sleep is:
Item 2 Do you have difficulty falling asleep each night?
Item 3 Do you have difficulty staying asleep?
Item 4 Do you have restlessness of legs or arms at night or in the evening causing disruption of sleep?
Item 5 Do you fidget in bed?
Item 6 Do you suffer from distressing dreams at night?
Item 7 Do you suffer from distressing hallucination at night (seeing or hearing things that you are told do not exist)?
Item 8 Do you get up at night to pass urine?
Item 9 Do you have incontinence of urine because you are unable to move due to "off" symptoms?
Item 10 Do you experience numbness or tingling of your arms or legs which wake you from sleep at night?
Item 11 Do you have painful muscle cramps in your arms or legs whilst sleeping at night?
Item 12 Do you wake early in the morning with painful posturing of arms or legs?
Item 13 On waking do you experience tremor?
Item 14 Do you feel tired and sleepy after waking in the morning?
Item 15 Have you unexpectedly fallen asleep during the day?

For question 1: Awful = 0, Excellent = 10. For question 15: Frequently = 0, Never = 10. For the remaining of the questions: Always = 0, Never = 10.

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Excessive daytime sleepiness and sleep episodes in Japanese patients with Parkinson's disease

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Abstract

In Parkinson's disease (PD), sudden unexpected sleep episodes and excessive daytime sleepiness (EDS) while driving and engaging in social activities are important problems. We conducted a multi-center study to clarify the prevalence and contributing factor of EDS and sleep episodes in Japanese patients with PD. We evaluated 188 patients with PD (85 men, 103 women) and 144 age-matched controls for sleepiness. EDS was defined as an Epworth sleepiness scale (ESS) score of ≥ 10. ESS score was significantly higher (6.6 ± 4.2 vs. 5.6 ± 3.8) and prevalence of sleep episodes was higher in PD than in controls (6.4% vs. 0.7%). PD patients with EDS were more likely to have sleep episodes (22.5% vs. 2.0%), higher score for disease severity and depressive symptoms, and on higher dose of dopaminergic agents than those without EDS. However, there were no differences in nocturnal disturbances between the two groups. ESS score was not different between patients taking ergot and non-ergot dopamine agonists. Logistic regression analysis demonstrated that mental state, total dose of dopaminergic agents, and ESS score were significant predictors of sleep episodes. ESS score of ≥ 10 had 75% sensitivity and 82.4% specificity for sleep episodes. These results suggest that sleepiness in PD is dependent on disease itself and dopaminergic treatment rather than nocturnal disturbances.

Keywords: Parkinson's disease; Excessive daytime sleepiness; Sleep episodes; Dopaminergic treatment; Disease severity

1. Introduction

Sleep disorders are common problems in Parkinson's disease (PD) [1]. Since 1999, when sudden-onset sleep with motor vehicle accidents in PD patients treated with non-ergot dopamine agonists (ropinirole and pramipexole) were reported [2], the relationship between excessive daytime sleepiness (EDS) and dopaminergic drugs has attracted attention. The reported prevalence of EDS in PD ranges from 15 to 50% [3–5]. Although the etiology of EDS is still debated, the disease process itself [6–8], medication effect [9], nocturnal disturbances [10], aging [11], and primary sleep disorders (sleep apnea syndrome, periodic limb

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movement disorder) [12,13] are thought to be contributing factors. Sudden-onset sleep episodes, or "sleep attacks", are reported to occur in 3–40% of PD patients [5,14,15]. However, the concept of a sleep attack, which occurs without warning unexpectedly and irresistibly, is not supported by sleep physiology, because sleep attacks are not included in the sleep disorders classified by the American Sleep Disorders Association [16] and the term "sleep attack" has been abandoned even in patients with narcolepsy because of lack of evidence that episode of sleep occurs without warning [17]. Similarly, sleep attack in PD has been suggested to be preceded by somnolence, and thus the term "sleep attacks" may be better described as "sleep episodes" [18-21]. However, the reason that some PD patients report falling asleep without warning or prodrome may be due to amnesic state for sleepiness or habituation to the sensation of chronic tiredness preceding the event [19,21].

In Asian PD patients, the pathogenesis of sleepiness is still unclear because there are only a few studies on EDS and sleep episodes [22,23]. We performed a multi-center study to assess the prevalence and contributing factors to EDS and sleep episodes in PD. This study is part of an epidemiological study on non-motor symptoms in PD [24].

2. Patients and methods

A total of 251 patients with PD consulted the participating medical schools of several universities and various medical university hospitals in the Kanto area of Japan during the period from April to December 2005. The current population of Kanto area is approximately 43 million (34.3% of all Japan). The area is called the metropolitan area and includes the city of Tokyo. Semi-structured, questionnaire-based interviews were conducted among the 251 patients. Of the 251 patients, 63 were excluded from this study (30 men and 33 women); 36 gave incomplete answers to the questionnaire, 1 was bedridden, 1 was less than 40 years of age and had juvenile PD, and 25 had dementia. Cognitive function was evaluated by the Mini Mental State Examination (MMSE) and a score of less than 24 points was regarded as indicative of dementia [25]. The participating patients were 85 men and 103 women with a mean age of 66.4± 8.7 years (±SD) and disease duration of 6.9±5.3 years. For comparison, we included 144 age-matched healthy control subjects (65.1±6.8 years, 64 men and 80 women) who lived in the Kanto area of Japan. The control subjects had no history of ischemic heart diseases, painful joint diseases, neurologic diseases (including stroke), chronic obstructive airway disease or psychiatric diseases and took no hypnotic drugs and antidepressants.

