**Magnetic Susceptibility Changes in the Basal Ganglia and Brain Stem of Patients with Wilson’s Disease: Evaluation with Quantitative Susceptibility Mapping**

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**Objectives:** Wilson’s disease (WD) is characterized with the accumulation of copper in the liver and brain. The objective of this study is to quantitatively measure the susceptibility changes of basal ganglia and brain stem of pediatric patients with neurological WD using quantitative susceptibility mapping (QSM) in comparison to healthy controls.

**Methods:** Eleven patients with neurological WD (mean age 15 ± 3.3 years, range 10–22 years) and 14 age-matched controls were prospectively recruited. Both groups were scanned on a 1.5 Tesla clinical scanner. In addition to T₁- and T₂-weighted magnetic resonance (MR) images, a three dimensional (3D) multi-echo spoiled gradient echo (GRE) sequence was acquired and QSM images were derived offline. The quantitative measurement of susceptibility of corpus striatum, thalamus of each hemisphere, midbrain, and pons were assessed with the region of interest analysis on the QSM images. The susceptibility values for the patient and control groups were compared using two-sample t-test.

**Results:** One patient with WD had T₁ shortening in the bilateral globus pallidus. Another one had hyperintensity in the bilateral putamen, caudate nuclei, and substantia nigra on T₂-weighted images. The rest of the patients with WD and all subjects of the control group had no signal abnormalities on conventional MR images. The susceptibility measures of right side of globus pallidus, putamen, thalamus, midbrain, and entire pons were significantly different in patients compared to controls (P < 0.05).

**Conclusion:** QSM method exhibits increased susceptibility differences of basal ganglia and brain stem in patients with WD that have neurologic impairment even if no signal alteration is detected on T₁- and T₂-weighted MR images.

**Keywords:** quantitative susceptibility mapping, magnetic resonance imaging, Wilson’s disease, copper

**Introduction**

Wilson’s Disease (WD) is an inherited copper metabolism disorder resulted from a mutation to ATP7B gene acting in the transportation of the copper. Due to defective transportation, copper accumulates particularly in the liver and brain. This is assumed to be the main mechanism responsible for the pathogenesis of the disease. Thus, the spectrum of clinical presentations consists of hepatic, neurological, or neuropsychiatric disturbances. Main neurological symptoms associated with WD include dizziness, lack of strength, excessive salivation, slurred speech, ataxia, dystonia, bradykinesia, personality changes, and headache. The diagnosis of clinically suspected patients is based on the combinations of the presence of Kayser-Fleischer ring detected by slit lamp examination, decreased level of serum ceruloplasmin, increased excretion of urinary copper, and eventually detection of excessive copper deposition in the liver biopsy.

Conventional magnetic resonance imaging (MRI) sequences may exhibit abnormal signal intensities in basal ganglia and brain stem in the course of WD. The most...
common finding reported in patients with WD is the hyperintensity of globus pallidus, striatum, and brain stem on \( T_2 \)-weighted images that well correlates with the neurologic symptoms.""\] Conversely, the shortening of \( T_1 \) relaxation time of bilateral lentiform nuclei may be detected prior to neurologic deterioration and is attributed to hepatic dysfunction rather than copper accumulation of affected brain sites.\(^6\)\(^,\)\(^7\)

Quantitative susceptibility mapping (QSM) is a relatively new post-processing technique to create susceptibility maps of the brain and has been reported to be highly effective in differentiation of intracranial calcification and hemorrhage.\(^8\)\(^,\)\(^9\) It was also utilized in quantification of iron levels in the human brain.\(^10\)\(^,\)\(^11\) A recent study has shown that copper (II) compounds could result in a measurable difference in susceptibility maps in patients with WD compared to healthy controls at magnetic field strength of 7 Tesla.\(^17\) To the best of our knowledge, this study was the first and only study that focuses on the quantification of the copper accumulation in the basal ganglia with the implementation of QSM technique.

In the current study, we hypothesized that susceptibility changes throughout the basal ganglia and brain stem of patients with neurological WD could be detected prior to any alterations on \( T_1 \) and \( T_2 \)-weighted MRI images using QSM method. We aimed to quantitatively measure the susceptibility changes of basal ganglia and brain stem in patients with WD using QSM technique obtained from a 1.5 Tesla clinical scanner.

