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Original Article

Brain iron deficiency in idiopathic restless legs syndrome measured by quantitative magnetic susceptibility at 7 tesla

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ABSTRACT

Objectives: Altered brain iron homeostasis with regional iron deficiency has been previously reported in several studies of restless legs syndrome (RLS) patients. Inconsistencies still exist, however, in the reported iron changes in different brain regions and different RLS phenotypes. The purpose of this study was to assess differences in brain iron concentrations between RLS patients and healthy controls and their relation to severity of disease and periodic limb movement during sleep (PLMS).
Methods: Assessment of brain iron was done using quantitative magnetic susceptibility measurement, which has been shown to correlate well with the tissue iron content in brain's gray matter. Thirty-nine RLS patients and 29 age-matched healthy controls were scanned at 7 T. Magnetic susceptibilities in substantia nigra (SN), thalamus, striatum, and several iron-rich gray matter regions were quantified and compared with related clinical measures.
Results: Compared with healthy controls, RLS patients showed significantly decreased magnetic susceptibility in the thalamus and dentate nucleus. No significant difference was found in the SN between RLS patients and healthy controls, but a significant correlation was observed between magnetic susceptibility in SN and the PLMS measure.
Conclusions: Using quantitative magnetic susceptibility as an in vivo indicator of brain iron content, the present study supports the general hypothesis of brain iron deficiency in RLS and indicates its possible link to PLMS.

1. Introduction

Restless legs syndrome (RLS) is a sensorimotor disorder with a prevalence of 5–10% of the population [1]. It is characterized by unpleasant sensations mainly in the legs and an urge to move while sitting or resting in the later part of the day [2]. The exact pathophysiology of idiopathic RLS is still unclear, but previous studies suggest that an alteration in dopaminergic function and also in iron homeostasis is involved in the disease [3]. A general hypothesis of brain iron deficiency in RLS has been supported by previous cerebrospinal fluid (CSF), brain autopsy, and imaging studies [4–7]. In particular, two studies of CSF in RLS patients found a decrease in the main iron storage protein (ferritin) along with an increase in transferrin even though the serum ferritin and transferrin levels did not differ significantly from healthy controls [4,8]. Histological and immunoblot studies using brain autopsy tissues from RLS and controls also showed a decrease in iron and H-ferritin, but no difference in L-ferritin in the substantia nigra (SN) [5], in the epithelial cells in choroid plexus, and in the brain microvasculature [9]. These autopsy findings indicated the presence of altered iron acquisition and utilization in the RLS brain. In vivo measurements of brain iron concentrations in RLS using magnetic resonance imaging (MRI) relaxometry [6,7,10–13], that is, measuring the magnetic resonance (MR) transverse relaxation rate R2 and the effective transverse relaxation rate R2* or R2′ (R2′ = R2* – R2), and very recently using MR phase imaging [14] have supported the hypothesis of decreased brain iron in RLS.

Despite the commonly accepted general hypothesis of brain iron deficiency in RLS, discrepancies exist in the available MRI studies on RLS in terms of the affected brain regions and the possible changes in different RLS phenotypes. Among all the studies, SN is the most

Abbreviations: IRLSS, international restless legs syndrome score; PLMS, periodic limb movement during sleep; QSM, quantitative susceptibility mapping; RLS, restless legs syndrome; ROI, region of interest; SN, substantia nigra; SWI, susceptibility weighted imaging.

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consistently reported region affected by possible iron deficiency in RLS [6,7,11–14], together with thalamus [10,14]. Thus, these areas were the primary ones analyzed in this study. The involvement of the other brain regions in RLS-related tissue iron changes still remains unclear and was therefore included in this study as secondary, exploratory areas of interest, that is, red nucleus, cerebellar dentate nucleus, caudate nucleus, putamen, globus pallidus, and thalamic pulvinar. In addition, brain iron changes in the two main RLS phenotypes, that is, early-onset versus late-onset RLS, remain quite elusive. In one prior study using R2′ as a brain iron measure [7], lower brain iron content in the SN was found only in early-onset RLS patients but not in late-onset RLS patients. Some later studies, however, reported lower SN iron concentrations in late-onset RLS [11,12], but not in early-onset RLS, either using R2 [15] or R2′ [12]. Moreover, there is one study reporting no change in the MRI-based iron index (R2* weighted signal intensity) in RLS [16] and another reporting an increased iron index (R2) in early-onset RLS without medical treatment [15]. Besides biological variations, small sample sizes, uncertain differential diagnosis of RLS and controls, and possible treatment-induced brain iron changes [17], such discrepancies may be attributed to the different MRI techniques used for measuring tissue iron contents with different sensitivities and methodological limitations [18]. For example, it is known that R2-based relaxometry is affected by tissue water content that is not related to iron and R2* or R2′ may be contaminated by background field gradients. MR phase or field shift measures are nonlocal, meaning that they are affected by the susceptibility values of the surrounding tissue, causing a dependence on the subject's head orientations relative to the magnetic field [19,20]. In comparison, quantitative measurement of tissue magnetic susceptibility is believed to be a more accurate and probably more specific measure of tissue iron content, especially in gray matter. Recent development of quantitative susceptibility mapping (QSM) techniques together with the availability of high-field MRI have made it possible to directly map, with high spatial resolution (sub-millimeter), the brain tissue magnetic susceptibility [21–26], which has been shown to correlate well with tissue iron concentration in most of brain's gray matter regions [27–31].