The diagnosis of PD was based on the UK Parkinson's Disease Society Brain Bank clinical diagnostic criteria [26]. In other words, PD patients were defined as having bradykinesia and at least one of the following three symptoms: resting tremor, muscular rigidity, and/or postural instability. Parkinsonism, such as that induced by chemical or vascular insults, was excluded from disease history and imaging diagnosis. Furthermore, all patients were assessed by a neurologist and confirmed to be free of progressive supranuclear palsy, multiple system atrophy, corticobasal degeneration and other forms of atypical parkinsonism. The disease severity was evaluated in PD patients by the Unified Parkinson's Disease Rating Scale (UPDRS) [27] and Hoehn and Yahr (H&Y) stage [28]. The mean values of H&Y stage and UPDRS of all PD patients were 2.5±0.8 and 32.9±18.1, respectively.

With regard to medications, 148 patients had taken levodopa with decarboxylase inhibitor (levodopa/DCI) and 130 patients had taken dopamine agonists (DA) with a mean equivalent levodopa dose of 456.3±281.6 mg/day [29].

Symptoms of depression were assessed using the Zung Self-Rating Depression Scale (SDS) [30,31]. All patients were evaluated for sleep disturbances using Parkinson's disease sleep scale (PDSS)-Japanese version [24,32] and Pittsburgh Sleep Quality Index (PSQI) [33]. The PDSS, which is a visual analog scale type questionnaire, consists of 15 individual items of sleep disorders and nocturnal problems associated with PD [34] (see Appendix A). The PSQI is a self-administered questionnaire to assess the subject's sleep and includes seven components, and has been used widely for evaluation of insomnia [33]. A sleep disorder was diagnosed when the PSQI global score was more than 5. The following seven sub-items were also estimated (range of subscale scores, 0–3): C1, sleep quality; C2, sleep latency; C3, sleep duration; C4, habitual sleep efficiency; C5, sleep disturbances (e.g., early-morning awakening, arousal during sleep, nocturia, feeling of smothering, bouts of nocturnal coughs, loud snoring, feeling cold or hot, nightmares, and aches); C6, use of sleeping medications; and C7, daytime dysfunctions. Daytime sleepiness was measured by the Epworth sleepiness scale (ESS), which is a self-administered questionnaire designed to evaluate excessive sleepiness in different situations [35]. An ESS score of 10 or greater was defined as EDS. Sleep episodes were defined as "an event of falling asleep unexpectedly and irresistibly while driving, eating meals, engaging in a social activity during the past one month and more than 3 times a week" using question 8 of the PSQI sub-item. The presence or absence of a warning sign with sleep episodes was not evaluated in this study.

The study was approved by the institutional review boards appropriate for each investigator and all study participants gave written informed consent.

2.1. Statistical analysis

Comparison between two groups was made with the unpaired Student t (Mann–Whitney U) test for continuous variables and Chi-square test for categorical variables. One-way analysis of variance (ANOVA) was used for comparison of seven groups classified by difference in dopaminergic treatment. When a significant difference was detected by

Table 1
Clinical characteristics of patients with Parkinson's disease and control subjects

	PD (n=188)	Control (n=144)	p value*
Age (years)	66.4±8.7	65.1±6.8	0.889
Men/women	85/103	64/80	0.165*
Body mass index (kg/m^2)	21.8±3.4	23.0±2.8	0.001
SDS	43.4±9.6	35.4±8.2	<0.001
Sleep episodes, n (%)	12 (6.4%)	1 (0.7%)	0.008*
ESS	6.6±4.2	5.6±3.8	0.025
PDSS	112.8±25.4	126.6±17.8	<0.001
PSQI global score	6.7±4.5	4.1±2.8	<0.001
PSQI component score			
Sleep quality	1.3±0.8	0.7±0.8	<0.001
Sleep latency	0.9±1.0	0.7±0.9	0.163
Sleep duration	1.2±1.0	1.1±0.9	0.275
Habitual sleep efficiency	0.6±1.0	0.2±0.6	<0.001
Sleep disturbances	1.0±0.6	0.8±0.5	<0.001
Use of sleeping medication	0.8±1.2	0.2±0.7	<0.001
Daytime dysfunction	0.9±0.9	0.3±0.5	<0.001

*By unpaired t test. *Chi-square test.
PD: Parkinson's disease, SDS: Zung Self-Rating Depression Scale, ESS: Epworth sleepiness scale, PDSS: Parkinson's disease sleep scale, PSQI: Pittsburgh Sleep Quality Index.

ANOVA, *post hoc* test (Bonferroni test) was conducted. Prediction of sleep episodes in PD patients used a backward logistic regression model, including age, gender, disease duration, ESS, SDS, H&Y stage, UPDRS I (mental state), UPDRS II (activities of daily living), UPDRS III (motor performance), UPDRS IV (complications of treatment), and mean daily levodopa dose equivalents. Variables with a log-likelihood ratio of $p>0.05$ were excluded in a stepwise procedure until each remaining variable was statistically significant. Regression results were also presented as odds ratio (OR) and their respective 95% confidence interval (CI).

