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# Alterations in spontaneous brain activity of maintenance hemodialysis patients with restless legs syndrome: a cross-sectional case-control study

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## Abstract

**Objective** Through resting state functional magnetic resonance imaging (rs-fMRI) we evaluate the spontaneous brain activity changes of maintenance hemodialysis (MHD) patients with restless legs syndrome (RSL) and analyzed the imaging features and related mechanisms of RLS in patients with MHD.

**Method** We select 27 MHD patients with RLS and 27 patients without RSL matched by age, gender, cognitive function. Both groups underwent neuropsychological tests and MRI scans. MRI data analysis was performed to obtain and compare the amplitude of low-frequency fluctuation (ALFF), fractional amplitude of low-frequency fluctuations (fALFF), and regional homogeneity (ReHo) values, which were mALFF, mfALFF, and mReHo. Clinical data were collected and compared. Differentiated indicators and RLS scores conduct Pearson correlation analysis.

**Result** Compared with the MHD-nRLS group, the MHD-RLS group showed significantly lower mALFF values in the left precentral, right precentral gyrus, and right postcentral gyrus, lower mfALFF values in the left precentral gyrus, right precentral gyrus, left calcarine fissure, left lingual gyrus, left postcentral gyrus, and right postcentral gyrus, and lower mReHo values in the left precentral gyrus, right precentral gyrus, left calcarine fissure, left lingual gyrus, left postcentral gyrus, and right postcentral gyrus, and right postcentral gyrus ($P < 0.05$). The MHD-RLS group exhibited lower hemoglobin levels ($P = 0.001$), higher total iron-binding capacity levels ($P = 0.011$), and higher folic acid levels ($P = 0.022$). The above indicators were correlated with RLS scores using Pearson correlation analysis, and it was found that the mfALFF value of the right precentral gyrus and the right postcentral gyrus, and the mReHo values of the right precentral gyrus and right postcentral gyrus were negatively correlated with the RLS score ($r = -0.567$, $P = 0.002$;$r = -0.705$, $P < 0.001$;$r = -0.414$, $P = 0.032$; $r = -0.410$, $P = 0.034$), and the hemoglobin concentration was negatively correlated with the RLS scores ($r = -0.394$, $P = 0.042$).

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**Conclusion** Patients with MHD-RLS exhibit abnormal spontaneous brain activity in the right precentral gyrus and right postcentral gyrus within the sensorimotor network, along with lower hemoglobin levels, which may be associated with the pathogenesis and severity of MHD-RLS.

**Keywords** Hemodialysis, Resting-state functional magnetic resonance imaging, Restless legs syndrome

## Introduction

Patients with end-stage renal disease (ESRD) undergoing maintenance hemodialysis (MHD) may experience various complications, among which restless legs syndrome (RLS) is one of the common neurological complications in MHD patients [1]. RLS is a common sensorimotor disorder characterized by an urge to move, usually occurring in the evening or at night, worsening during rest or inactivity, and improving or disappearing with movement or activity. The prevalence of RLS among MHD patients varies significantly across different regions, with a global prevalence rate of approximately 27.2%[2]. These patients often suffer from insomnia, cognitive impairment, cardiovascular events, anxiety, and depression. RLS symptoms severely affect the patients' quality of life, leading to poor prognosis and increased mortality rate [2].

At present, although neuroimaging techniques are increasingly applied to the functional and metabolic changes of the central nervous system, the pathophysiology of RLS in MHD patients is still unclear. Among them, resting-state functional magnetic resonance imaging (rs-fMRI) can evaluate the neural network state by measuring blood oxygen level-dependent information during rest, and it has been extensively utilized to investigate neurological pathological changes in MHD patients [3]. Amplitude of low-frequency fluctuation (ALFF), fractional amplitude of low-frequency fluctuations (fALFF), and regional homogeneity (ReHo) are three commonly used methods for quantifying neural activity in rs-fMRI. Among them, ALFF and fALFF reflect the signal of each voxel in the 0.01–0.08 Hz frequency band, indicating the functional intensity of local brain regions [4], while ReHo reflects the regional coherence of neural activity between adjacent voxels [5].

In previous researches of MHD patients, rs-fMRI was used to explore the changes in spontaneous brain activity in MHD patients, and it has been found that the reduction of ALFF/fALFF/ReHo values in the default mode network (DMN) region is associated with cognitive decline [6]. Moreover, in studies on RLS patients, by applying the rs-fMRI method and comparing diffusion tensor imaging (DTI) and ReHo index between individuals with and without RLS, it was found that RLS patients exhibit higher ReHo in the striatum, thalamus, and limbic system, suggesting that emotional processing, motor control, and cognition in the cortico-striato-thalamo-cortical circuit may be the areas of functional impairment in RLS patients [7]. Previous research, through studying dynamic and static ALFF, functional connectivity, and Granger causality analysis, has discovered that abnormalities in the cerebellum-basal ganglia-sensorimotor cortex circuit might constitute the potential neuropathological basis of RLS [8]. Previous research found that after transcranial magnetic stimulation treatment, there was a significant increase in ALFF in multiple sensorimotor and visual regions [9]. Previous studies had detected changes in gray matter (GM) volume and white matter (WM) microstructure characterization in patients with MHD-RLS by using voxel-based morphology (VBM) analysis and tractography atlas-based analysis (TABS) through DTI and T1-weighted imaging, and found that patients with MHD-RLS have abnormal white matter microstructure of the corticospinal tract relative to healthy people. It is hypothesized that this structural alteration may be an imaging marker associated with the severity of motor dysfunction in patients with ESRD-RLS [10].

The severity of RLS symptoms significantly impacts the quality of life of MHD patients, shortens their survival period, and imposes a heavy burden on the patients' families and society. However, current research on the pathogenesis of RLS or MHD-RLS using imaging technology primarily involves comparisons between RLS or MHD-RLS patients and healthy individuals. These studies have not excluded brain structure and function changes caused by MHD and tend to focus more on structural or microstructural abnormalities rather than brain function changes. Currently, there is a paucity of research comparing neural activity changes between MHD patients with RLS and those without RLS (nMHD-RLS). Therefore, to elucidate the alterations in brain function and the potential pathogenesis associated with MHD-RLS, further in-depth investigations are warranted.