The aim of the present study was to determine regional brain iron concentrations in RLS using QSM and to test possible correlations between the measured brain tissue magnetic susceptibility and RLS clinical features.

2. Materials and methods

2.1. Subjects

Thirty-nine consenting subjects with idiopathic RLS and 29 consenting age- and gender-matched healthy control subjects were recruited through the Center for Restless Legs Syndrome at the Johns Hopkins University School of Medicine. Subjects were excluded if they had significant medical or neurological disorders or were taking medications that would disturb sleep or if they had significant sleep disorders other than RLS, for example, insomnia not related to RLS, narcolepsy, and sleep apnea. Since transcranial magnetic stimulation studies were included in this study (reported separately), subjects were also excluded if they did not have a strong hand preference or had significant activities involving training hand movements, for example, musicians and artists.

All of the RLS and control patients were diagnosed by an expert clinician using the validated structured diagnostic interview (Hopkins Diagnostic Telephone Interview) modified to exclude mimics and meet the updated diagnostic criteria for RLS [32]. All RLS patients were off any medications for RLS at least 10 days prior to admission to the Hopkins Clinical Research Center, where they had two consecutive full nights of standard polysomnography following the standards established by the American Academy of Sleep Medicine, including six channels of electroencephalogram and two of anterior tibialis electromyography for recording periodic leg movements [33]. Sleep breathing was evaluated only on the first night and subjects with sleep disordered breathing rates >15/h were excluded from the study. Sleep staging was scored using the American Academy of Sleep Medicine criteria [33]. Periodic limb movements during sleep (PLMS) were scored using the World Association of Sleep Medicine criteria [34]. The final clinical assessment and MRI scan were preformed after the second night of sleep, at least 12 days off any RLS medications. The severity of RLS symptoms was assessed using international restless legs syndrome score (IRLSS) [35,36]. In addition, a morning fasting blood sample of all subjects was tested for serum ferritin after the first night in the Clinical Research Center. The institutional review boards at the Johns Hopkins University School of Medicine and Kennedy Krieger Institute approved the protocols. Written informed consent was obtained from all participants before the study.

2.2. MR imaging protocol

All the subjects underwent MRI scans in the morning after the second night sleep study. Due to the circadian rhythm of the disease, patients scanned in the morning were either free of or exhibited only minimum RLS symptoms. In addition, during the MRI scans foam pads and straps were used to restrict head movement of the patients. MRI scans were done using a 7 T Achieva scanner (Philips Healthcare, Best, The Netherlands) equipped with a 32-channel head coil (Novamedical). MR phase measurements used for QSM calculation were acquired using a 3D single-echo gradient echo sequence with 0.8 mm isotropic resolution, $220 \times 220 \times 140$ mm^3 field of view, axial slab orientation, repetition time/echo time = 20/12 ms, flip angle 10°, bandwidth 169 Hz/px, coil sensitivity encoding factor of $2.5 \times 1 \times 2$, and scan time 4′38″.

2.3. Image analysis

MR phase data were used for QSM calculation. First, phase unwrapping was performed using a Laplacian-based phase unwrapping method [37]. The unwrapped phase images were then divided by the corresponding echo time to obtain an image of the frequency shift. Subsequently, the background field was removed using the sophisticated harmonic artifact reduction for phase data approach with the variable spherical kernel size (V-SHARP) method [28,38,39]. For this, we used a variable spherical kernel size with a maximum radius of 4 mm and a singular value decomposition threshold of 0.05. After that, dipole inversion was calculated to obtain susceptibility images using a least square with the QR factorization-based minimization method [37]. In addition, to compare with a previous iron study in RLS using phase imaging [14], a high-pass filtered phase was calculated using a similar susceptibility weighted imaging (SWI) method [40] with a 2D k-space filter with each axial slice of size 18×18.

For automated image segmentation, all QSM images were coregistered to the QSM atlas created in the Johns Hopkins University using linear and large deformation diffeomorphic metric mapping transformation [31]. After that, the brain parcellation map was transformed to the subject space and inspected by a neuroradiologist (H.L.). Manual adjustment of the brain segmentation of the selected regions of interest was then made after inspection. The central CSF region in the lateral ventricles was selected for each subject as a reference region for susceptibility quantification. Choroid plexus regions that showed obvious hypointensity in the QSM image possibly due to calcium deposition were excluded from the CSF reference region. Quantitative susceptibility values were then referenced to the mean susceptibility values of all the voxels in this reference region. For group comparison and correlation analysis, mean magnetic susceptibility values