**Methods**

**Patient selection**

Sixty five patients that were being followed up with the diagnosis of WD by the pediatric gastroenterology and neurology clinics of our institution were recruited in this institutional review board approved the study. All patients had either symptomatic or asymptomatic liver involvement. The diagnosis of WD was based on combinations of presence of Kayser-Fleischer rings detected with slit lamp examination, increased urinary copper excretion, decreased serum ceruloplasmin level, and quantification of copper with liver biopsy.\(^5\)\(^,\)\(^18\) Patients were evaluated for the presence of any neurologic findings by two pediatric neurologists. Subsequently, 54 patients that had no neuropsychiatric findings (hepatic form) were excluded and 11 patients with neurologic impairment (neurological form) were recruited. The control group consisted of 14 children who came to the pediatric neurology clinic with the symptom of headache but otherwise healthy after the clinical evaluations. The mean age for the patients and the control group were 15 ± 3.3 years (four males, range 10–22 years) and 13.2 ± 2.4 years (six males, range 8–16 years), respectively. The control group had normal levels of serum ceruloplasmin and urinary copper excretion. The subjects and their parents were informed about the study concept and written informed consent was obtained. Two pediatric neurologists who were blinded to cranial MRI findings reviewed the clinical data of the patients in consensus and recorded.

**MR Imaging and QSM Analysis**

The cranial MRI examinations were obtained on a 1.5T clinical scanner (Magnetom Aera, Siemens, Erlangen, Germany) using a 20-channel neuro coil with the following protocol; a) coronal and axial \( T_2 \)-weighted turbo spin echo, b) three dimensional (3D) \( T_1 \)-weighted Magnetization Prepared Rapid Acquisition Gradient Echo (MPRAGE) c) axial 3D multi-echo spoiled gradient echo (GRE) sequence (\( T_2^* \)). The protocol parameters for the multi-echo spoiled GRE sequence were as follows: flip angle = 25°, repetition time (TR) = 80 ms, number of echoes = 5, echo times (TE) = [8.3, 16.8, 24.7, 32.7, 40.7] ms, number of slices = 64, slice thickness (ST) = 2.5 mm, field of view (FOV) = 220 × 206 mm, Matrix: 192 × 154, bandwidth (BW) = 130 Hz/pixel, GRAPPA acceleration factor of 2, and acquisition time (\( T_{seq} \)) = 4 min 17s.

QSM images were reconstructed from multi-echo GRE images. The reconstruction routine involved phase unwrapping, background phase removal using projection onto dipole fields (PDF) approach\(^18\) and morphology-enabled dipole inversion (MEDI)\(^9\)\(^,\)\(^20\) to reconstruct the susceptibility maps.

Region of interest (ROI) analysis was performed on six regions of each hemisphere and two regions of the half of the brain stem: anterior and posterior of globus pallidus, anterior and posterior of putamen, caudate nucleus, thalamus, substantia nigra in the midbrain, and lastly posterior part of the pons adjacent to the cerebral aqueduct on QSM images. ROIs were drawn manually on a single slice where the region of interest is best visible, using ImageJ software\(^21\) by a pediatric radiologist together with an observer radiologist in consensus. Each ROI was delineated according to anatomical boundary of the interested region. An additional measurement was obtained from cerebrospinal fluid (CSF) using a circular ROI inside the fourth ventricle. The CSF measurement value was subtracted from the other measured values for referencing. Two-sample t-test was implemented to compare the susceptibility measurements between the patients and controls. A \( P \) value <0.05 was considered as significant.

The images of \( T_1 \), \( T_2 \), and \( T_2^* \) sequences of both the patient and the control group were qualitatively evaluated by two pediatric radiologists in consensus for the presence of any signal alterations throughout the basal ganglia and brain stem.

**Results**

The patients had neurologic symptoms including tremor, dystonia, dizziness, lack of strength, and headache. Kayser-Fleischer rings were detected in five (45%) of the patients. Elevated liver function tests were present in all patients but one, while all patients had clinic and radiologic findings of chronic liver failure. The mean duration of follow up was 48.2 ± 38.1 months and the patients had been treated with D-penicillamine and zinc since the definitive diagnosis was established (Table 1).
### Table 1. Features of study patients and magnetic resonance imaging findings