The purpose of this study is to use rs-fMRI technology to compare and evaluate the differences in ALFF/fALFF/ReHo in various brain regions between MHD-nRLS patients and MHD-RLS patients, analyze the correlation of these changes with the occurrence of RLS, and discuss the possible pathogenesis of MHD-RLS in combination with clinical indicators, as well as analyze its clinical significance.

## Objectives and methods

### Research subject

This study is a cross-sectional case study. A total of 27 MHD patients with RLS (MHD-RLS group) and 27 MHD patients without RLS (MHD-nRLS group) who matched

with age, gender, years of education, duration of dialysis, cognitive function, Kt/V, diabetes and hypertension. Patients who were treated at the Third Affiliated Hospital of Nanjing Medical University from January 2023 to May 2024 were enrolled, and the inclusion criteria are shown in Table 1. Subjects completed the Psychological Scale Test and cranial MRI scan 2 h before short-interval dialysis. Blood samples were collected before dialysis. The patient who never experienced RLS symptoms was divided into MHD-nRLS group. Patient who met the diagnostic of RLS was categorized as MHD-RLS group [2].

This study was approved by the Medical Research Ethics Committee of the Third Affiliated Hospital of Nanjing Medical University (Ethics Number: KY032-01). Informed consent was obtained from all patients before the study.

## Methords

### *General clinical data and laboratory tests*

General clinical data such as gender, age, education level, duration of MHD, history of hypertension, and history of diabetes were collected. In subjects before short-interval dialysis, blood samples were collected, and laboratory auxiliary tests were completed, including: creatinine, blood urea nitrogen, uric acid, white blood cell count, red blood cell count, hemoglobin concentration, hematocrit, serum potassium, serum sodium, serum calcium, inorganic phosphorus, total cholesterol, triglycerides, total protein, albumin, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, aspartate transaminase, alanine transaminase, parathyroid hormone, serum iron, serum ferritin, total iron-binding capacity, transferrin saturation, folic acid, vitaminB12, dialysis adequacy (Kt/V), glomerular filtration rate, and high-sensitivity C-reactive protein.

**Table 1** Inclusion and exclusion criteria

| Inclusion Criteria | Exclusion Criteria |
|---|---|
| Age ≥ 18-year-old | RLS symptoms present before receiving Maintenance Hemodialysis Treatment |
| GFR < 15 ml/min/1.73 $m^2$ | Family history of RLS |
| Dialysis Duration ≥ 3 months, 4 h per session | Good control of RLS symptoms after RLS medication treatment |
| Right-handed | History of Neuroactive Drugs and Alcohol Dependence |
| Complete clinical data | Presence of anxiety, depression, and sleep disorders |
| No contraindications for Imaging Examination | MRI Head movement during MRI scan affects image evaluation quality |
| | Suffering from neurological or psychiatric disorders, including brain tumor, brain injury, stroke, epilepsy, schizophrenia, Parkinsons disease, and Alzheimers disease. |

### *Assessment of RLS*

Subjects undergoing short-interval dialysis 2 h before dialysis, according to the updated diagnostic criteria of the International Restless Legs Syndrome Study Group (IRLSSG) to determine whether the subjects have RLS [11]. The following 4 symptoms are sufficient: (1) There is an urge to move the legs, which usually but not always accompanies or causes discomfort in the legs; (2) In a state of rest or inactivity (such as lying down or sitting), the subject's impulse to move his legs or the accompanying discomfort will appear or worsen; (3) the subject's urge to move his legs and the accompanying discomfort can be partially or completely relieved by exercise (such as walking or stretching), or during activity; (4) when at rest or inactivity, the subject's urge to move his legs and the accompanying discomfort only appear or worsen at night; (5) The occurrence of the above characteristics cannot be entirely explained by other medical or behavioral conditions (such as myalgia, venous stasis, leg edema, arthritis, leg cramps, positional discomfort, habitual foot tapping) as the primary symptoms. According to the International Restless Legs Syndrome Rating Scale (IRLS) [11], the MHD-RLS group subjects RLS symptom severity was scored and evaluated. See supplementary material Table 1 for details.

### *Neuropsychological assessment*

Subjects underwent short-interval dialysisand received the Montreal Cognitive Assessment (MoCA) evaluation 2 h before dialysis [12]. This assessment primarily encompasses cognitive dimensions such as visuospatial abilities, executive function, memory, naming, attention, language, abstract thinking, and orientation. The modified scale has been extensively utilized for the screening and evaluation of clinical cognitive impairment. See supplementary material Fig. 1 for details.

### *MRI acquisition and data analysis*

Before short-interval dialysis, complete the cranial MRI scan 2 h prior. This study used the GE Discovery MR 750 W 3.0T scanner for scanning [12]. In order to eliminate the effect of circadian rhythms, all subjects were scanned in the morning, and no RLS symptoms occurred during the scan. MRI scanning was assisted by a radiology technician who was unaware of the group assignments. During the scan, subjects were in a supine position with their heads stabilized by a sponge pad to prevent artifacts caused by movement. Rubber earplugs were used to reduce discomfort from noise. During the scan, subjects quietly closed their eyes but remained awake to avoid falling asleep. This study selected T2-FLAIR, excluding organic lesions, and 3D brain volume imaging (3D-BRAVO) sequences to obtain

high-resolution anatomical T1-weighted images with the following parameters:

[Repetition Time (TR)=7.5ms, Echo Time(TE)=2.5ms, Inversion Time (TI)=450ms, Slice Gap=1 mm, Flip Angle(FA)=15°, Field of View (FOV)=240 mm x 240 mm, Slice Thickness=1 mm, Number of Slices=154. The total scan time was 3 min 51 s].