Table 2
Profile of PD patients

	PD with EDS	PD without EDS	p value*
N	40 (21.3%)	148	
Age (years)	65.5±8.6	66.6±8.7	0.509
Men/women	23/17	62/86	0.078*
Body mass index (kg/m^2)	21.3±3.4	21.9±3.5	0.369
Disease duration	9.5±5.8	6.3±4.9	0.001
SDS	48.2±9.0	42.2±9.4	0.001
Sleep episodes, n (%)	9 (22.5%)	3 (2.0%)	<0.001*
H&Y stage	2.8±0.8	2.4±0.8	0.021
UPDRS	40.8±18.2	30.7±17.5	0.002
UPDRS I	2.0±2.4	0.9±1.3	<0.001
UPDRS II	12.2±6.7	8.7±6.0	0.001
UPDRS III	23.4±10.3	19.4±11.3	0.036
UPDRS IV	3.2±3.4	1.8±2.8	0.002
Daily levodopa dose equivalents (mg/day)	595.0±335.3	418.8±253.8	0.004

*By Mann–Whitney U test. *Chi-square test.
PD: Parkinson's disease, EDS: excessive daytime sleepiness, SDS: Zung Self-Rating Depression Scale, H&Y stage: Hoehn and Yahr stage, UPDRS: Unified Parkinson's Disease Rating Scale.

Table 3
The PSQI global and component scores in Parkinson's disease

	PD with EDS (n=40)	PD without EDS (n=148)	p value*
Sleep quality	1.4±0.9	1.2±0.79	0.290
Sleep latency	0.6±0.9	1.0±1.1	0.031
Sleep duration	1.2±1.1	1.3±1.0	0.720
Habitual sleep efficiency	0.6±1.1	0.6±1.0	0.399
Sleep disturbances	1.2±0.7	1.1±0.6	0.361
Use of sleeping medication	0.8±1.3	0.8±1.2	0.766
Daytime dysfunction	1.4±1.1	0.7±0.8	<0.001
PSQI	7.1±4.8	6.6±4.4	0.404
PSQI >5, n (%)	15 (37.5%)	56 (37.8%)	0.969*

*By Mann–Whitney U test. *Chi-square test.
PD: Parkinson's disease, EDS: excessive daytime sleepiness, PSQI: Pittsburgh Sleep Quality Index.

Significance of differences was defined as two-tailed $p<0.05$. SPSS II Windows Ver 11.0 (SPSS Japan Inc.) was used for statistical analyses. All data are expressed as mean± standard deviation.

3. Results

Table 1 shows the clinical characteristics of PD and control subjects. PD had significantly higher ESS and PSQI scores, and lower PDSS scores, relative to the controls. In the sub-items of PSQI, the score was more commonly impaired in PD than controls except for sleep latency and sleep duration. The frequency of sleep episodes was significantly higher in PD compared with controls (6.4% vs. 0.7%).

Table 4
Total PDSS score and scores of individual items in Parkinson's disease

	PD with EDS	PD without EDS	p value*
Total	103.0±33.3	115.5±22.2	0.054
Item 1	5.8±3.9	6.6±3.2	0.370
Item 2	7.9±3.4	7.2±3.4	0.097
Item 3	5.0±4.1	5.8±3.6	0.274
Item 4	8.0±3.3	8.7±2.3	0.953
Item 5	8.1±2.9	8.5±2.6	0.551
Item 6	7.3±3.4	8.0±2.8	0.544
Item 7	7.5±3.6	8.8±2.5	0.055
Item 8	4.3±3.8	4.2±3.8	0.797
Item 9	7.8±3.4	9.0±2.0	0.114
Item 10	7.4±3.7	8.6±2.6	0.397
Item 11	7.8±3.2	8.7±2.1	0.446
Item 12	8.3±3.0	8.6±2.6	0.537
Item 13	7.1±3.7	7.5±3.3	0.530
Item 14	6.3±3.9	7.3±3.1	0.409
Item 15	4.4±3.7	8.0±2.7	<0.001

*By Mann–Whitney U test.
PDSS: Parkinson's disease sleep scale, PD: Parkinson's disease, EDS: excessive daytime sleepiness.

Table 5
Dopaminergic agents used by patients with PD and sleep episodes

Medication	Patients (n)
Levodopa alone	1
Levodopa and DA	3
Levodopa and 2DA	7
Levodopa and 3DA	1

PD: Parkinson's disease, DA: dopamine agonist(s).

EDS was noted in 21.3% of PD patients. PD in patients with EDS was associated with significantly longer disease duration, higher score on disease severity and depressive symptoms, and higher dose of dopaminergic agents relative to PD patients without EDS, whereas there was no difference in age, gender, and body mass index between the groups (Table 2). The prevalence of sleep episodes was significantly higher in PD patients with EDS than in those without EDS (22.5% vs. 2.0%). Among patients with sleep episodes, an ESS score of <10 was observed in 3 of 12 (25.0%) patients.

There was no difference in PSQI total score, sleep duration, habitual sleep efficiency, sleep disturbances, and the use of hypnotic drugs between PD patients with EDS and those without EDS. The sleep latency was significantly shorter and daytime sleepiness was more common in PD with EDS than in those without EDS (Table 3). Similarly, there were no differences in PDSS total score and scores of sub-items between PD patients with EDS and those without EDS, except for item 15 (daytime sleepiness) (Table 4).

