CSF iron, ferritin and transferrin levels in restless legs syndrome

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SUMMARY The aim of this study is evaluating iron, ferritin, and transferrin in both serum and CSF in patients of restless legs syndrome (RLS), based on the hypothesis that iron deficiency in the central nervous system (CNS) causes the symptoms as a result of the dysfunction of dopaminergic systems. These parameters, polysomnographic sleep measures, and subjective evaluation of the sleep quality were compared in 10 patients of idiopathic RLS (RLS group) and 10 age-matched patients of psychophysiological insomnia without RLS symptoms (non-RLS group). With sleep patterns, sleep latency was longer and sleep efficiency was lower in the RLS group than those in the non-RLS group. Periodic leg movement index in the RLS group was higher than that of the non-RLS group. With serum examination, there were no significant differences for the iron, ferritin, and transferrin values between the both groups. With CSF examination, the iron and ferritin values were lower and the transferrin values were higher in the RLS group than those in the non-RLS group. There was positive correlation between the serum and CSF ferritin levels in the both groups, but the slope of the regression lines for the RLS group was lower than that for the non-RLS group. These results indicate low brain iron concentration caused by the dysfunction of iron transportation from serum to CNS in patients with idiopathic RLS.

KEYWORDS cerebrospinal fluid, dopamine, ferritin, periodic leg movement, polysomnogram, transferrin

INTRODUCTION

Restless legs syndrome (RLS) is a well defined sleep-related disorder characterized by abnormal sensations in the legs at rest, associated with an urge to move the affected legs. The symptoms become worse at night, and the patients often develop difficulty with sleep initiation and halfway awakening (Ekbom, 1944). Although the mechanism of development is unknown, there is circumstantial evidence for a role of the dopaminergic system and iron status in the pathophysiology of RLS (Allen and Earley, 2001). Tyrosine hydroxylase is the rate-limiting enzyme in the production of dopamine and requires iron as a cofactor for hydroxylation of tyrosine (Cooper et al., 1991). Therefore, iron deficiency may affect dopamine production indirectly, while dopaminergic agents are effective in therapy for idiopathic RLS (Brodeur et al., 1988; Chesson et al., 1999; Collado-Seidel et al., 1999; Earley et al., 1998; Hening et al., 1999; Montplaisir et al., 1999). Several clinical studies have reported a higher incidence of RLS in pregnancy and iron deficiency anemia, suggesting a relationship between low serum iron levels and RLS (Ekbom, 1960, 1970; Parrow and Werner, 1966).

Although serum iron levels, in contrast to tissue iron levels, are highly variable and may be affected by diet, stress, sleep behavior, and individual circadian rhythms, serum ferritin is regarded as a more reliable indicator of iron deficiency. The majority of the iron in the brain is found in ferritin, which serves as a storage protein for intracellular iron (Tarquini, 1978). O’Keeffe et al. (1994) investigated the relationship between ferritin status and RLS in 18 elderly patients with RLS and 18 age-matched control subjects. They reported that serum ferritin levels were reduced in the RLS patients compared with control subjects. They also maintained that whether or not anemia is present, low serum ferritin status is an important contributor to the development of RLS because serum iron levels and hemoglobin levels did not differ between
two groups. Sun et al. (1998) also investigated the relationship between serum ferritin levels and RLS severity. They reported that most of the patients with severe RLS had ferritin levels ≥50 μg L⁻¹ and showed significantly more periodic limb movements in sleep with arousal. Nordlander (1953) reported that iron supplementation can also bring about marked improvement in some patients with RLS, although the majority of the patients had normal iron status before this treatment. These results may indirectly suggest abnormalities in iron transportation from serum to CNS in patients of RLS.

Earley et al. (2000) measured the relationship between serum and CSF iron, ferritin, and transferrin in patients with idiopathic RLS, and reported that a 65% decrease in CSF ferritin and 300% increase in CSF transferrin was detected in RLS patients compared with age-matched healthy controls, despite normal serum levels of ferritin and transferrin in both groups. They also reported that a correlation between serum and CSF ferritin was found for both groups; however, the slope of the regression line for the RLS patients was reduced compared with that of the controls. They emphasized the low level of CSF ferritin and high level of CSF transferrin in RLS patients, in spite of a level of serum ferritin and transferrin similar to normal controls. Overall, the data would suggest that the capacity for the iron transport to the CNS is abnormal in idiopathic RLS. To determine whether brain iron status was altered in idiopathic RLS, we evaluated serum and CSF iron, ferritin, and transferrin concentrations in patients with RLS and patients of psychophysiological insomnia without RLS symptoms, who had been complaining insomnia as much as RLS patients.