Preprocessing of 3D-BRAVO data using SPM12 and RESTPLUS: (1) Remove the first 10 time points of data for each subject. (2) Perform slice timing correction. (3) Perform head motion correction, ensuring that the translation distance of each subjects head does not exceed 3 mm and the rotation angle is not greater than 3°. (4) Conduct spatial normalization with the following steps: (a) Align each subjects T1 structural image to the mean BOLD functional image of that subject; (b) Segment the transformed structural image into gray matter, white matter, and cerebrospinal fluid; (c) Register the segmented gray matter image to the tissue probability map in MNI standard space using a nonlinear method; (d) Apply the nonlinear registration parameters obtained in the previous step to each filtered volume to obtain the functional image of each subject in MNI standard space, and resample the spatially normalized functional image to a voxel size of 3 mm × 3 mm × 3 mm. 5) Perform spatial smoothing with a Gaussian smoothing FWHM of 6 mm. 6) Conduct subsequent denoising steps, including detrending and regression of noise covariates. The noise covariates include head motion parameters, specifically Friston24, white matter signal, cerebrospinal fluid signal, and other confounding variables. 7) Calculate the ALFF and fALFF metrics, setting the frequency band range of interest to 0.01–0.08 Hz, and perform mean normalization on the calculated metrics. 8) Building upon step 5, proceed with subsequent denoising steps, which include detrending, regression of noise covariates, and filtering. The noise covariates encompass head motion parameters (i.e., Friston24), white matter signal, cerebrospinal fluid signal, and other confounding variables. The filtering range is 0.01–0.08 Hz. 9) Calculate the ReHo index, and then perform demeaning and standardization on the calculated index. Apply spatial smoothing to the standardized index, with the Gaussian smoothing FWHM set to 6 mm. 10) Utilize the standardized data for statistical analysis.

#### *Statistical analysis*

The general data, laboratory tests, MoCA scores between the two groups were compared using SPSS version 26.0software for baseline data statistical analysis. qualitative data were analyzed using the $\chi^2$ test; quantitative data following a normal distribution were analyzed using the independent sample T test, expressed as (mean±SD); quantitative data following a non-normal distribution were analyzed using the Mann-Whitney U test, expressed as M (Q1, Q3).

Regional homogeneity, amplitude of low-frequency fluctuation and fractional amplitude of low-frequency fluctuations indicators were analyzed using SPM12 and Matlab2018a with a two-sample T-test to compare the differences between the MHD-RLS group and the MHD-nRLS group. The initial *P*-value was set to 0.05. For the MHD-RLS group and the MHD-nRLS group, Pearson correlation analysis was conducted on mReHo, mALFF, and mfALFF indicators and clinical variables with IRLS scores that showed significant differences. $P<0.05$ was considered statistically significant.

## Results

#### Results of mALFF in brain regions between the two groups

Compared with the MHD-RLS group, the nMHD-RLS group exhibited significantly higher mALFF values in the left precentral gyrus, right precentral gyrus, and right postcentral gyrus. It was no statistically significant differences in other paired groups. See Table 2; Fig. 1 for details.

#### Results of mfALFF in brain regions between the two groups

Compared to the MHD-RLS group, the nMHD-RLS group showed significantly higher mfALFF values in the left precentral gyrus, right precentral gyrus, left calcarine fissure, left lingual gyrus, left postcentral gyrus, and right postcentral gyrus. There were no statistically significant differences in other paired groups. See Table 3; Fig. 2 for details.

#### Results of mReHo in brain regions between the two groups

Compared with MHD-RLS group, nMHD-RLS group showed significantly higher mReHo values in the right precentral gyrus, right postcentral gyrus, and left precentral gyrus. There were no statistical differences in other paired groups. See Table 4; Fig. 3 for details.

**Table 2** Results of brain regions with statistically significant differences in mALFF

| Brain region | MNI (mm) | | | Voxel size ($mm^3$) | T value | *P* value |
|---|---|---|---|---|---|---|
| | X | Y | Z | | | |
| left precentral pyrus | -39 | -6 | 51 | 1028 | -3.21 | < 0.05 |
| right precentral gyrus | 41 | -8 | 52 | 1002 | -5.14 | < 0.05 |
| right postcentral gyrus | 41 | -25 | 53 | 1138 | 41 | < 0.05 |

MNI: Montreal Neurological Institute

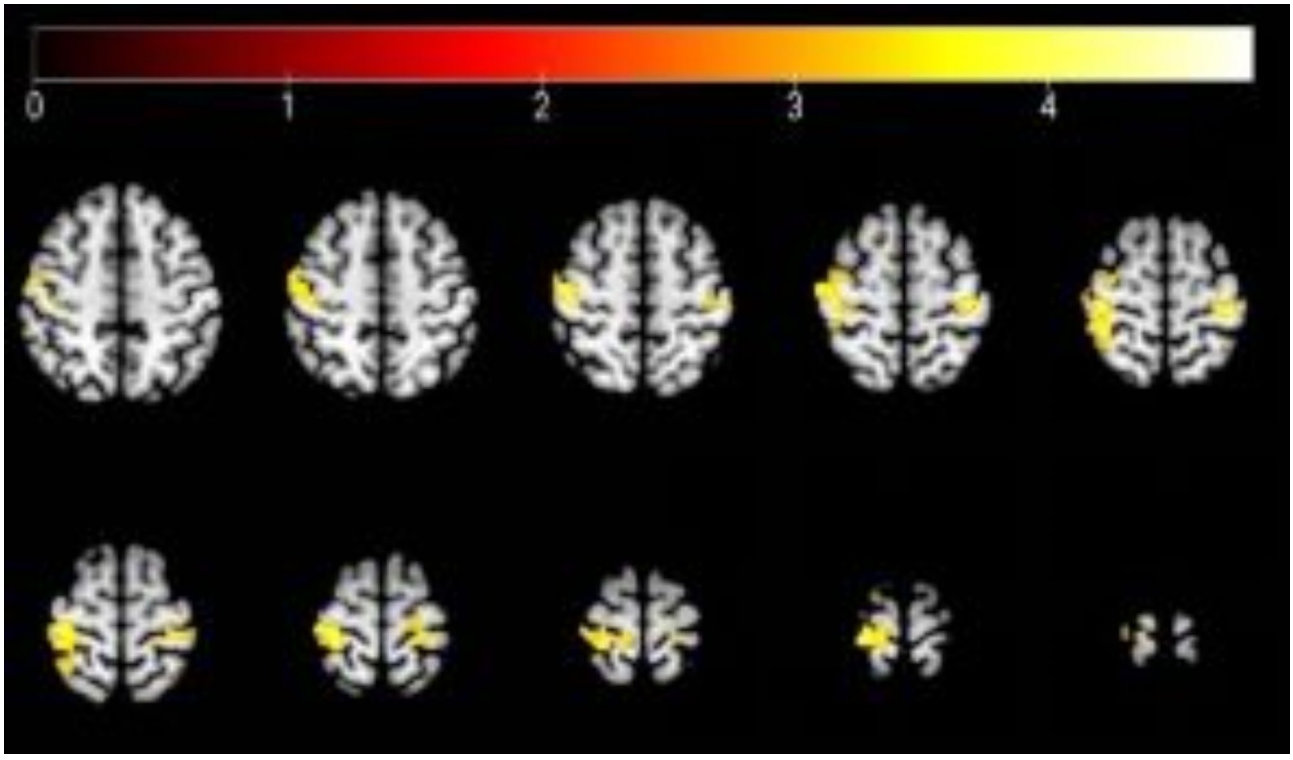


**Fig. 1** Comparison of mALFF differences between MHD-RLS group and MHD-nRLS group. The coloured areas indicate significantly higher mALFF values in the MHD-nRLS group compared to the MHD-RLS group

