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ORIGINAL RESEARCH

Compromised Dynamic Cerebral Autoregulation in Patients with Restless Legs Syndrome

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Background: Restless legs syndrome (RLS) is a prevalent sensorimotor nervous system disorder in patients accompanied with insomnia, blood pressure fluctuation, and sympathetic dysfunction. These symptoms may disrupt cerebral hemodynamics. Dynamic cerebral autoregulation (dCA) describes the temporary response of cerebrovascular system to abrupt fluctuations in blood pressure, which keep cerebral blood flow stable and serve as a marker of cerebrovascular system ability.

Objective: This research aimed to assess dCA in RLS patients.

Methods: In this study, RLS patients were recruited and subsequently classified into four groups (mild, moderate, severe, and very severe) based on the International RLS Rating Scale (IRLS). Healthy controls matched for age and sex were enrolled. All participants were evaluated dCA by assessing phase difference (PD). A portion of patients with RLS was reassessed for dCA after one month of medication therapy (pramipexole [0.125 mg/day] and gabapentin [300 mg/day]).

Results: There were altogether 120 patients with RLS and 30 controls completed the polysomnography and dCA assessment. PD was lower in the moderate, severe, and very severe RLS groups than that in the controls and mild RLS groups. Periodic limb movement index (PLMI), arousal index, and IRLS all showed a linear correlation with PD in RLS patients. Additionally, PD increased in RLS patients after therapy.

Conclusion: The dCA was compromised in moderate, severe, and very severe RLS patients and was negatively correlated with the IRLS, arousal index, and PLMI. After 1 month of therapy, dCA improved in RLS patients.

Keywords: restless legs syndrome, dynamic cerebral autoregulation, autonomic nerve dysfunction, dopaminergic

Introduction

Restless legs syndrome (RLS) is a sensorimotor nervous system disorder marked by extreme discomfort in both lower limbs, with irresistible movement impulses in the evening or when it is quiet, and continuous movement could partially or completely alleviate this impulse.[1] It may cause insomnia, fatigue, blood pressure fluctuation, and sympathetic dysfunction.[2] A series of trends appeared suggesting that the risk variables for stroke and RLS interacted.[3] Several studies have found that people with RLS who had periodic limb movements in sleep (PLMS) have cerebral hemodynamic disturbances.[3,4] Byun et al used near-infrared spectroscopy to find that there were no changes in the low-frequency oscillations between oxyhemoglobin concentration and deoxyhemoglobin concentration during the different sleep stages, which shows that RLS patients with PLMS had cerebral hemodynamic disturbances, and sympathetic hyperactivity has been suggested as the underlying mechanism.[4] RLS patients are prone to erectile dysfunction, and the risk increases with a higher frequency of RLS symptoms.[5,6] Shneyder et al found that more autonomic complaints were present in RLS patients, mainly in sialorrhea, heat intolerance, dizziness on standing, early abdominal fullness, and constipation.[7] Furthermore, patients with RLS have been shown to have decreased nitric oxide (NO) levels, elevated nitrite oxide synthase expression, compromised vascular endothelial function, and increased arterial stiffness,[8–11] which may disturb

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cerebral hemodynamics and increase the risk of cerebrovascular disease.[12] However, it is not easy to determine the underlying functional alterations of cerebrovascular disease.

Dynamic cerebral autoregulation (dCA), is the brain's natural ability to sustain cerebral blood flow under conditions of rapid and significant variations in arterial blood pressure. It is defined as the instantaneous rate of change of cerebral blood flow to cerebral perfusion pressure.[13] Among the evaluation parameters were phase difference (the primary metric, indicates the phase change angle between 0° and 90°), gain (the variation in arterial blood pressure and cerebral blood flow velocity amplitude), and the coherence function (the signal-to-noise ratio) were among the evaluation parameters.[14] A decreased phase difference often reflects a compromised dCA. The mechanism of dCA is complex and still unclear; four theories are widely accepted: endothelial, myogenic, metabolic, and neurogenic mechanisms.[13] The dCA could be vital to sustaining steady cerebral blood flow in patients with RLS because RLS has potential effects on cerebral hemodynamics; however, few studies have assessed dCA in RLS patients.

One of the primary theories for the etiology of RLS is a dopaminergic deficiency in the central nervous system (CNS).[15] The most widely used medication is dopamine agonists (DAs), which lessen both PLMS and the main symptoms of RLS.[16] Pramipexole, a high-affinity D3 receptor agonist, enhances objective sleep parameters and subjective RLS symptoms.[17] Winkelman et al found that pramipexole significantly decreased periodic limb movement index (PLMI) and increased total sleep time and sleep efficiency in RLS patients.[18] Trenkwalder et al demonstrated a highly significant deterioration of subjective RLS indicators in the group that stopped pramipexole after six months of treatment in a withdrawal experiment.[19] Pramipexole may diminish autonomic responses and re-establish appropriately responsive sympathovagal homeostasis by acting on the same D3 receptors in the dorsal and intermediolateral gray matter of the spinal cord, which may affect dCA. The voltage-dependent calcium channel's α2δ-1 subunit is highly-affinity bound by gabapentin, an analog of gamma aminobutyric acid, which additionally inhibits the release of neurotransmitters and postsynaptic excitability.[20] Gabapentin improved sleep architecture and reduced PLMS score and symptoms across all rating scales as compared to placebo in a double-blind, cross-over trial.[21] Gabapentin inhibits the release of serotonin, norepinephrine, and dopamine via engaging in a voltage-dependent calcium channel interaction with the α2δ-1 subunit.[22] These neurotransmitters may affect the dCA.