METHODS

We enrolled 10 non-treated patients with idiopathic RLS (six female and four male; mean age ± SD, 71.3 ± 5.5 years) who complained of all four symptoms of RLS used by the International RLS Study Group as minimal diagnostic criteria (Walters and the International Restless Legs Syndrome Study Group, 1995): (a) desire to move the extremities, often associated with paresthesias/dysesthesias, (b) motor restlessness, (c) worsening of symptoms at rest, with at least temporary relief by activity, and (d) worsening of symptoms during the evening or the night; in addition, these patients had at least a 1-year history of daily RLS symptoms. Ten age-matched patients of psychophysiological insomnia diagnosed by the criteria of International Classification of Sleep Disorders (Diagnostic Classification Steering Committee, 1990) were used on the control subjects (five female and five male; mean age ± SD, 70.5 ± 10.1 years) who had never developed RLS symptoms. Subjects with renal/metabolic disorders, iron deficiency anemia, neuropathy, brain or spinal code injuries, chronic inflammatory disease, or chronic pain syndromes were excluded. Those taking iron supplements, non-steroidal anti-inflammatory agents, H₂-blockers, hypnotics, neuroleptics, dopaminergic, or opiate agents were also excluded.

All subjects had a standard all-night clinical polysomnogram (PSG), which included recording sleep electroencephalogram (EEG) (C3-A2, O1-A2), bilateral eye movements, respiratory effort (abdominal and thoracic), airflow (oral and nasal), O₂ saturation of arterial blood, submental electromyogram (EMG), and bilateral anterior tibialis EMG. Sleep stage was scored according to the standard criteria (Rechtschaffen and Kales, 1968), and the periodic leg movement (PLM) index was identified using the modified Guilleminault criteria (Coleman, 1982). Time in bed, total sleep time, percentage sleep efficiency, and PLM index were obtained independently. The Japanese version of the Pittsburgh Sleep Quality Index (PSQI-J; Doi et al., 2000) was also carried out to all the subjects in order to investigate a subjective sleep quality. The highest score is 21 and the higher scores mean more severe state of sleep disturbance. Using a cut-off point of 5.5 in the global score, estimations of sensitivity and specificity provide high enough for sleep disorders.

The lumbar puncture and venous blood extraction were performed at 10:00 hours on an inpatient basis. Six milliliters (mL) of CSF and 5 mL of venous blood were stored at −80 °C until assayed for each valuables.

We measured iron by the nitroso-PSAP method (Autoanaly-alyzer 7170-type; Hitachi Ltd, Japan), ferritin by the two-step sandwich-enzyme immunoassay (EIA) method (Automated Chemiluminescent Enzyme Immunoassay Analyzer LS-2000; Eiken Chemical Co. Ltd, Tokyo, Japan) and transferrin by the nephelometry method (Behring Nephelometer Analyzer; Dade Behring Marburg Co. Ltd, Marburg, Germany) following the manufacturer’s instructions in both serum and CSF.

All subjects gave informed written consent before undergoing this study. The medical ethics committee of the psychiatric division, that is the subsystem of Shimane Medical University, approved this study.

Data analysis

Differences in non-ordered categorical variables were tested using the chi-square test or Fisher’s exact test. Differences in continuous variables between both groups were tested using an unpaired t-test. We explored the relationship between serum and CSF values for each of the three indices by Pearson’s coefficient correlation for each group separately. The differences of the slopes for regression lines between the both groups were elevated by an analysis of covariance (Snedecor and Cochran, 1967). The procedure is as follows: each residual sum of squares was calculated by the null hypothesis to determine whether the slopes of both regression lines were equal, and the F-value was calculated by each residual sum of the square. Finally, the significance in the difference of the slopes of both lines was evaluated by reference to a F-value distribution table of 1%.