**Table 3** Results of br\ain regions with statistically different mfALFF

| Brain region | MNI (mm) | | | Voxel size ($mm^3$) | T value | *P* value |
|---|---|---|---|---|---|---|
| | X | Y | Z | | | |
| left precentral gyrus | -39 | -6 | 51 | 1028 | -3.00 | < 0.05 |
| right precentral gyrus | 41 | -8 | 52 | 1002 | -4.73 | < 0.05 |
| left calcarine fissure | -7 | -79 | 6 | 648 | -2.57 | < 0.05 |
| left lingual gyrus | -15 | -68 | -5 | 660 | -1.83 | < 0.05 |
| left postcentral gyrus | -42 | -23 | 49 | 1159 | -2.78 | < 0.05 |
| right postcentral pyrus | 41 | -25 | 53 | 1138 | -4.73 | < 0.05 |

MNI: Montreal Neurological Institute

### Results of clinical data between the two groups

There were no statistically significant differences in age, gender, years of education, duration of dialysis, cognitive function, Kt/V, diabetes and hypertension between the two groups ($P>0.05$). The hemoglobin level in the MHD-nRLS group was higher than that in the MHD-RLS group (110.0 (103.0, 122.0) vs. 101.0 (77.0, 107.0), $P=0.001$), the total iron-binding capacity in the MHD-nRLS group was lower than that in the MHD-RLS group (32.6±10.1 umol/L vs. 40.0±10.7 umol/L, $P=0.011$), and the folate level in the MHD-nRLS group was lower than that in the MHD-RLS group (4.6 (3.5, 9.1) vs. 6.9 (4.8, 20.0), $P=0.022$). There were no statistically significant differences in other clinical indicators between the two groups of patients ($P>0.05$). See Table 5 for details ( The table is reflected after the reference).

### Results of correlation analysis

The mfALFF value of the right anterior central gyrus was negatively correlated with the RLS scores ($r$ = -0.567, $P=0.002$), and the mALFF value of the right postcentral gyrus was negatively correlated with the RLS scores ($r$ = -0.705, $P<0.001$), the mReho values of the right anterior central gyrus and right postcentral gyrus were negatively correlated with RLS scores ($r$ = -0.414, $P=0.032$; $r$ = -0.410, $P=0.034$), and hemoglobin concentration was negatively correlated with RLS scores ($r$=-0.394, $P=0.042$). There was no correlation between the remaining brain regions and clinical indicators and RLS scores ($P>0.05$). See Fig. 4 for details.

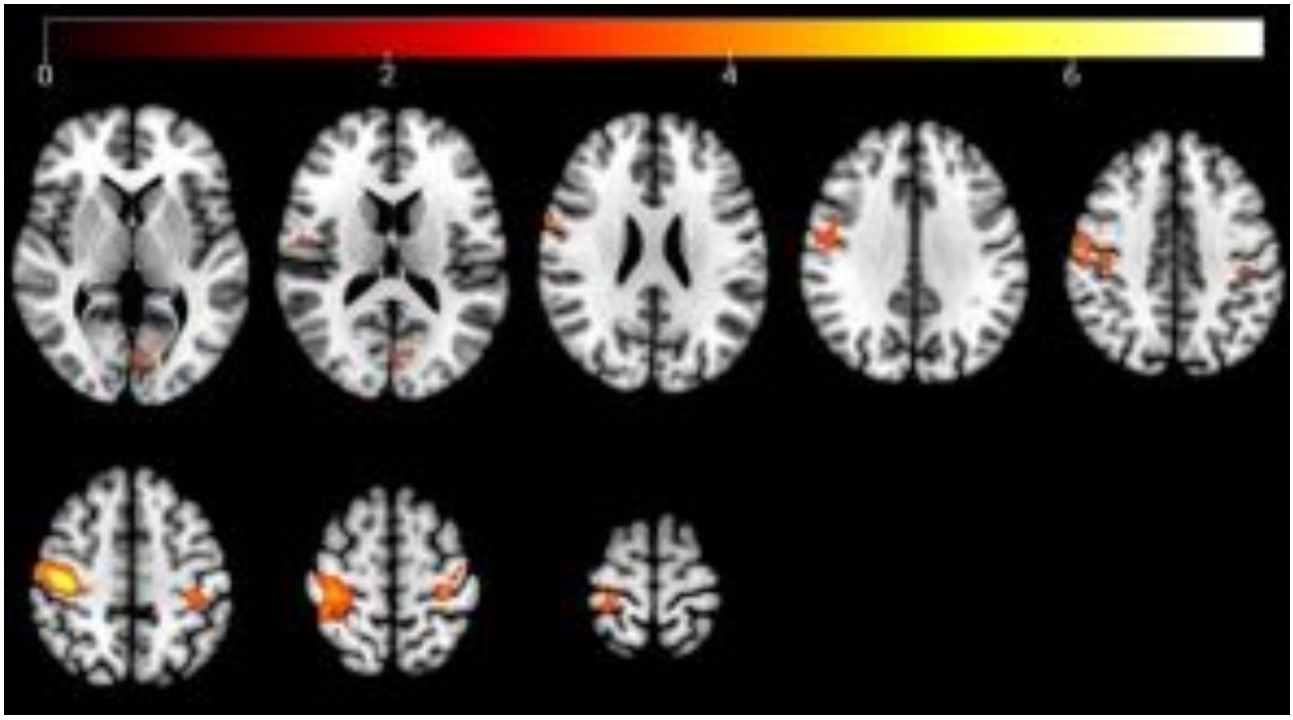


**Fig. 2** Comparison of mfALFF differences between MHD-RLS group and MHD-nRLS: group The coloured areas indicate significantly higher mfALFF values in the MHD-nRLS group compared to the MHD-RLS group