Autoregulatory parameters of patients with RLS were obtained using transfer function analysis (TFA) to assess dCA function in this study. We speculate that RLS patients have compromised dCA, and find the relationship between dCA parameters and clinical data, and access the function of pramipexole and gabapentin in dCA of RLS after therapy, providing more evidence for evaluating RLS and accompanying symptoms.

Methods

Participants

The study recruited participants from the Department of Neurology at the First Hospital of Jilin university between March 2020 and November 2022. The following were the inclusion criteria for each of the groups: Control group: age- and sex-matched individuals without neurological disease and sleep disorders and the IRLS scores were zero; RLS group: according to the International Restless Legs Syndrome Study Group (IRLSSG), the following were the diagnostic criteria for RLS: an irresistible urge to move the legs, typically but not constantly accompanied by unpleasant and uncomfortable feelings in the legs; symptoms that start or get worse during lying down or sitting down; Movement could either totally or partially relieve symptoms; symptoms are either exclusive to the evening or night and worsen there than during the day; and the presence of the aforementioned characteristics cannot be completely explained by the fact that they are signs of a different medical or behavioral condition;[23] excellent bilateral temporal window penetration. The IRLS was used to further categorize the patients into four categories: mild, moderate, severe, and very severe, Mild ranging from 1 to 10, moderate ranging from 11 to 20, severe ranging from 21 to 30, and very severe ranging from 31 to 40. Exclusion criteria were: age more than 65 years; medical or neurological disorders (including diabetes mellitus, liver or kidney disease, Parkinson's disease, stroke, epilepsy, narcolepsy, other movement disorder and psychiatric disease); hyperthyroidism, arrhythmia, and other hemodynamic factors; dopaminergic, adrenergic, or cholinergic medication for up to 4 weeks before enrollment; and vascular ultrasound (EMS-9 PB, Delica, Shenzhen, China) with the diagnosis of

intracranial and extracranial vascular stenosis or occlusion. If symptoms are moderate or above, pharmacological therapy was required. Non-dopaminergic drugs like gabapentin are the first-line treatment, and avoid augmentation. Dopaminergic therapies may develop augmentation syndrome and dose-related adverse effects (such as dizziness, drowsiness, nausea, or headache), so a lowest-effective dose was prescribed for DAs in patients with RLS.[24] Since DAs and gabapentin utilize distinct pharmacological processes via which they achieve their therapeutic effects, combination treatment can be more effective at controlling symptoms than monotherapy.[23,24] Gabapentin is preferential for pain and paresthesia, while dopaminergic therapy is primarily used to improve motor symptoms in patients with RLS.[24] Considering co-morbidities and symptom intensity of patients with moderate or above symptoms, we chose pramipexole and gabapentin. It is advised to take 0.125 mg of pramipexole once day, 2–3 h before bedtime, the advised conventional dosage for gabapentin is 300mg given 2–3 h before bedtime.[25]

Age, sex, medical history, Cambridge-Hopkins questionnaire for RLS, IRLS, and neurological examination were obtained from all participants. All participants underwent polysomnography (PSG), dCA measurements, and blood tests, including serum iron, ferritin, creatinine, urea nitrogen, alanine aminotransferase, and aspartate aminotransferase levels. The dCA was examined again after one month of therapy with pramipexole (0.125 mg/day) and gabapentin (300 mg/day) in some patients with RLS.

Questionnaires

The aim of the Cambridge-Hopkins questionnaire for RLS (CH-RLSq) is to evaluate RLS symptoms using patient-administered diagnostic questionnaires, which includes 6 items for diagnostic symptoms and 1 item for differential diagnosis. The CH-RLSq was validated in the general population by Allen et al, with 87.2% sensitivity and 94.4% specificity for ascertainment of RLS.[26]

As the first-choice method to assess the subjective severity of RLS, IRLS was developed by the IRLSSG through questions posed to its membership, consisting of 10 elements scored from 0 (none) to 4 (very severe), concerning the frequency, severity, and effects of RLS symptoms on mood, sleep, and daily activities.[27] IRLS categories have been defined as follows: mild (0–10), moderate (11–20), severe (21–30), and very severe (31–40). The IRLS was validated in 20 centers from six countries by Walters et al, possessing high levels of convergence, reliability for test-retests over an interval of 2–4 weeks, internal coherence, and inter-examiner reliability.[28]

Polysomnography

All subjects were required to be monitored for at least 8 hours in a typical sleep-laboratory room using PSG recording (Compumedics, Abbotsford, Australia). Caffeinated beverages were not allowed for participants in the afternoon before the recordings. The research adhered to the American Academy of Sleep Medicine (AASM) with regards to electro-encephalography, electrooculography, chin muscle electromyography, electrocardiography, nasal pressure, finger oximetry, and respiratory bands on the chest and abdomen. The AASM version 2.3 were applied by professional PSG technologists to assess the PSG findings.

Dynamic Cerebral Autoregulation Assessment and Analysis

For at least 12 hours before measurement, participants were instructed to refrain from using nicotine, alcohol, and caffeinated drinks, and physical activity. The experiments were carried out at a consistent temperature of 22–24°C in a dedicated laboratory. An expert operator conducted all measurements. Before the baseline arterial blood pressure and heart rate were measured (Omron 711; Omron, Kyoto, Japan, the participants were required to spend 15 minutes lying down in a comfortable supine position). We spontaneously recorded beat-to-beat arterial blood pressure (Finometer Model 1; Finapres Medical Systems, Enschede, Netherlands) and Continuous bilateral middle cerebral artery blood flow velocity (MultiDop X2, DWL, Sipplingen, Germany) for 10 minutes. We further analyze dCA through the obtained data. A facemask connected to a nasal tube and a capnograph were used to measure end-tidal CO_2 levels. Every participant was asked to remain awake throughout.