RESULTS

Table 1 shows the comparison of demographic composition and hematological variables between the two groups. There were no significant differences for age, gender, and the values
of complete blood count, such as RBC or hemoglobin between the groups. Table 2 shows the sleep patterns, PLM index, and PSQI-J global score of the both groups. Sleep latency was longer, and the sleep efficiency was lower in the RLS group than in the non-RLS group. The PLM index of the RLS group was higher than that of the non-RLS group. There was no significant difference in the PSQI-J global score between the both groups. Table 3 shows the comparison of serum and CSF variables between the groups. There were no significant differences for the serum iron, ferritin, or transferrin values. The CSF iron and ferritin values of the RLS group were lower and the CSF transferrin values of the RLS group were higher than those of the non-RLS group. Fig. 1 shows a linear regression plot of the relationship between serum and CSF ferritin for both groups. The slope for the regression line of the RLS group was significantly lower than that of the non-RLS group. RLS, restless leg syndrome; NS, not significant.

### Table 1: Comparison of demographic composition and hematological values

<table>
<thead>
<tr>
<th></th>
<th>RLS group</th>
<th>Non-RLS group</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>10</td>
<td>10</td>
<td>NS</td>
</tr>
<tr>
<td>Age</td>
<td>71.3 ± 5.5</td>
<td>70.5 ± 10.1</td>
<td>NS</td>
</tr>
<tr>
<td>Sex (female : male)</td>
<td>6 : 4</td>
<td>5 : 5</td>
<td>NS</td>
</tr>
<tr>
<td>RBC (10^6 µL⁻¹)</td>
<td>445.8 ± 35.1</td>
<td>429.6 ± 24.4</td>
<td>NS</td>
</tr>
<tr>
<td>Hb (g dL⁻¹)</td>
<td>14.08 ± 0.75</td>
<td>13.70 ± 1.24</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are given as mean ± SD. There was no significant difference between the groups.

### Table 2: Comparison of sleep patterns, PLM index, and PSQI-J global score

<table>
<thead>
<tr>
<th></th>
<th>RLS group</th>
<th>Non-RLS group</th>
<th>P-value</th>
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</thead>
<tbody>
<tr>
<td>Time in bed (min)</td>
<td>471.8 ± 24.2</td>
<td>444.7 ± 44.6</td>
<td>NS</td>
</tr>
<tr>
<td>Sleep latency (min)</td>
<td>103.3 ± 26.8</td>
<td>59.2 ± 30.5</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Total sleep time (min)</td>
<td>299.4 ± 24.3</td>
<td>338.3 ± 59.2</td>
<td>NS</td>
</tr>
<tr>
<td>Sleep efficiency (%)</td>
<td>63.4 ± 3.3</td>
<td>75.8 ± 8.6</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>PLM index</td>
<td>23.2 ± 5.79</td>
<td>1.1 ± 1.20</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>PSQI-J global score</td>
<td>10.3 ± 1.77</td>
<td>8.8 ± 2.62</td>
<td>NS</td>
</tr>
</tbody>
</table>

PLM, periodic leg movement; PSQI-J, Japanese version of Pittsburgh Sleep Quality Index; NS, not significant.

Values are given as mean ± SD, unpaired t-test. Sleep latency was longer and percentage sleep efficiency was lower in the RLS group than in the non-RLS group. The PLM index of the RLS group was higher than that of the non-RLS group. There was no significant difference in the PSQI-J global score between the both groups.

### Table 3: Comparison of serum and CSF valuables

<table>
<thead>
<tr>
<th></th>
<th>RLS group</th>
<th>Non-RLS group</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>[1.50 ± 0.71, 3.00 ± 2.01] &lt; 0.05</td>
<td>[121.4 ± 29.8, 114.8 ± 42.2] NS</td>
<td></td>
</tr>
<tr>
<td>Ferritin (ng mL⁻¹)</td>
<td>[6.68 ± 0.93] &lt; 0.01</td>
<td>[138.1 ± 35.9, 111.7 ± 26.9] NS</td>
<td></td>
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<tr>
<td>Transferrin (mg dL⁻¹)</td>
<td>[243.0 ± 44.7] NS</td>
<td>[243.1 ± 28.1] NS</td>
<td></td>
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<tr>
<td>CSF</td>
<td>[2.18 ± 0.70, 1.60 ± 0.39] &lt; 0.05</td>
<td>[1.50 ± 0.71, 3.00 ± 2.01] &lt; 0.05</td>
<td></td>
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</table>

RLS, restless leg syndrome; NS, not significant.