We performed subset analysis to assess the correlation between dopaminergic medication and ESS score. Patients taking multiple dopamine agonists (n=1), and patients taking bromocriptine and levodopa (n=4) were excluded from the analysis. ESS score was significantly higher in patients taking 2 dopamine agonists plus levodopa (10.1±6.9, n=16, p<0.05) compared to other medication group, but there was no difference in ESS score among patients taking pergolide plus levodopa (6.6±4.3, n=35), cabergoline plus levodopa (5.8±3.0, n=37), pramipexole plus levodopa (7.7±5.1, n=12), levodopa monotherapy (6.2±3.4, n=46), dopamine agonist monotherapy (6.2±3.4, n=26), and no dopaminergic medication (4.6±3.1, n=11).

Logistic regression analysis revealed ESS (OR, 1.242; 95% CI, 1.051–1.467; p=0.011), mean equivalent levodopa dose (OR, 1.003; 95% CI, 1.000–1.005; p=0.025), and UPDRS 1 (mental state) (OR, 1.559; 95% CI, 1.014–2.396; p=0.043) were significant predictor of sleep episodes, while age, gender, disease duration, SDS, H&Y stage, UPDRS II (activities of daily living), UPDRS III (motor performance), and UPDRS IV (complications of treatment) were not. Table 5 lists the dopaminergic agents used in patients with sleep episodes. None of the patients was untreated. Receiver operating characteristic curve analysis showed that an ESS score of ≥10 had 75.0% sensitivity and 82.4% specificity for sleep episodes.

4. Discussion

In this study, the scores of ESS and PSQI in the PD group were significantly higher and the scores of PDSS in the PD group were significantly lower than those in the control group. These results indicate impaired daytime sleepiness, sleep conditions and nighttime symptoms in PD patients relative to the control group. In a study on the incidence of sleep episodes in Caucasian PD patients, Hobson et al. [5] reported that sleep episodes occurred in 3.8% of 420 PD patients while driving a car. Meanwhile, Paus et al. [14] reported that sleep episodes occurred in 6.0% of 2952 patients. On the other hand, Tan et al. [22] reported that sleep attacks were observed in 13.9% of 201 Asian PD patients, suggesting that genetic or racial differences may play an etiological role. In our study, sleep episodes were found in 6.4% of PD, which was similar to the data of the Caucasian PD patients. The differences in the incidence among these studies may be due to differences in the design of individual studies, including our setting in which sleep episodes were defined as those which had occurred 3 times a week during the past month. We established this definition in order to use a short time lag between background factors, such as dose of dopaminergic treatment, severity, or sleep conditions, and the period of a sleep episode, and to exclude sleep episodes that developed at low frequency with no repeatability due to bad physical conditions on the day or bad sleep conditions on the previous night.

In this study, significantly higher disease severity, higher dose of dopaminergic treatment and a tendency for depression were observed in the group of patients with EDS, compared with the non-EDS group, and thus the effects of the disease itself and dopaminergic treatment were considered. Furumoto [23] reported that EDS in 53 Japanese PD patients was closely related with the disease severity (UPDRS) and disease duration, but not with dopaminergic treatment. However, there was no description regarding nighttime symptoms or sleep disturbance. We reported previously that PD patients with H&Y of 4 exhibited significantly lower scores of PDSS with more impaired nighttime symptoms, compared with those of H&Y of 1–3 [24]. In the present study, however, there was no significant difference between the EDS and non-EDS groups with regard to all PDSS items except for the sub-item 15 (daytime sleepiness). In addition, no difference was found in PSQI except for significantly shorter sleep latency and significantly higher scores in daytime sleepiness in the EDS group.

The results of the present study indicate that EDS may be an independent symptom related to daytime wakefulness, and might not reflect nighttime symptoms or sleep conditions. However, we did not perform maintenance of wakefulness test to detect such abnormality. It has been

reported that daytime sleepiness occurs prior to clinical symptoms, and EDS is associated with a risk of PD [36]. In addition, no changes were observed in the findings of polysomnography (PSG) in a narcolepsy-like PD group with short sleep latency confirmed by the multiple sleep latency test, compared with a non-narcolepsy-like group [37]. No differences were also found in PSG findings between PD patients with and without sleep episodes [38], suggesting that daytime sleep episodes may occur in PD patients independent of their nighttime sleep conditions.

With regard to the factors that affect wakefulness in PD, previous studies reported degeneration of dopaminergic neurons of the mesocorticolimbic system that modulate thalamocortical arousal from the ventral tegmental area (VTA) [6,7], and reduction of neurotransmitters related to the ascending reticular activating system, such as serotonin, noradrenaline and acetylcholine [8]. Autopsy studies also reported significant reduction of dopaminergic neurons in the VTA, and diminished secretion of serotonin, noradrenaline, and acetylcholine in PD patients with depression and dementia [39]. Tandberg et al. [3] reported significant cognitive decline in the EDS group. In the present study, cognitive impairment and depressive state were related to sleep episodes and EDS, respectively. Other dopaminergic systems related to sleep and wakefulness include midbrain dopaminergic neurons, which are involved in the sleep–wakefulness cycle via the mesothalamic system [40], and wake-active dopaminergic neurons found in the ventral periaqueductal gray matter [41]. Studies in PD patients with EDS showed no reduction in orexin concentrations in cerebrospinal fluid [42,43]; however, in autopsy cases of PD, few orexin cells were seen in the hypothalamus and their numbers correlated with the disease progression [44]. These results suggest a link between EDS and orexin neurons in PD patients.