The dCA data were processed using MATLAB software (MathWorks, Natick, MA, USA). TFA was used to analyze dCA. The cross-correlation function was used to eliminate the delay between the Doppler cerebral blood flow velocity

and the arterial blood pressure, and align the signal with heart rate beat by beat. The signal was filtered using a third-order Butterworth low-pass filter (with a cutoff frequency of 0.5 Hz) and reduced to 1Hz. TFA was used to analyze and calculate the quotient of the cross-correlation spectrum and autocorrelation spectrum between the cerebral blood flow velocity (CBFV) and arterial blood pressure (ABP), and derive relevant parameters for evaluating dCA function in the low-frequency domain (0.06–0.12 Hz).[29] The degree of a linear relationship between *oscillations* in CBFV and ABP was reflected by coherence. When the coherence was > 0.5, statistical analysis was performed.[30]

Statistical Analysis

The SPSS software (version 23.0) was used to analyze data statistics. The normal distribution of continuous variables was evaluated using the Shapiro–Wilk test. The mean and standard deviation were used to represent measurement data with a normal distribution, which included IRLS, mean arterial pressure (MAP), iron, ferritin, PD, heart rate, end-tidal CO_2, total sleep time (TST), sleep efficiency (SE), sleep onset latency (SOL), stage 1 non-REM (stage N1), stage 2 NREM (stage N2), stage 3 NREM (stage N3), rapid eye movement (REM) sleep, arousal index, periodic limb movement index (PLMI) and apnea-hypopnea index (AHI). Male sex, age, body mass index (BMI), IRLS, hypertension, hyperlipidemia, and smoking were examples of categorical variables that were reported as percentages and absolute numbers. The variations between various independent samples (IRLS, PD, iron, ferritin, age, BMI, MAP, heart rate, end-tidal CO_2, TST, SOL, SE, stage N1-N3, REM sleep, arousal index, AHI, PLMI, male sex, hypertension, hyperlipidemia, and smoking) were compared using one-way analysis of variance or the chi-square test. The Spearman-test was used to analyze the correlations between PD and IRLS, arousal index, and PLMI. An independent sample *t*-test was used to assess the differences in age, TST, SOL, SE, stage N1-N3, REM sleep, arousal index, AHI, PLMI, and PD, and the chi-square test was used to assess the differences in IRLS and male between the RLS group with ferritin < 45 μg/L and the RLS group with ferritin ≥ 45 μg/L. Pre- and post- therapy PD in RLS patients were compared using a non-parametric Wilcoxon signed-rank test. Univariate and multivariate linear regressions were used to assess the association between PD and clinical parameters, including male sex, age, BMI, MAP, heart rate, hypertension, hyperlipidemia, smoking, end-tidal CO_2, IRLS, iron, ferritin, TST, SOL, SE, stage N1-N3, REM sleep, arousal index, AHI, and PLMI. The relationship between PD and clinical factors, including male, age, BMI, MAP, heart rate, hypertension, hyperlipidemia, smoking, end-tidal CO2, IRLS, iron, ferritin, TST, SOL, SE, stage N1–N3, REM sleep, arousal index, AHI, and PLMI, was examined using univariate and multivariate linear regressions. The adjusted P-value was calculated using Bonferroni corrected post hoc analysis. The statistical significance level was set at $P < 0.05$.

Results

Participant Characteristics

In total, there were 142 RLS patients and 43 age- and sex-matched normal controls were included, and 120 RLS patients (30 with mild, 30 with moderate, 30 with severe RLS, and 30 with very severe) and 30 controls completed PSG and dCA assessments. All parameters from all groups were compared (see Table 1). Male sex, age, BMI, hypertension, hyperlipidemia, and smoking were very similar among the five groups and were not statistically different. MAP, heart rate, end-tidal CO_2, and iron and ferritin levels among the groups showed no statistically significant differences.

PSG Parameters

Among the PSG parameters, TST, SOL, SE, REM sleep, stage N1, stage N3, arousal index, and PLMI showed significant variations in the RLS groups and controls ($P = 0.004, < 0.001, < 0.001, = 0.005, < 0.001, < 0.001, < 0.001, < 0.001$, respectively). Regarding TST, the level was significantly decreased in patients with severe (337.80 ± 70.04) and very severe RLS groups (330.27 ± 90.18) compared to controls (400.40 ± 34.72); sleep onset latency was significantly longer in severe (22.41 ± 9.14) and very severe RLS groups (27.89 ± 10.85) than those in controls (15.53 ± 3.03), mild RLS group (15.53 ± 3.33) and moderate RLS groups (17.11 ± 4.10), with statistically significant difference between severe and very severe RLS groups; sleep efficiency was significantly decreased in patients with moderate (71.06 ± 13.37), severe (63.34 ± 11.44), and very severe RLS groups (58.13 ± 14.19) than those in controls (86.50 ± 8.72), and sleep efficiency was statistically different in very severe RLS groups than those

Table 1 Clinical Characteristics and Phase Difference in Mild, Moderate, Severe, Very Severe RLS Patients and Controls