**Table 4** Results of brain regions with statistically significant mReHo

| Brain region | MNI | | | Voxel size ($mm^3$) | Z value | *P* value |
|---|---|---|---|---|---|---|
| | X | Y | Z | | | |
| left precentral gyrus | -39 | -6 | 51 | 1028 | -2.85 | < 0.05 |
| right precentral gyrus | 41 | -8 | 52 | 1002 | -3.03 | < 0.05 |
| right postcentral gyrus | 34 | -39 | -20 | 760 | 0.59 | < 0.05 |

MNI: Montreal Neurological Institute

## Discussion

RLS not only has a negative impact on the mental and emotional well-being of patients, reduces the quality of life, causes cardiovascular and cerebrovascular risk events, but also increases the burden on families and society [14, 15]. The mechanism of RLS is still unclear, and the iron deficiency hypothesis, the abnormal dopamine metabolism hypothesis, the neurotransmitter and pathway abnormality hypothesis, and the neuroanatomical abnormality hypothesis are widely accepted [16, 17]. Cerebral iron deficiency is one of the biological markers of RLS, and in the iron deficiency hypothesis, it is believed that central and peripheral iron deficiency may affect oxygen transport, and then activate the hypoxic pathway, which can affect the regulatory transport mechanism of iron across the blood-brain barrier [18]. Salminen et al. found that RLS patients have certain peripheral hypoxia manifestations, which are closely related to the severity of RLS [19], and studies have confirmed that the probability of iron deficiency anemia in RLS patients is 20 times higher than that of the normal population, and the severity of RLS symptoms is positively correlated with the severity of anemia [20]. Duddin J et al. have shown that anemia can reduce the oxygen-carrying capacity of the blood, which in turn reduces the oxygenation level of cerebral supplementation [21], and this change can cause cerebral blood volume reduction, which in turn causes hypoxia, further aggravates the iron metabolism disorder related to cerebral oxidative metabolism, and leads to neurodegenerative changes [22]. Salminen et al. confirmed a strong correlation between peripheral hypoxia and RLS severity in a case-control study measuring the partial pressure of oxygen and carbon dioxide in the legs, and confirmed a strong pathophysiological link between peripheral hypoxia and RLS symptoms. This is further supported by dopaminergic therapy that simultaneously reverses hypoxia and discomfort [23]. In this study, the total iron-binding capacity and tretinoic acid levels in the nMHD-RLS group were lower than those in the MHD-RLS group and the hemoglobin group in the nMHD-RLS

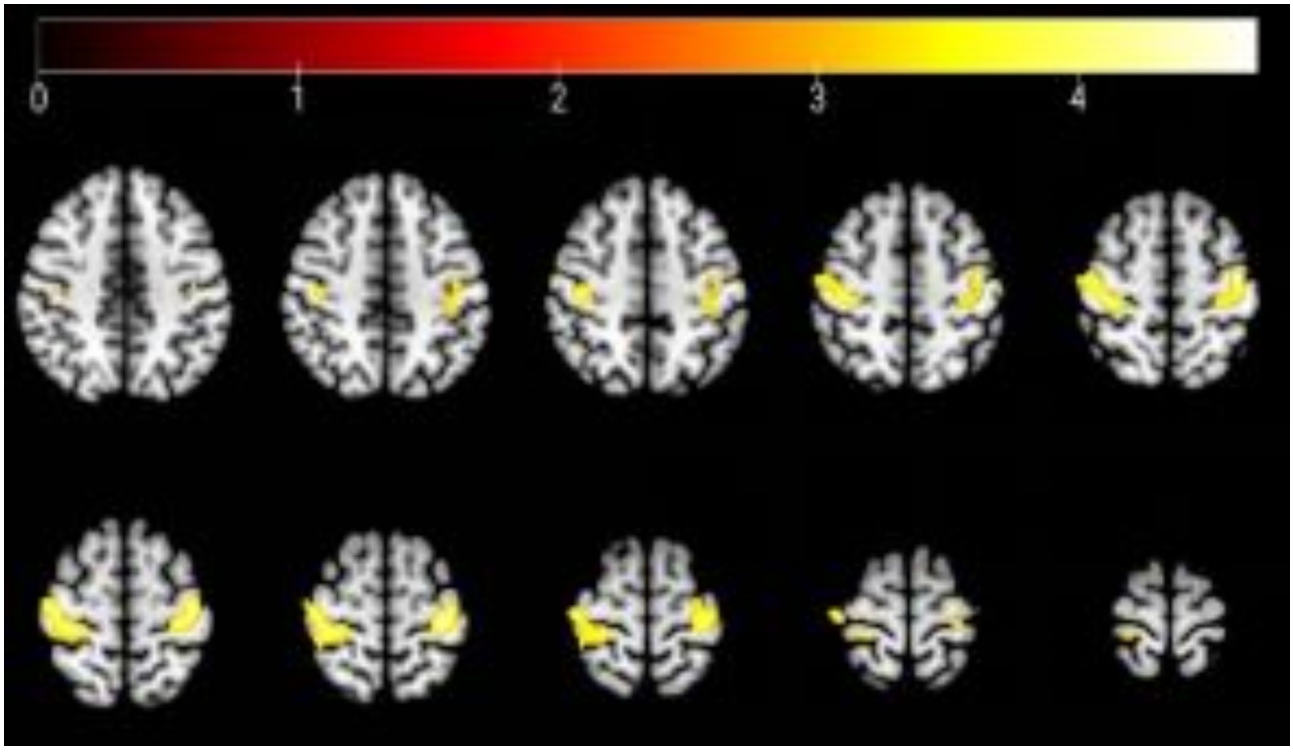


**Fig. 3** Comparison of mReHo differences between MHD-RLS group and MHD-nRLS group: The coloured areas indicate significantly higher mReHo values in the MHD-nRLS group compared to the MHD-RLS group

group compared with the MHD-RLS group. Changes in total iron-binding capacity and hemoglobin suggest a more severe iron deficiency state in the MHD-RLS group, while higher folate levels are thought to be due to folic acid supplementation in clinical anemia. After conducting a Pearson correlation analysis with RLS scores, it was found that hemoglobin levels are negatively correlated with RLS scores, indicating that the lower the hemoglobin level, the higher the severity of RLS in MHD-RLS group patients, which is consistent with the findings of Allen et al. [20].