ESS score of ≥ 10 is generally considered to reflect pathological sleepiness [45]. We demonstrated that PD patients with an ESS score ≥ 10 exhibited significant risk for sleep episodes. Logistic regression analysis revealed that ESS score, total amount of dopaminergic treatment, and UPDRS 1 (mental state) were significant predictors of sleep episodes. The relationships among sleepiness, higher score of ESS and higher dose of dopaminergic agents were consistent with the previous reports of Brodsky et al. [21] and Tan et al. [22]. Analysis of ESS according to the type of dopamine agonists showed no differences in the ESS scores among such agonists, similar to the results of previous reports [4,14]. Only those patients who were treated with 2 DA plus levodopa exhibited higher scores of ESS, compared to other medication groups.

In conclusion, we speculate that the severity of PD, including mental state and dopaminergic treatment, could be involved in sleepiness. In PD, EDS may result from disturbance of sleep–wakefulness mechanism(s) as an independent symptom from nocturnal disturbances.

Appendix A. Aspects of nocturnal disability in PD measured by PDSS (from Chaudhuri et al. [34])

Item 1: Overall quality of nocturnal sleep
Items 2/3 Sleep onset and maintenance insomnia
Items 4/5: Nocturnal restless legs
Items 6/7: Nocturnal psychosis and REM behavior disorder
Items 8/9: Nocturia and off-related incontinence
Items 10–13: Nocturnal akinesia and motor symptoms
Item 10: Early morning dystonia
Item 14: Sleep refreshment
Item 15: Daytime sleepiness

Scores for a given individual item range from 0 to 10, and 10 represents the best while 0 represents the worst score. The maximum total score for PDSS is 150 (patient is free of symptoms associated sleep disorders).

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INTERNAL MEDICINE

□ ORIGINAL ARTICLE □

Predictors of a Prolonged Clinical Course in Adult Patients with Herpes Simplex Virus Encephalitis

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Abstract

Objective Herpes simplex virus encephalitis (HSVE) patients occasionally follow a prolonged course despite standard antiviral treatment. The purpose of this study was to analyze clinical variables to identify predictors of a prolonged course.

Methods A series of 23 HSVE patients treated with acyclovir (ACV) during the acute stage were selected and divided into 2 groups: the non-prolonged group (n = 15), with improvement within 2 weeks after initial ACV treatment; and the prolonged group (n = 8), without improvement within 2 weeks. Differences in clinical variables, including age, duration from onset to initial ACV treatment, Glasgow coma scale (GCS) score, corticosteroid administration, detection of abnormal lesions on initial cranial computed tomography (CT) and magnetic resonance imaging, detection of periodic lateralized epileptiform discharges on electroencephalogram, and clinical outcome, were compared between the groups.

Results There were significant differences in GCS score, clinical outcome, and detection of lesions on CT between the non-prolonged and prolonged groups [$p = 0.021$, $p = 0.041$ (Mann-Whitney's U test), respectively, and $p = 0.027$ (Fisher's exact test)]. Four of the eight patients with a prolonged course had a poor outcome despite treatment with additional drugs.

Conclusion A lower GCS and a higher rate of lesions on CT were identified as predictors of a prolonged course for HSVE. These predictors are in accordance with the conventional predictors of poor outcome for HSVE. This study suggests that the initial ACV treatment was insufficient for HSVE patients with these predictors at the acute stage. The initial treatment may need to be modified for such patients.

Key words: outcome, prolonged, predictor, herpes simplex virus encephalitis

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Introduction

Herpes simplex virus (HSV) is a human herpes virus that can cause HSV encephalitis (HSVE), the most common, serious, sporadic, viral encephalitis in humans (1). HSVE patients who do not receive antiviral treatment have an extremely high mortality rate (about 70%), and fewer than 3% of survivors return to normal function (1, 2). Of the common central nervous system (CNS) viral infections, HSVE has a disproportionately high mortality compared with encephalitis due to other viruses. The introduction of acyclovir (ACV) has dramatically improved mortality and morbidity for patients with HSVE (2, 3); mortality rates for HSVE have decreased to 19 - 28% (2, 3). Although ACV treatment for HSVE is highly effective, the rate of poor outcome including advanced sequelae remains high, at 30 - 50%, and the rate of return to normal living is less than 50% (1-3). Thus, the morbidity and mortality remain significantly high for HSVE despite standard ACV treatment at the acute stage.

Conventional predictors of poor outcome for HSVE have been reported to include the following 5 factors: age over 30 years, duration of more than 4 days from onset to the initiation of antiviral treatment, Glasgow Coma Scale (GCS) score of 6 points or less, detection of abnormal lesions on

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cranial computed tomography (CT) at initiation of antiviral treatment, and the detection of more than 100 copies/mL of HSV-DNA by polymerase chain reaction (PCR) in the initial cerebrospinal fluid (CSF) (2, 4-6). Management of patients with these predictors of poor outcome is difficult, and some patients with HSVE follow a prolonged course even with appropriate ACV treatment. Thus, further improvements in therapeutic regimens are needed for patients with HSVE.