	Controls (n = 30)	Mild RLS (n = 30)	Moderate RLS (n = 30)	Severe RLS (n = 30)	Very Severe RLS (n = 30)	χ^2/F	*P*
Male, n (%)	12 (40.0)	12 (40.0)	13 (43.3)	10 (33.3)	10 (33.3)	1.019	0.907
Age (years)						8.124	0.775
< 35	3 (10.0)	1 (3.3)	3 (10.0)	1 (3.3)	3 (10.0)		
35 <= Age < 45	4 (13.3)	4 (13.3)	4 (13.3)	7 (23.3)	3 (10.0)		
45 <= Age < 55	15 (50.0)	10 (33.3)	10 (33.3)	10 (33.3)	12 (40.0)		
≥ 55	8 (26.7)	15 (50.0)	13 (43.3)	12 (40.0)	12 (40.0)		
BMI (kg/m^2)						14.449	0.071
< 18.5	2 (6.67)	2 (6.67)	1 (3.33)	0 (0.0)	0 (0.0)		
18.5 <= BMI < 24	13 (43.3)	13 (43.3)	24 (80.0)	17 (56.7)	17 (56.7)		
≥ 24	15 (50.0)	15 (50.0)	5 (16.7)	13 (43.3)	13 (43.3)		
MAP (mmHg)	85.03 ± 14.90	84.00 ± 9.42	89.10 ± 9.70	88.23 ± 7.44	87.17 ± 8.38	1.300	0.273
Heart rate (beats/min)	74.5 ± 8.1	73.6 ± 7.5	74.7 ± 9.9	75.7 ± 6.6	72.6 ± 7.3	0.640	0.635
IRLS	—	6.73 ± 2.45	15.96 ± 2.81	26.30 ± 2.73	33.73 ±2.53	—	—
Hypertension, n (%)	5 (16.7)	4 (13.3)	4 (13.3)	5 (16.7)	6 (20.0)	0.694	0.952
Hyperlipidemia, n (%)	4 (13.3)	4 (13.3)	5 (16.7)	4 (13.3)	3 (10.0)	0.577	0.966
Smoking, n (%)	3 (10.0)	2 (6.67)	4 (13.3)	5 (16.7)	3 (10.0)	1.725	0.786
Iron (μmol/L)	20.10 ± 4.83	18.72 ± 4.78	19.14 ± 4.04	16.38 ±7.53	17.97 ± 8.33	1.544	0.193
Ferritin (μg/L)	130.25 ± 52.62	128.65 ± 57.45	116.56 ± 77.99	109.43 ± 38.52	109.52 ± 73.59	0.801	0.526
Phase difference (degree)	52.37 ± 9.39[a]	50.58 ± 10.00[a]	39.34 ± 10.11[b]	39.54 ± 7.20[b]	37.48 ± 7.82[b]	18.353	< 0.001*
End-tidal CO_2 (mmHg)	35.65 ± 4.74	35.84 ± 4.20	35.00 ± 4.61	35.81 ± 4.61	35.64 ± 5.70	0.364	0.834

Notes: **P* value < 0.05 (statistically different in one-way analysis of variance and the chi-square test). Similar superscript letters indicate no significant difference, while different superscript letters indicate significant difference in Bonferroni's post-hoc test.
Abbreviations: RLS, restless legs syndrome; BMI, body mass index; MAP, mean arterial pressure; IRLS, International restless legs syndrome severity scale.

in mild and moderate RLS groups. The arousal index was significantly increased in the mild (16.01 ± 4.86), moderate (22.56 ± 11.65), severe (19.92 ± 8.47), and very severe RLS groups (25.10 ± 7.71) versus controls (6.00 ± 3.58), and the arousal index in mild patients was significantly increased compared to those in moderate and very severe RLS groups. PLMI was significantly increased in mild (16.89 ± 6.40), moderate (18.93 ± 5.86), severe (35.70 ± 2.83) and very severe RLS groups (79.73 ± 25.08) versus controls (0.89 ± 0.77), and PLMI was significantly increased in very severe RLS groups versus mild to severe RLS groups, and PLMI was significantly increased in the severe RLS group compared to the moderate RLS group (Table 2).

DCA Parameters

We used the average values in the following analyses because there were no discernible differences in the right and left dCA parameters (PD). The PD was significantly decreased in the moderate (39.34 ± 10.11), severe (39.54 ± 7.20), and very severe RLS groups (37.48 ± 7.82) versus controls (52.37± 9.39, $P < 0.001$) and mild RLS groups (50.58 ± 10.00, $P < 0.001$) (Table 1, Figure 1).

Comparison of IRLS, PD, and PSG Parameters Between RLS Patients with Ferritin < 45 μg/L and Ferritin ≥ 45 μg/L

A total of 120 RLS patients were separated into ferritin < 45 μg/L (25 patients) and ≥ 45 μg/L (95 patients) groups in accordance with ferritin levels. The IRLS, PD, and PSG parameters of the two groups are shown in Table 3. There was a statistically significant difference in IRLS between the two groups ($P = 0.003$). The group with ferritin < 45 μg/L had a significantly higher arousal index (24.89 ±12.76) than the group with ferritin > 45 μg/L (19.85 ± 7.56, $P = 0.013$) (Table 3).