Neuroimaging studies provide important reference data for clarifying the changes in neuroanatomy and function of RLS patients through non-invasive techniques, and are of great significance in exploring the pathophysiology of RLS. The precentral gyrus, also known as the primary motor cortex (M1), is part of the primary motor cortex and controls the movement of various parts of the body [24], mainly related to the control of RLS patients motor symptoms [14]. As the Primary Somatosensory Cortex, the Postcentral Gyrus shows significant morphological changes in the somatosensory systemin RLS patients [25]. The occurrence of RLS is associated with microstructural changes in the Motor Cortex, which can cause abnormalities in the Sensorimotor Pathway. These changes are related to the Severity of RLS symptoms. The alterations in the brains Sensorimotor Pathway provide Imaging Evidence for the Pathological Mechanism of RLS [26]. MRI examination indicates that patients with RLS exhibit structural changes in the thalamus, sensorimotor cortex, and cerebellum [27], however, there is some disagreement regarding cortical thickness changes, Lee et al. found that RLS patients have significantly reduced white matter-in the somatosensory pathways of the corpus callosum-postcentral gyrus. However, Stefani et al. discovered through VBM measurements that, compared to healthy individuals, RLS patients exhibit a significant increase in white matter volume in the bilateral primary sensory cortex, left premotor cortex, and right inferior parietal lobule. Additionally, the duration of the disease is significantly associated with changes in the white matter of the postcentral gyrus and precentral gyrus. In terms of treatment, Lanza et al. have confirmed that electrical stimulation of both sides of the RLS patientsmainlyM1leg area, primary somatosensory cortex, and left primary motor cortex can alleviate the RLS subjective symptoms and severity scale scores of patients, and this effect can last for several weeks [15, 28]. Similarly, high-frequency repetitive transcranial magnetic stimulation (rTMS) applied to the Primary motor cortex or Supplementary motor cortex, and low-frequency rTMS applied to the Primary somatosensory cortex, seems to have a transient beneficial effect [29]. RLS patients show a decrease in functional connectivity (FC) of the right salience network

**Table 5** Comparison of general data, laboratory tests, MoCA scores

| | MHD-nRLS (*n* = 27) | MHD-RLS (*n* = 27) | t/Z/χ2 | *P* value |
|---|---|---|---|---|
| Age(years) | 48.4 ± 7.9 | 52.8 ± 10.4 | 1.731 | 0.089[a] |
| Gender(male/female) | 14/13 | 12/15 | 0.297 | 0.586[c] |
| Education(years) | 9.0(6.0,12.0) | 9.0(6.0,12.0) | -0.638 | 0.523[b] |
| HD duration(months) | 31.0(24.0,43.0) | 36.0(24.0,60.0) | -0.663 | 0.507[b] |
| Hypertension(n%) | 23(85.2) | 23(85.2) | 0.000 | 1.000[c] |
| Diabetes(n%) | 16(59.3) | 20(74.1) | 1.333 | 0.248 [c] |
| MoCA(score) | 27.0(26.0,27.0) | 26.0(26.0,27.0) | -0.793 | 0.950[b] |
| Kt/V (ml-s-l/1.73m$^2$) | 1.4(1.3,1.5) | 1.4(1.3,1.5) | -0.063 | 0.276[b] |
| BUN(mmol/L) | 19.9(15.9,23.7) | 22.3(18.7,25.4) | -1.168 | 0.243[b] |
| UA(umol/L) | 344.9 ± 96.4 | 347.4 ± 133.5 | 0.938 | 0.938[a] |
| Cr(umol/L) | 1184.0 ± 185.9 | 1199.3 ± 193.2 | 0.767 | 0.767[a] |
| $K^+$(mmol/L) | 4.3 ± 1.0 | 4.7 ± 0.7 | 0.147 | 0.147[a] |
| $Na^{2+}$(mmol/L) | 140.2 ± 3.7 | 140.5 ± 3.8 | 0.745 | 0.745[a] |
| $Ca^{2+}$(mmol/L) | 2.2 ± 0.3 | 2.2 ± 0.2 | 0.540 | 0.591[a] |
| P(mmol/L) | 1.9 ± 0.5 | 1.9 ± 0.6 | -0.072 | 0.943[a] |
| WBC($10^9$/L) | 5.9(5.4,5.9) | 5.9(5.2,6.1) | -0.273 | 0.785[b] |
| CRP(mg/L) | 4.2(2.1,16.5) | 5.5(1.8,16.5) | -0.225 | 0.822[b] |
| RBC($10^{12}$/L) | 3.4 ± 0.8 | 3.3 ± 0.9 | -0.356 | 0.723[a] |
| Hb(g/L) | 110.0(103.0,122.0) | 101.0(77.0,107.0) | -3.281 | 0.001[b] |
| Hct(%) | 30.2 ± 6.2 | 30.8 ± 7.1 | 0.323 | 0.748[a] |
| TP(g/L) | 61.6 ± 10.3 | 65.2 ± 8.1 | 1.409 | 0.165[a] |
| Alb(g/L) | 36.8 ± 6.6 | 37.9 ± 5.9 | 0.630 | 0.532[a] |
| TC(mmol/L) | 3.9(3.0,4.7) | 4.2(3.3,4.8) | -0.579 | 0.563[b] |
| TG(mmol/L) | 1.5 ± 0.6 | 1.6 ± 0.6 | -0.571 | 0.610[a] |
| AST(U/L) | 13.0(11.0,19.0) | 14.5(11.3,18.0) | -0.069 | 0.945[b] |
| ALT(U/L) | 9.7(6.7,13.1) | 11.0(10.0,12.9) | -1.524 | 0.127[b] |
| HDL-C(mmol/L) | 1.1 ± 0.3 | 0.9 ± 0.2 | -1.757 | 0.051[a] |
| LDL-C(mmol/L) | 1.8(1.5,2.6) | 1.9(1.5,2.5) | -0.536 | 0.592[b] |
| ALP(U/L) | 74.0(65.0,97.0) | 83.0(64.0,98.6) | -0.917 | 0.359[b] |
| PTH(g/L) | 239.0(138.8,423.7) | 221.0(158.4,430.8) | -0.026 | 0.979[b] |
| Serum iron(umol/L) | 10.5 ± 3.6 | 9.7 ± 3.0 | -0.859 | 0.394[a] |
| TIBC(umol/L) | 32.6 ± 10.1 | 40.0 ± 10.7 | 2.646 | 0.011[a] |
| Serum ferritin(ng/mL) | 75.4(61.1,174.4) | 99.4(47.3,158.5) | -0.043 | 0.966[b] |
| Folate(ng/mL) | 4.6(3.5,9.1) | 6.9(4.8,20.0) | -2.282 | 0.022[b] |
| VitB12(pg/mL) | 578.3 ± 363.3 | 733.5 ± 292.9 | 1.729 | 0.090[a] |