Several previous reports have described relapses of HSVE in pediatric (7-10) and adult patients (3, 11-15), but there have been no previous studies involving adult HSVE patients who had a prolonged course and did not improve significantly despite standard ACV treatment. Therefore, the details of the clinical course were studied in adult HSVE patients with a prolonged course. The present study is the first to evaluate the clinical predictors in HSVE patients with a prolonged course despite standard ACV treatment.

Patients and Methods

The subjects consisted of HSVE patients treated with ACV during the acute stage. A series of patients with HSVE were selected from among all HSVE patients admitted to Nihon University Itabashi Hospital in Tokyo, Japan, between 1996 and 2007. To evaluate predictors of a prolonged course in adult HSVE patients, diagnostic and therapeutic protocols were established in advance, as reported previously (16). In this diagnostic protocol, the etiological diagnosis of HSVE was based on positive results from the following three laboratory tests: nested or real-time PCR; specific intrathecal HSV antibody synthesis; and chemiluminescence assay (17). The patients were treated according to the clinical guideline of herpes simplex encephalitis in Japan, which consisted of intravenous ACV (30 mg/kg/day) for 14 days from the time of admission (18). The therapeutic protocol permitted the use of corticosteroids at the discretion of the patients' treating physicians, although it did not specify the dosage or duration of corticosteroid treatment. When used, corticosteroids were started at the same time that ACV treatment was started. The selected patients were treated according to our therapeutic protocol, which consisted of intravenous ACV for 14 days starting at the time of admission. HSVE patients who were not treated with ACV in the acute stage were excluded. The selected patients were divided into 2 groups: the non-prolonged group, which was defined as patients who showed improvement within 2 weeks after initiation of ACV treatment without deterioration; and the prolonged group, which was defined as patients without any neurological improvement at the time of completion of the administration of ACV for 14 days. When the standard treatment failed, the patients were given additional treatment, such as extended ACV treatment, or adenine arabinoside (Ara-A), or combination therapy with both ACV and Ara-A. The clinical, neuroradiological, and neurophysiological parameters were compared between these groups in order to identify the predictors of a prolonged course in HSVE patients.

To assess differences between the groups, several parameters were studied. Clinical parameters that were extracted from the medical records of adult HSVE patients were: 1) sex (m = male, f = female); 2) age (years); 3) presence or absence of a prolonged course (absent = 0, present = 1); 4) duration from onset of HSVE to initiation of ACV treatment (days); 5) GCS at the start of ACV treatment; 6) corticosteroid administration (given = 0, not given = 1); and 7) clinical outcome. The clinical outcome was classified into five groups as reported previously (19), and categorized as follows: complete recovery = 0; mild sequelae = 1, for patients with minor neuropsychological deficits; moderate sequelae = 2, for patients with limitations due to motor, speech, memory, or seizure disorders; severe sequelae = 3, for patients requiring supportive care; and death = 4. The clinical outcomes were assessed three months after the completion of ACV treatment in the same way as described previously (19).

Neuroradiological and neurophysiological parameters included: 8) detection of focal lesions on initial cranial CT (initial CT examinations were performed in all subjects within 24 hours after admission; absent = 0, present = 1); 9) detection of abnormal lesions on initial magnetic resonance imaging (MRI; absent = 0, present = 1); and 10) detection of periodic lateralized epileptiform discharges (PLEDs) on the initial electroencephalogram (EEG; absent = 0, present = 1).

All continuous variables are expressed as minimum, mean, median, and maximum, and differences between groups were assessed using Mann-Whitney's U test. All categorical variables are expressed as percentages, and differences were assessed using Fisher's exact test. Values of $p < 0.05$ were considered statistically significant. Statistical analyses were performed using SPSS™ for Windows software, version 15 (SPSS, Chicago, IL, USA).

All of the patients and/or their families gave their written informed consent to participate in the study according to a protocol approved by the Ethics Committee for Human Studies at Nihon University Itabashi Hospital.

Results

A series of 23 HSVE patients was selected from a total of 32 HSVE patients. The 23 patients included 14 men and 9 women, and their ages ranged from 17 to 77 years (average age, 46.4±19.9 years). Nine patients who were treated with Ara-A therapy and did not receive ACV during the acute stage were excluded. The clinical data, including treatments and clinical outcomes, of the 23 patients are shown in Table 1. The patients were divided into the non-prolonged group [n=15 (65.2%); patients 1 to 15 in Table 1] and the prolonged group [n = 8 (34.8%); patients 16 to 23 in Table 1]. The prolonged group patients received additional antiviral therapies: 1 patient was treated with Ara-A (patient 17); 3 patients were treated with extended ACV treatment (patients 18, 20, and 21); and 4 patients were treated with

Table 1. Clinical Data of the 23 Patients with Herpes Simplex Virus Encephalitis Included in the Present Study