Table 2 Polysomnography Parameters in Mild, Moderate, Severe, Very Severe RLS Patients and Controls

	Controls (n = 30)	Mild RLS (n = 30)	Moderate RLS (n = 30)	Severe RLS (n = 30)	Very Severe RLS (n = 30)	F	P
Total sleep time (min)	400.40 ± 34.72^{a}	377.99 ± 89.39^{ab}	386.96 ± 114.52^{ab}	337.80 ± 70.04^{b}	330.27 ± 90.18^{b}	4.069	0.004*
Sleep onset latency (min)	15.53 ± 3.03^{c}	15.53 ± 3.33^{c}	17.11 ± 4.10^{c}	22.41 ± 9.14^{b}	27.89 ± 10.85^{a}	18.240	< 0.001*
Sleep efficiency (%)	86.50 ± 8.72^{a}	73.46 ± 15.66^{b}	71.06 ± 13.37^{b}	63.34 ± 11.44^{bc}	58.13 ± 14.19^{c}	19.977	< 0.001*
Stage N1 (%)	4.42 ± 1.24^{c}	19.06 ± 6.68^{b}	24.47 ± 9.04^{b}	24.47 ± 8.69 ^{b}	31.43 ± 11.77^{a}	45.086	< 0.001*
Stage N2 (%)	50.47 ± 6.97	51.29 ± 10.06	50.82 ± 7.22	51.93 ± 10.53	48.63 ±12.70	0.490	0.743
Stage N3 (%)	21.20 ± 5.02^{c}	11.92 ± 7.46^{c}	6.68 ± 4.82^{c}	6.02 ± 3.92 ^{b}	3.43 ± 3.12^{a}	57.748	< 0.001*
REM sleep (%)	23.90 ± 8.59^{b}	17.38 ± 7.49^{b}	16.82 ± 7.78^{b}	16.81 ± 9.77^{b}	16.52 ± 10.02^{a}	3.861	0.005*
Arousal index (events/h)	6.00 ± 3.58^{c}	16.01 ± 4.86^{b}	22.56 ± 11.65^{ab}	19.92 ± 8.47^{a}	25.10 ± 7.71^{a}	27.559	< 0.001*
AHI (events/h)	2.12 ± 1.61	2.31 ± 0.99	2.56 ± 1.37	2.30 ± 1.39	2.22 ± 1.23	0.437	0.781
PLMI (events/h)	0.89 ± 0.77^{d}	16.89 ± 6.40^{bc}	18.93 ± 5.86^{c}	35.70 ± 2.83^{b}	79.73 ± 25.08^{a}	75.534	< 0.001*

Notes: *P value < 0.05 (statistically different in one-way analysis of variance). Similar superscript letters indicate no significant difference, while different superscript letters indicate significant difference in Bonferroni's post-hoc test.

Abbreviations: RLS, restless legs syndrome; REM, rapid eye movement; Stage N1, stage 1 non-REM; Stage N2, stage 2 non-REM; Stage N3, stage 3 non-REM; AHI, apnea-hypopnea index; PLMI, periodic limb movement index.

Correlation Analysis for IRLS with PD, Arousal Index, and PLMI in RLS

Spearman correlation analysis revealed a significant relationship between the PD value of RLS patients and IRLS (r = - 0.343, P = 0.0001), arousal index (r = - 0.213, P = 0.0193), and PLMI (r = - 0.230, P = 0.0113) (Figure 2).

Univariable and Multivariable Analysis of dCA-Influencing Factors

Table 4 reports the univariate and multivariate regression analysis of PD. In the univariate model, there was a positive correlation between PD and IRLS stage N3 (β = 0.292, P = 0.001), and a negative correlation between PD and IRLS (β = - 0.359, P < 0.001), sleep onset latency (β = - 0.336, P < 0.001), stage N1 (β = - 0.183, P = 0.046), arousal index (β = - 0.218, P = 0.017), and PLMI (β = - 0.240, P = 0.008). Clinical data including IRLS, TST, SOL, SE, stage N1, stage N3, arousal index, and PLMI with P < 0.1 in the univariate analysis were analyzed in the multivariate model, and no factors associated with dCA.

DCA Parameters of 35 Patients with RLS Before and After Treatment

Out of 120 patients with RLS, only 35 patients (12 with moderate, 11 with severe, and 12 with very severe RLS) were examined with IRLS and dCA after one month of standardized therapy with pramipexole (0.125 mg/day) and gabapentin (300 mg/day). The IRLS decreased after treatment (24.91 ± 8.27 versus 20.26 ± 8.89, P < 0.001), the PD increased after the treatment [36.41 (33.05–40.45) versus 48.56 (39.99–54.35), P < 0.001] (Figure 3).

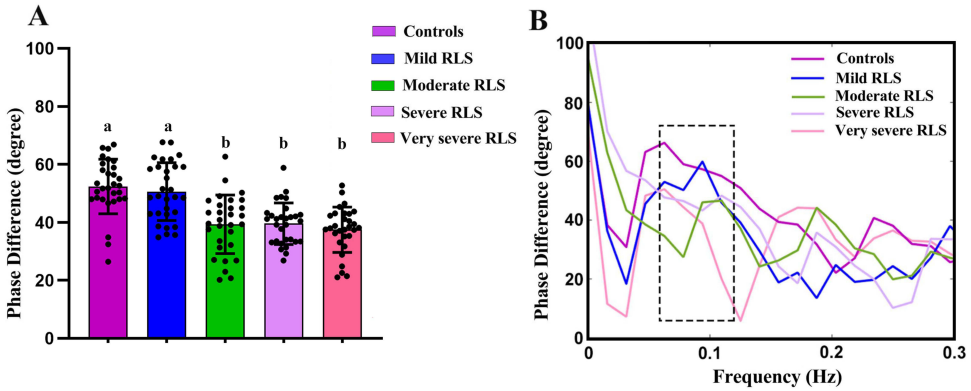

Figure 1 The autoregulatory parameter and statistical distributions in each group. Statistical distributions of average phase difference (**A**) and its transfer function (**B**) in each group. Similar lowercase letters indicate no significant difference, while different lowercase letters indicate significant difference (P < 0.001) (One-way analysis of variance and Bonferroni's post-hoc test). The dashed frame represents a specific frequency domain (0.06–0.12Hz).