[a] Independent Samples t-test; [b] Mann-Whitney U-test; [c] Chi-square test

HD: duration: Hemodialysis duration; MoCA: Montreal Cognitive Assessment; BUN: Blood urea nitrogen; UA: Uric acid; WBC: White blood cell; RBC: Red blood cell; TC: Total cholesterol; TP: Total protein; ALP: Alkaline phosphatase; HDL-C: High-density lipoprotein cholesterol: LDL-C: Low-density lipoprotein cholesterol; PTH: Parathyroid hormone; TIBC: Total iron binding force;; Kt/V: Urea clearance index

(SN), with an increase in FC in the bilateral orbitofrontal cortex and the right postcentral gyrus SN FC. This suggests that RLS patients SN FC changes during asymptomatic periods. This may affect the processing of salient information, particularly sensory information processing and inhibitory mechanisms [30].

MHD treatment and renal failure can affect the structure and function of the brain through blood perfusion, toxin deposition, anemia, electrolyte imbalances, and abnormal iron metabolism [31], which can be captured by MRI technology. Luo et al. discovered that compared to healthy individuals, the short functional connectivity (SFC) DC values in MHD patients were reduced in the right middle frontal gyrus. MHD patients often have lower ALFF values in the default mode network regions compared to healthy individuals, with significantly reduced ALFF values in the left superior parietal lobule, left inferior parietal lobule, and left precuneus [32]. Serum urea and creatinine levels are negatively correlated with ALFF in some default mode network regions, while hemoglobin is positively correlated with ALFF in the bilateral precuneus, precentral gyrus, and supplementary motor area [33].

MHD-RLS patients also exhibit changes in brain microstructure related to motor functions [34]. Mu and others found that patients with ESRD-RLS, compared