Patient No.	Sex	Age (years)	Prolonged course	Days from onset to initial ACV	GCS score at initial ACV	Initial CSF leukocyte cell count (/μL)	Initial CSF protein (mg/dL)	Additional treatment	Corticosteroid administration	Detection of lesions on initial brain CT	Detection of abnormal lesions on initial brain MRI	Detection of PLEDs on EEG	Outcome score
A. Non-prolonged group													
1	m	74	0	2	6	52	33	None	0	1	1	1	2
2	m	19	0	10	7	149	120	None	0	1	1	1	0
3	f	20	0	3	10	9	33	None	0	0	0	1	1
4	f	24	0	4	12	362	40	None	1	0	0	0	0
5	f	34	0	5	15	820	398	None	1	0	0	0	0
6	m	30	0	10	10	16	88	Ara-A	0	0	1	0	0
7	f	53	0	7	11	458	189	Extend ACV	1	1	1	1	2
8	m	56	0	2	12	503	37	None	0	0	1	1	1
9	m	77	0	1	12	571	580	None	0	0	1	0	1
10	f	77	0	3	4	7	88	None	0	1	1	0	2
11	m	27	0	4	15	21	45	None	1	1	1	0	0
12	m	32	0	5	13	253	171	None	0	0	0	0	0
13	m	54	0	4	11	9	38	Extend ACV	0	0	1	0	1
14	f	76	0	6	6	7	24	Extend ACV	1	0	1	0	2
15	f	61	0	3	15	315	97	None	0	0	0	0	0
B. Prolonged group with good clinical outcome													
16	m	28	1	5	12	236	115	Extend ACV +Ara-A	1	0	1	0	0
17	m	17	1	4	7	139	104	Ara-A	0	1	1	1	1
18	f	28	1	3	3	4	77	Extend ACV	0	1	1	1	1
19	f	53	1	1	6	45	72	Extend ACV +Ara-A	0	1	1	1	1
C. Prolonged group with poor outcome													
20	m	66	1	5	3	56	85	Extend ACV	1	1	1	0	3
21	m	57	1	7	3	944	410	Extend ACV	1	1	1	0	4
22	m	59	1	2	12	5	40	Extend ACV +Ara-A	0	1	1	1	4
23	m	45	1	7	4	3	70	Extend ACV +Ara-A	1	1	1	1	4

GCS, Glasgow coma scale; CT, computed tomography; MRI, magnetic resonance imaging; ACV, acyclovir; Ara-A, adenine arabinoside; PLEDs, periodic lateralized epileptiform discharges; EEG, electroencephalogram; m, male; f, female; Corticosteroid administration: 0 = given; 1 = not given; Detection of lesions on initial brain CT and MRI: 0 = absent; 1 = present; Detection of PLEDs on EEG: 0 = absent; 1 = present; Outcome score: 0 = complete recovery; 1 = mild sequelae; 2 = moderate sequelae; 3 = severe sequelae; 4 = death.; Prolonged course: 0 = absent; 1 = present; Additional treatment gives the name of the antiviral drug that was administered after ACV treatment for 14 days. Extend ACV indicates extension of the duration of ACV administration.

both (patients 16, 19, 22, and 23). Of these 8 patients, 2 (patients 22 and 23) of the 4 patients who received both additional treatments after initial ACV died, and 2 (patients 20 and 21) of the 3 patients with extended ACV treatment had poor outcomes (Table 1). Despite the administration of additional antiviral treatments, the clinical outcome was poor in 50% (4 patients, Nos. 20 to 23) of prolonged group patients, including 3 patients (patients 21 to 23) who died. Patient 21 died of HSVE, and patients 22 and 23 died of multiple organ failure. However, the 4 remaining patients (patients 16 to 19) had a good outcome.

Differences in the clinical characteristics between the non-prolonged and prolonged groups and the results of the statistical analyses are shown in Table 2. The mean GCS score at the time of the initial ACV treatment was 6.3 in the prolonged group and 10.5 in the non-prolonged group; the GCS score was significantly lower in the prolonged group than in the non-prolonged group ($p = 0.021$, Mann-Whitney's U test). The rate of abnormal lesions on cranial CT at the time of the initial ACV treatment was 87.5% in the prolonged group and 33.3% in the non-prolonged group; thus, abnormal lesions on cranial CT were significantly more common in the prolonged group than in the non-prolonged group ($p = 0.027$, Fisher's exact test). The mean clinical outcome score was 2.25 in the prolonged group and 0.8 in the non-prolonged group; thus, the clinical outcome score was significantly worse in the prolonged group than in the non-prolonged group ($p = 0.041$, Mann-Whitney's U test). No other variables showed significant differences between the groups. The prolonged group was thus characterized as having a lower GCS score at the start of ACV treatment, a higher rate of abnormal lesions on initial cranial CT, and a poorer outcome score.

Discussion

In the present study, compared to HSVE patients in the non-prolonged group, HSVE patients in the prolonged group had a lower GCS score at the start of ACV treatment, a higher rate of abnormal lesions on initial cranial CT, and a poorer clinical outcome score. A lower GCS score and a higher rate of abnormal lesions on initial cranial CT in the

Table 2. **Baseline Clinical Characteristics by Patient Group (Prolonged or Non-prolonged Course)**

	Prolonged course (n = 8)	Non-prolonged course (n = 15)	p
(1) Male (%)	75.0	53.3	1.00
(2) Age (minimum, mean, median, and maximum: years)	17.0, 44.1, 49.0, 66.0	19.0, 47.6, 53.0, 77.0	0.693
(3) Days from onset to initiation of ACV (minimum, mean, median, and maximum)	1.0, 4.5, 5.0, 7.0	1.0, 4.6, 4.0, 10.0	0.776
(4) GCS score at initiation of ACV (minimum, mean, median, and maximum)	3.0, 6.3, 5.0, 12.0	4.0, 10.5, 11.0, 15.0	0.021*
(5) Corticosteroid administration (%)	50.0	66.7	0.657
(6) Detection of lesions on initial CT (%)	87.5	33.3	0.027*
(7) Detection of lesions on initial MRI (%)	100.0	66.7	0.112
(8) Detection of PLEDs on EEG (%)	62.5	33.3	0.221
(9) Clinical outcome score (0 = complete recovery; 1 = mild sequelae; 2 = moderate sequelae; 3 = severe sequelae; 4 = death) (minimum, mean, median, and maximum)	0, 2.25, 2.0, 4.0	0, 0.8, 1.0, 2.0	0.041*