Table 3 The IRLS, Phase Difference and Polysomnography Parameters in RLS Patients with Ferritin <45 μg/L and Ferritin ≥45 μg/L

	Ferritin < 45 μg/L (n=25)	Ferritin ≥ 45 μg/L (n=95)	χ^2/t	*P*
Male, n (%)	10 (40.0)	35 (36.8)	0.084	0.772
Age (years)	42.8 ± 8.7	53.9 ± 8.0	−6.095	< 0.001*
IRLS, n (%)			14.198	0.003*
1–10	4 (16.0)	56 (44.8)		
11–20	8 (32.0)	22 (17.6)		
21–30	3 (12.0)	28 (22.4)		
31–40	10 (40.0)	19 (15.2)		
Total sleep time (min)	364.92 ± 122.11	356.50 ± 86.50	0.395	0.694
Sleep onset latency (min)	22.14 ± 9.41	20.37 ± 8.80	0.885	0.378
Sleep efficiency (%)	69.70 ±15.59	66.19 ± 14.69	1.049	0.296
Stage N1 (%)	25.50 ± 10.11	24.49 ±10.17	0.358	0.721
Stage N2 (%)	50.70 ±7.06	50.66 ±10.98	0.019	0.985
Stage N3 (%)	6.82 ± 5.14	7.07 ± 6.12	− 0.118	0.851
REM sleep (%)	16.46 ± 6.27	16.70 ± 9.30	− 0.274	0.784
Arousal index (events/h)	24.89 ±12.76	19.85 ± 7.56	2.530	0.013*
PLMI (events/h)	41.99 ± 24.07	36.71 ± 29.69	0.820	0.414
Phase difference (degree)	41.07 ± 9.13	41.91 ± 10.49	− 0.364	0.717

Notes: *P value < 0.05 (statistically different in one-way analysis of variance and the chi-square test).
Abbreviations: RLS, restless legs syndrome; IRLS, International restless legs syndrome severity scale; REM, rapid eye movement; Stage N1, stage 1 non-REM; Stage N2, stage 2 non-REM; Stage N3, stage 3 non-REM; PLMI, periodic limb movement index.

Discussion

The current study showed that dCA was compromised in patients with moderate, severe, and very severe RLS and was negatively correlated with the IRLS severity scale, arousal index, and PLMI in PSG. After 1 month of medical therapy with pramipexole (0.125 mg/day) and gabapentin (300 mg/day), the dCA improved in patients with RLS.

Studies have reported cerebral hemodynamic changes in patients with RLS. The case of a father and daughter with familial RLS was reported in 1998 by San et al; hyperperfusion in the thalamus and anterior cingulate and hypoperfusion in the caudate nuclei were found using positron emission tomography and single-photon emission computed tomography.[31] Byun et al reported cerebral hemodynamic disorder in RLS patients and PLMS using near-infrared spectroscopy, which could be an important mechanism that increase the risk of cerebrovascular disease.[4] Our results

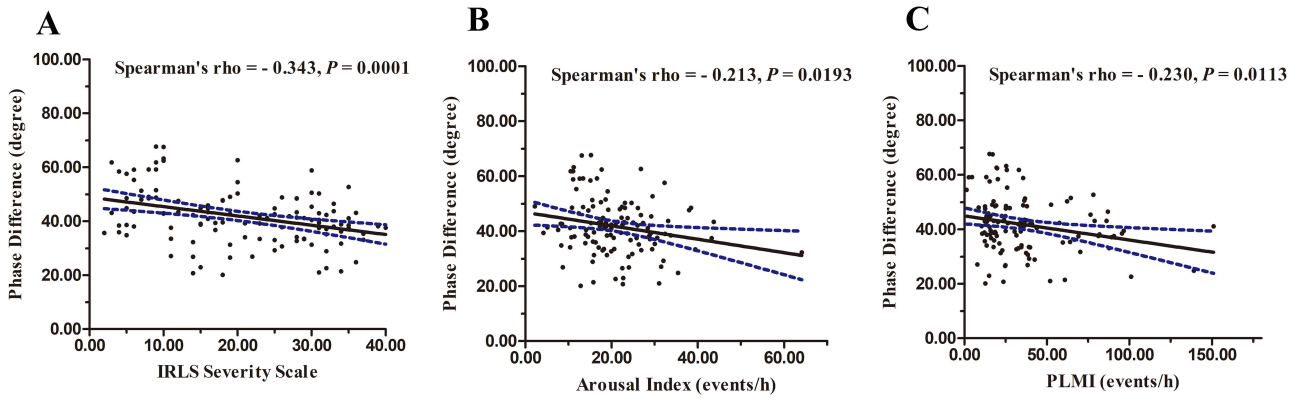

Figure 2 Relations between phase difference and IRSL (**A**), arousal index (**B**), and PMLI (**C**) in patients with RLS (Spearman correlation test).

Table 4 Univariable and Multivariable Analysis for the Phase Difference

Factors	Phase Difference, Degree			
	Univariable Analysis		Multivariable Analysis	
	β	P	β	P
Gender	0.086	0.349		
Age	− 0.046	0.576		
BMI	0.011	0.894		
MAP	− 0.119	0.195		
Heart Rate	0.013	0.886		
Hypertension	0.033	0.721		
Hyperlipidemia	0.103	0.263		
Smoking	− 0.096	0.297		
End-tidal CO_2	0.036	0.390		
IRLS	− 0.359	< 0.001[ab]	− 0.193	0.140
Iron (μmol/L)	− 0.032	0.730		
Ferritin (μg/L)	0.118	0.198		
Total Sleep Time (min)	0.160	0.081[a]	0.064	0.5443
Sleep onset latency (min)	− 0.336	< 0.001[ab]	− 0.164	0.129
Sleep Efficiency (%)	0.172	0.061[a]	0.014	0.896
Stage N1 (%)	− 0.183	0.046[ab]	− 0.002	0.983
Stage N2 (%)	0.029	0.756		
Stage N3 (%)	0.292	0.001[ab]	0.142	0.164
REM Sleep(%)	0.027	0.769		
Arousal Index (events/h)	− 0.218	0.017[ab]	− 0.091	0.324
AHI (events/h)	0.070	0.444		
PLMI (events/h)	− 0.240	0.008[ab]	0.021	0.854

Notes: [a]Nominally significant values ($P < 0.1$) included in the multivariable model; [b]P value < 0.05 (statistically different).