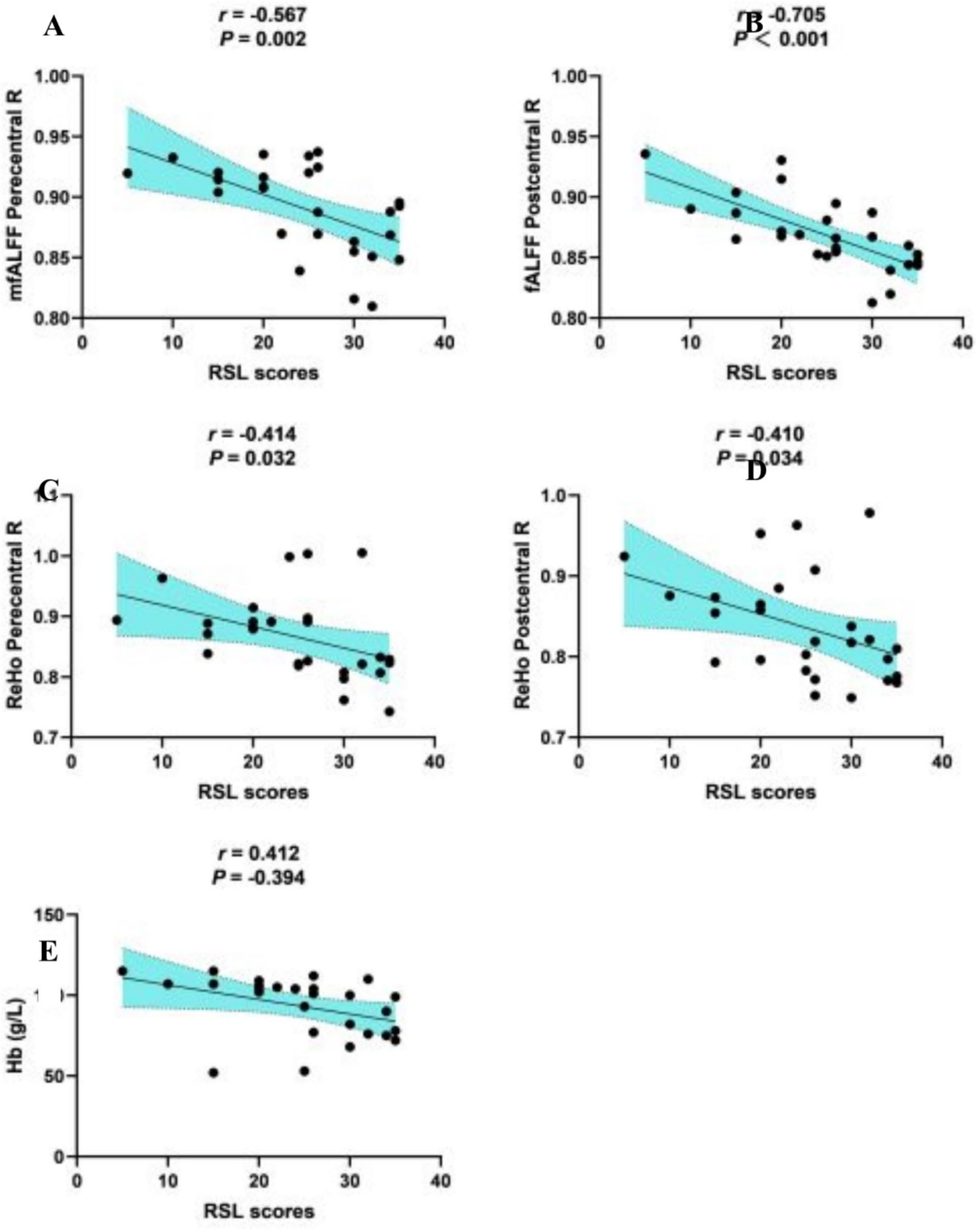


**Fig. 4** Correlation analysis. **A**: The mfALFF value of the right precentral gyrus in MHD-RLS patients is negatively correlated with the RLS scores ($r$ =- 0.567, $P$ = 0.002); **B**: The mALFF value of the right postcentral gyrus in MHD-RLS patients is negatively correlated with the RLS scores ($r$ = -0.705, $P$ < 0.001); **C**: The mReho value of the right precentral gyrus in MHD-RLS patients is negatively correlated with the RLS scores ($r$ = -0.414, $P$ = 0.032); **D**: The mReho value of the right postcentral gyrus in MHD-RLS patients is negatively correlated with the RLS scores ($r$ = -0.410, $P$ = 0.034); **E**: The hemoglobin concentration in MHD-RLS patients is negatively correlated with the RLS scores ($r$ = -0.394, $P$ = 0.042)

to healthy individuals, exhibited abnormalities in the motor-related brain regions of the bilateral superior frontal gyrus, precentral gyrus, and putamen GM volume, with significant differences in the diffusion characteristics of the posterior limb of the internal capsule, indicating that the motor-related brain structures of ESRD-RLS have undergone changes. They proposed that microstructural abnormalities might be imaging markers related to the severity of motor dysfunction in ESRD-RLS [10]. Using functional near-infrared spectroscopy and a graph theory approach, Park et al. demonstrated differences in functional connectivity between ESRD-RLS and nESRD-RLS [35]. Li and colleagues found that MHD-RLS patients, compared to nMHD-RLS patients, exhibited reduced left precentral gyrus cerebral blood flow (CBF), which is an independent risk factor for MHD patients developing RLS. They suggested that the abnormal CBF changes in the left precentral gyrus might be involved in the MHD-RLS patients potential neurological Pathological Mechanism [27].

These findings are consistent with the results that we found that MHD-nRSL patients had higher mfALFF values in the right anterior central gyrus and right postcentral gyrus, and mReHo values in the right anterior central gyrus and right postcentral gyrus than in MHD-RSL patients, and were negatively correlated with the RSL severity scores of MHD-RSL patients.

However, there are some limitations in this study. Firstly, this study is a cross-sectional study, which may not allow for clear causal inferences. Second, the diagnosis of RLS in patients in the MHD-RLS group was done through questionnaires and clinical presentations, and there was a lack of polysomnography recordings, which may have ignored cases of mild RLS in study participants. According to previous literature, ferritin is also considered a clinical risk factor for the occurrence of RLS, which our results did not reflect. This might be related to insufficient sample size or individual heterogeneity. Finally, the sample size of this study is relatively small, which may lead to some bias in the evaluation of the prevalence of RLS in MHD patients and the related clinical risk factors. It is interesting that in previous studies, there was disagreement about which hemisphere of the brain is altered. here were studies demonstrated changes in the left [9, 32], right [36] and both sides [8, 10, 25, 26] of sensorimotor area. In this study, although there were differences in both the left and right anterior central gyrus and posterior central gyrus, however, only the alteration in spontaneous brain activity of right brain were proved to be correlated with the severity of RLS symptom after Pearson correlation analysis. Except for statistical bias, we considered this could be due to the reduce of the WM integrity of right-hemispheric thalamus (posterior ventral lateral nucleus) among RLS patients, which altered cerebral sensorimotor pathways of right hemicerebrum more significantly [26]. And also limited by the sample size, both patients who did not received medical treatment and patients whose symptoms did not improve after the use of drugs were included in MHD-RLS group without further classified. Therefore, an increased sample size is needed to further clarify the results.

## Conclusion

There were abnormal changes in spontaneous brain activity in MHD-RLS patients, among which the mfALFF values of right precentral gyrus and right postcentral gyrus, mReHo values of right precentral gyrus and right postcentral gyrus, and hemoglobin levels were negatively correlated with RLS severity scores, suggesting that sensorimotor networks may be the key areas affecting the occurrence of RLS in MHD patients, and the changes in spontaneous brain activity and hemoglobin levels in the above brain regions may affect the severity of RLS in MHD patients. The above results may provide a basis for exploring the pathogenesis of RLS in MHD patients.

### Abbreviations

| | |
|---|---|
| ALFF | Amplitude of low-frequency fluctuations |
| DMN | Default mode network |
| DTI | Diffusion tensor imaging |
| ESRD | End-stage renal disease |
| fALFF | Fractional amplitude of low-frequency fluctuations |
| GM | Gray matter |
| IRLSSG | International Restless Legs Syndrome Study Group |
| MHD-nRLS | Maintenance hemodialysis patient without restless legs syndrome |
| MHD-RLS | Maintenance hemodialysis patient with restless legs syndrome |
| MHD | Maintenance hemodialysis |
| ReHo | Regional homogeneity |
| RLS | Restless legs syndrome |
| rs-fMRI | Resting state functional magnetic resonance imaging |
| TABS | Tractography atlas-based analysis |
| VBM | Voxel-based morphometry |
| WM | White matte |

## Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12883-024-03985-6.

Supplementary Material 1

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### Author contributions

Author contributions included conception and study design (TL and HS), data collection or acquisition (DW, YT and WL), statistical analysis (DW, LX and FZ), interpretation of results (DW, WZ and YT), drafting the manuscript work or revising it critically for important intellectual content (TL and DW) and approval of final version to be published and agreement to be accountable for the integrity and accuracy of all aspects of the work (All authors).

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**Data availability**
The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

## Declarations

**Ethical approval and consent to participate**
This study was approved by the Ethics Committee of the Third Affiliated Hospital of Nanjing Medical University, Changzhou, Jiangsu, China ([2022] KY005-01). The study was conducted in accordance with the Declaration of Helsinki and its later amendments. Informed consent was obtained from all participants and their legal guardians in the study.

**Consent for publication**
Not Applicable. All of our identifying images and other personal or clinical details of participants are presented that compromise anonymity.

**Competing interests**
The authors declare no competing interests.

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