Values are given as mean ± SD, unpaired t-test. CSF concentrations of iron and ferritin were lower and of transferrin were higher in the RLS group than in the non-RLS group. There was no significant difference in the serum valuables between the groups.

**DISCUSSION**

In this study, we discovered two matters characteristic of the RLS patients, one of those is a sleep patterns and another is CSF findings.

With regard to the sleep patterns, sleep latency was longer and sleep efficiency was lower in the RLS group than that of the non-RLS group. These findings supported by the PSG are consistent with the characteristics of this syndrome. In contrast to PSG investigations, there was no difference between the groups at subjective sleep quality index such as the PSQI-J. The PSG examinations are indispensable, in order to evaluate sleep patterns objectively and to distinguish this syndrome from other sleep disturbances. The PLM index of the RLS group was higher than that of the non-RLS group, which also fulfilled the objective criteria for RLS (Montplaisir et al., 1998). They explained that using a cut-off point of 11 or more...
in the PLM index of two consecutive PSG evaluations, estimations of sensitivity and specificity provided 81% for idiopathic RLS. Considering the patients of the RLS group met the standard criteria of this syndrome, these subjects are considered in a genuine group of idiopathic RLS and our findings of PLM index are not contradictory with their conclusion.

With regard to CSF findings, we found that the CSF iron and ferritin levels in the RLS group were significantly lower and the CSF transferrin levels in the RLS group were significantly higher than those in the non-RLS group. CSF ferritin, which is the major iron storage protein in the brain, reflects a variation of total brain iron, and CSF transferrin, which is the principal iron transport protein in the brain, reflects a necessity for iron in the brain (Conner and Menzies, 1995). For example, the amount of ferritin in iron deficiency rats, decrease in the brain and the amount of transferrin increase on a compensatory target (Chen et al., 1995). Therefore, our findings, such as low CSF levels of ferritin and high CSF levels of transferrin in the RLS group despite the normal serum levels of those substances, show that total brain iron concentrations in RLS patients are lower than those in psychophysiological insomnia patients without RLS.

In order to take iron into the brain, active transportation of iron across the blood–brain barrier (BBB) must work (Bradbury, 1997). The cerebral capillary transferrin receptor in the BBB is an important iron-controlling protein in the interface between serum and CSF. Transferrin receptors are found in high concentration in the endothelial cells that make up a part of the BBB (Moos, 1996). The transferrin–transferrin receptor complex is considered a primary candidate for moving iron across the BBB. In this study, we also explored the correlation between serum and CSF ferritin in both groups; however, the slope of regression line for the RLS group was significantly reduced compared with that of the psychophysiological insomnia patients without RLS, despite similar levels of serum ferritin in the groups. These results would suggest a dysfunction of iron transportation at the BBB in idiopathic RLS patients. Earley et al. also reported the same differences between RLS patients and healthy controls. Furthermore, the characteristic point of this study is that the control group consisted of the psychophysiological insomnia patients. This control group is instructive for excluding the sleep loss as a confounding variable.

Regional distribution of iron in the brain is not homogeneous. The substantia nigra, red nucleus, deep cerebellar nucleus, and corpus striatum contain more iron than other areas of the brain in healthy subjects (Bartzokis et al., 1993). Magnetic resonance imaging (MRI) techniques to quantify brain iron concentration in each area showed that RLS patients have lower iron concentrations in the substantia nigra and in the putamen (Allen et al., 2001). The results of this MRI study suggest that idiopathic RLS patients may have a dysfunction of dopamine production induced by the iron deficiency in a dopamine-related specific area of the brain, and support the iron-dopamine model of this syndrome (Allen and Earley, 2001).

Using CSF analysis of iron-related proteins such as ferritin and transferrin, it was shown that a reduction in brain iron concentration occurs in RLS patients. There are probably several mechanisms related to this syndrome; however, we believe that the iron reduction in the brain is one of the important pathogenesis. Further studies to assess the relationship between brain iron and RLS symptoms may be helpful to elucidate the pathogenesis of RLS.

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