Abbreviations: BMI, body mass index; MAP, mean arterial pressure. IRLS, International restless legs syndrome severity scale; REM, rapid eye movement; Stage N1, stage 1 non-REM; Stage N2, stage 2 non-REM; Stage N3, stage 3 non-REM; AHI, apnea-hypopnea index; PLMI, periodic limb movement index.

appear to be consistent with those of other studies on RLS, supporting an association between cerebral hemodynamics and RLS.[4,31] We recruited patients with RLS in the absence of known risk diseases (previous stroke, previous heart disease, arrhythmia, and sleep-disordered breathing). Our study showed no significant differences between the RLS

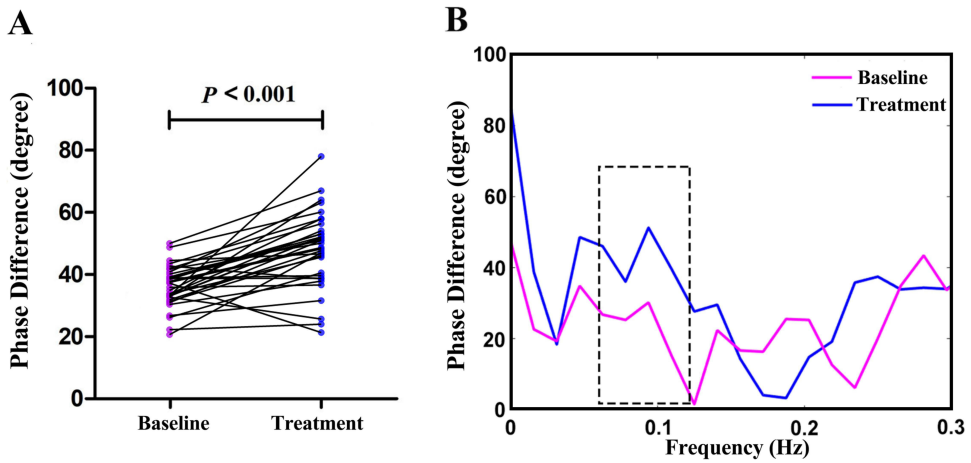

Figure 3 The autoregulatory parameter and statistical distributions in RLS patients before and after treatment. Statistical distributions of phase difference (**A**) and its transfer function (**B**) in RLS patients before and after treatment (Wilcoxon signed-rank test).

patients and the control group with regard to demographics and vascular risk factors (including hypertension, hyperlipidemia, and smoking), indicating that dCA was compromised in moderate to very severe patients with RLS, which suggests that RLS may be an influencing factor for preclinical evidence of cerebrovascular disease by affecting dCA.[4]

The potential mechanisms of impaired dCA in patients with RLS are unclear; however, there are theoretical explanations that patients with RLS have impaired dCA. First, sympathetic hyperactivity was widely present in patients with RLS. Chenini et al reported that untreated patients with RLS had more autonomic symptoms in evaluated cardiovascular, gastrointestinal, urinary, thermoregulatory, pupillomotor, and sexual dysfunctions using the SCOPA-AUT questionnaire, which were without effect of PLMS, sleep fragmentation and medication.[32] Izzi et al discovered that patients with RLS tend to have hypertension, the ability to regulate vasodilation or vasoconstriction was reduced and the autonomous nervous system was imbalanced.[33] In addition, PLMS is observed in up to 80% of RLS patients and is often accompanied by hyperarousal, sleep fragmentation, significant transient increases in heart rate and blood pressure, which suggests sympathetic activation and autonomic arousal exited in patients with RLS.[34,35] Sympathetic hyperactivity could modulate cerebral flow through vasodilation or vasoconstriction and has an important impact on dynamic cerebral autoregulation.[36]

Second, the disturbed sleep architecture and sleep continuity in RLS patients have been demonstrated in several studies.[35,37,38] Most patients with RLS reported sleep disturbances, including decreased total sleep time, difficulties in sleep onset and maintenance, reduced sleep efficiency, and frequent arousal in a questionnaire and PSG studies.[35] A study by Winkelman et al demonstrated that RLS patients exhibited higher arousal index and longer mean sleep latency in PSG, more continual RLS symptoms, and greater sleep latency than those without RLS.[37] The comparative observational study of Hornyak et al found that RLS patients experience a reduction in both REM and non-REM sleep, as well as a shorter total sleep time, longer sleep onset latencies, poorer sleep efficiency, a higher arousal index, and sleep stage changes. Furthermore, RLS patients showed substantially increased PLMS and sleep fragmentation indices compared to controls.[38] Our study discovered that RLS patients experienced disrupted sleep structure, which mainly manifests as increased arousal index, prolonged sleep latency, worse sleep efficiency, and decreased sleep efficiency, which was consistent with the above results. It is widely known that there may be a link between sleep loss and sleep disturbance and a higher risk of type 2 diabetes, obesity, hypertension, and coronary artery disease.[39–42] An elevated risk of stroke and cardiovascular disease was associated with sleep loss and disturbance.[43,44] The elevated activity of the sympathetic system, dysfunction of vascular endothelial cells, and dyslipidemia may be the underlying mechanisms,[45,46] which have a significant association with dCA. Our study found that dCA in RLS patients was correlated with the arousal index and PLMI in PSG, which may also suggest that the mechanics of impaired dCA in RLS patients may be involved in sleep-related arousal. The acute phase of a stroke was accompanied by changes in sleep structure, such as a decrease in total sleep time and sleep efficiency,[47,48] which may prove that the dCA impairment caused by disruption of sleep structure and sleep loss may be a crucial intermediary mechanism of stroke in RLS patients.