GCS, Glasgow coma scale; CT, computed tomography; MRI, magnetic resonance imaging; PLEDs, periodic lateralized epileptiform discharges; EEG, electroencephalogram. Continuous variables were compared using Mann-Whitney's U test. Categorical variables were compared using Fisher's exact test. *$p < 0.05$

prolonged group are also in accordance with the conventional predictors of poor outcome for HSVE (2, 4, 5). In the prolonged group, the average GCS score was 5.5 with a poor outcome and 7.0 with a good outcome. The GCS score of the prolonged group with a poor outcome tended to be lower than that with a good outcome. 87.5% of prolonged group patients had abnormal lesions on CT.

According to a previous study, abnormal lesions, such as a low density lesion in the temporal lobe, became distinct and spread as the clinical features progressed (20). Therefore, the prolonged group already had progression during the period of initial treatment compared to the non-prolonged group. MRI is reported to be a useful tool and superior to CT for early detection of abnormal lesions (21), and most HSVE patients in the present study showed abnormal lesions on MRI; this may explain why the detection rate of lesions on MRI was not significantly different between the two groups.

The therapeutic management of HSVE has been established (22), but it is still considered to be unsatisfactory in some patients. HSVE patients occasionally show a relapse or have a prolonged course; patients who had a relapse were not included in the present study. With respect to relapse, several studies have been reported (3, 7-15). In the previous studies, the frequency of relapse after HSVE ranged from 5% to 26% (8-10), with pediatric patients having higher relapse rates. In a report of a series of 27 children with acute HSVE, 7 patients (26%) had a relapse of HSVE, and 5 of the 7 improved with repeated, high-dose ACV (30 - 45 mg/kg/day) treatment, although 3 of the 5 patients developed moderate to severe sequelae (9). In other reports, 4 of 32 (13%) adult HSVE patients had a relapse. These 4 patients were treated with additional ACV (30 mg/kg /day) at the time of relapse, but 3 of the 4 patients developed moderate to severe sequelae (15). According to these studies, a low initial dose of ACV has been reported to be frequently associated with HSVE relapse (9, 15). Although the duration of the original ACV trial (2) was 10 days, most physicians currently continue therapy for 14 - 21 days to reduce the risk of relapse (3, 14, 22). A recently published clinician's guide for HSVE (22) recommends the continuous administration of ACV if HSV-DNA is still detected on PCR at the end of initial ACV treatment.

In contrast to relapses, HSVE patients with a prolonged course have not been studied previously. In the present study, 8 of 23 HSVE patients had a prolonged course despite standard ACV treatment (30 mg/kg/day for 14 days) at the time of admission, and additional treatment with antivi-

ral drugs (ACV, Ara-A, or both) was ineffective in 4 (patients 20 to 23 in Table 1) of the 8 patients, though it was effective in the remaining 4 (patients 16 to 19 in Table 1).

The pathophysiological findings associated with prolonged HSVE remain unclear. Prolongation of HSVE was considered to be introduced by insufficient HSV inhibition, secondary encephalitis, or both. Insufficient HSV inhibition resulted from an insufficient ACV dose and/or the presence of ACV-resistant HSV. Considering that 8 out of 23 patients had a prolonged course despite initial standard ACV treatment in the present study (18), this initial treatment might have been insufficient to inhibit HSV infection for patients with a prolonged course. On the other hand, it has been reported that levels of pro-inflammatory cytokines in the CSF of HSVE patients were high (23), and HSV was not always detected by PCR at the time of relapse in the CSF of relapsed pediatric patients with HSVE (9). These reports suggested that prolongation of HSVE might be also caused by secondary encephalitis based on the host immune response. In the present study, corticosteroid treatment was given to 50% of prolonged group patients and 66.7% of non-prolonged group patients. Corticosteroid treatment was not significantly different between the groups, but 10 of 14 patients given corticosteroids did not have a prolonged course. Therefore, corticosteroid treatment may have a beneficial protective effect against secondary encephalopathy based on the host immune response in HSVE (23). Since our previous retrospective study showed that corticosteroid treatment improved the outcome of HSVE (16), this suggested the need for further investigation to determine whether corticosteroid treatment had the potential to protect against HSVE progression.

The prolonged group patients had a poorer clinical outcome than the non-prolonged group patients. Therefore, initial antiviral treatment may need to be modified in patients who have the predictors for a prolonged course, and this may improve their clinical outcome. The possible modifications of the initial antiviral treatment may include high-dose ACV treatment or combination ACV and Ara-A therapy in patients with the predictors of a prolonged course.

In conclusion, 2 predictors of a prolonged course were identified in HSVE patients: a lower GCS score at the start of antiviral treatment and a higher rate of abnormal lesions on initial CT. Since the number of patients was small in the present study, further investigation is required to assess the predictors of clinical outcome using multivariate analysis in a larger number of HSVE patients.

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