Moreover, the impaired dCA in RLS patients was also explained by the vascular variables, including impaired cerebral vascular endothelial dysfunction and increased arterial stiffness.[3,4,8] Decreased NO levels, increased oxidative stress, and sympathetic activation are the underlying systemic pathophysiologies of impaired endothelial function in patients with RLS.[10,11] Reduced NO production could promote vascular endothelial dysfunction, which can result in reduced local blood flow and hypoxia,[49] which may affect dCA. Moreover, oxidative stress is associated with endothelial dysfunction, leading to impaired perfusion and/or vascular tone.[50,51] These findings suggest that the impaired dCA may be caused by these pathological alterations.

Furthermore, we found that the arousal index in RLS patients with ferritin < 45 μg/L was higher than that in RLS patients with ferritin ≥ 45 μg/L, which suggests that nocturnal arousal and sleep fragmentation are more prone to occur in patients with low ferritin levels. Ferritin levels are a reflection of iron supply; iron is a part of the rate-limiting enzyme tyrosine hydroxylase and a cofactor in dopamine synthesis.[52] It has been suggested that iron metabolism may influence the dopamine of the CNS and sympathetic nervous system and impact the potential effects of dCA. Although the results of IRLS and PLMI did not show any significant differences, there was an increasing trend in the ferritin < 45 μg/L group, which further supports the theory that iron deficiency correlates with both PLMI and the intensity of RLS symptoms. Cerebral iron deficiency is the critical pathophysiology of RLS rather than peripheral iron status, which may occur

despite normal peripheral iron stores.[53,54] However, we detected serum iron and ferritin levels rather than brain iron levels. Further work is required to examine brain iron levels and to analyze their relationship with dCA in RLS patients.

One of the main hypotheses for the etiology of RLS is a dopaminergic deficiency in the CNS.[55] As a monoamine neurotransmitter, dopamine affects cerebral circulation and vasomotor function by direct and intricate vasoactive actions,[56] which can modulate dCA. Several dopaminergic pathways have been found in the cerebrum, including the striatonigral, mesolimbic, mesocortical, tuberoinfundibular, and supraspinal A11 dopamine cell groups.[57] Patients with RLS may also have an increase in sympathetic impulses due to decreased dopamine levels, while decreased A11 dopaminergic diencephalospinal pathway function results in increased peripheral sympathetic outflow.[58] Medical therapies for RLS in this study included pramipexole and gabapentin. Pramipexole is a dopamine agonist with selective action upon dopamine D3 receptors, which can reverse RLS symptoms, reduce PLMI, and improve subjective sleep quality.[59] Gabapentin is a derivative of γ-aminobutyric acid that decreases glutamate release. These trials have demonstrated improvements for PLMS, sleep architecture, and RLS symptoms.[21,60] After 1 month of therapy, dCA was higher than that before treatment in 35 patients with RLS. We hypothesized that as a high-affinity D3 receptor agonist, pramipexole may diminish autonomic responses and re-establish appropriately responsive sympathovagal homeostasis by acting on the same D3 receptors in the dorsal and intermediolateral gray matter of the spinal cord, which may affect dCA. Gabapentin interacts with the α2δ-1 subunit of voltage-dependent calcium channels, which antagonizes the release of dopamine, norepinephrine, and serotonin.[22,61] These neurotransmitters may affect the dCA. The response of dCA to dopaminergic medications further demonstrated that dysfunction of the neurotransmitter dopamine is one of the pathogenesis of RLS. However, it's possible that the improvement in sleep structure disturbance is an underestimated factor in the elevated dCA in RLS patients after therapy. But we did not reexamine PSG monitoring for patients, we will focus on this in following days.

This study had several limitations. First, the duration of RLS plays an important role in cerebrovascular alertness; however, we failed to consider the exact duration of RLS. Second, the inability to successfully penetrate the temporal bone window in elderly patients may limit their inclusion. Third, we lack PSG data after therapy, which limited our analysis of possible factors for impaired dCA. In addition, we choose a ferritin of 45 μg/L rather than the international recommendation of 75 μg/L, and our sample size was relatively small, and there may be some data bias; future studies will be done with a larger sample size.

Conclusion

In conclusion, this study demonstrated that dynamic cerebral autoregulation was compromised in patients with moderate, severe, and very severe RLS, which was negatively correlated with the IRLS severity scale, arousal index, and PLMI in PSG. After 1 month of medication therapy, dCA improved in patients with RLS. Further studies are needed to probe the in-depth mechanisms of RLS, its relationship with dCA, and the mechanism of its therapeutic effect, providing a new approach for clinical diagnosis and treatment.

Data Sharing Statement

The deidentified data used and analyzed during this study are available from the corresponding author on reasonable request.

Ethics Approval and Consent to Participate

The First Hospital of Jilin University Ethics Committee has approved the study (Approval No: 2016-294) and followed the guidelines of the Declaration of Helsinki (1964). All the participants gave an informed consent.

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Disclosure

The authors declare that they have no competing interests in this work.

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