<table>
<thead>
<tr>
<th>Patient</th>
<th>Gender</th>
<th>Age at diagnosis (year)</th>
<th>Age at MRI acquisition (year)</th>
<th>Duration of follow-up (month)</th>
<th>Time of diagnosis-first neurologic symptom (month)</th>
<th>Presentation with neurologic symptoms</th>
<th>Kayser-Fleischer ring</th>
<th>Liver biopsy</th>
<th>T₁ hyper intensity</th>
<th>T₂ hyper intensity</th>
<th>T₁* hypo intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>13</td>
<td>15</td>
<td>15</td>
<td>–</td>
<td>Dystonia, tremor, ataxia</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>7</td>
<td>22</td>
<td>128</td>
<td>108</td>
<td>Dystonia, tremor, headache</td>
<td>+</td>
<td>–</td>
<td>Bilateral GP</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>11</td>
<td>16</td>
<td>45</td>
<td>36</td>
<td>Tremor, headache</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>6</td>
<td>14</td>
<td>82</td>
<td>36</td>
<td>Headache, dizziness</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>8</td>
<td>16</td>
<td>80</td>
<td>72</td>
<td>Headache, dizziness, syncope</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>5</td>
<td>12</td>
<td>78</td>
<td>60</td>
<td>Headache</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>10</td>
<td>11</td>
<td>16</td>
<td>–</td>
<td>Headache, sudden lack of strength</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>15</td>
<td>17</td>
<td>15</td>
<td>12</td>
<td>Tremor</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>7</td>
<td>10</td>
<td>24</td>
<td>26</td>
<td>Headache, dystonia</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>10</td>
<td>F</td>
<td>15</td>
<td>16</td>
<td>18</td>
<td>–</td>
<td>Tremor, ataxia, fever, vomiting</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>11</td>
<td>F</td>
<td>10</td>
<td>16</td>
<td>29</td>
<td>18</td>
<td>Headache, tremor</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

GP, globus pallidus; CN, caudate nucleus; SN, substantia nigra; P, putamen; Nuc, nucleus.
The magnetic resonance (MR) images of a 15-year-old male with Wilson’s disease (WD) revealed (a) increased signal intensity of bilateral caudate nuclei (dashed arrows) and putamen (white arrows) on T₂-weighted axial image. Note the central hypointensity of bilateral globus pallidus and putamen qualitatively (white arrows). The artifact, anterolateral to right putamen is also revealed (black arrow). In addition to basal ganglia involvement, tegmentum in the mesencephalon also has the increased signal on T₂-weighted image (arrows) (c). Although not as prominent as in T₂-weighted image, on QSM image (d) the increased susceptibility changes of bilateral tegmentum can be appreciated (arrows). QSM image (e) of age-matched (15-year-old, male) control patient shows relatively low susceptibility differences of bilateral globus pallidus and putamen. Note the artifact located on left globus pallidus (black arrow).

When T₁- and T₂-weighted MR images were solely evaluated, two patients with WD were detected to have signal abnormalities on basal ganglia and brain stem: One patient had increased signal intensity on T₂-weighted images involving bilateral symmetrical head of caudate nucleus, putamen, and midbrain. There was no accompanying T₁ shortening (Fig. 1a). On T₁-weighted images of other patient, the bilateral globus pallidus was revealed hyperintense, without any abnormal signal alterations on T₂-weighted images (Fig. 2a). The rest of the patients with WD and the control group did not have any signal changes or structural abnormalities on conventional MR sequences.

The qualitative assessment of T₁ images of patients with WD revealed reduced signal intensity of bilateral globus pallidus, substantia nigra of all patients. In nine patients, out of 11 (82%) the bilateral red nucleus was hypointense while the bilateral putamen and head of caudate nuclei displayed decreased signal intensity in two different patients (Table 1). In the control group, all patients had reduced signal intensity in the bilateral globus pallidus. The bilateral substantia nigra were detected with decreased signal intensity in 13 patients (13/14 patients, 93%) while the bilateral red nucleus was hypointense in four patients (4/14 patients, 28%).

We observed a signal loss artifact (the black arrow in Fig. 1b) on some QSM images that hindered ROI measurement from globus pallidus and/or putamen in about three subjects in each group. Its possible origin is discussed in the discussion. The susceptibility values between the patient and the control were found to be significantly different in the regions of globus pallidus, putamen, thalamus located on the right hemisphere (P < 0.05). The ROI analysis of bilateral caudate nuclei, posterior part of left globus pallidus, anterior part of left putamen, left thalamus did not reveal any significant difference in susceptibility values between the patients and the control group (P > 0.05). When brain stem was evaluated, the susceptibility changes of the substantia nigra and pons were more prominent in the patients than the control. However, susceptibility values were not significantly increased in the left substantia nigra (P = 0.11). The measured values of QSM maps are presented in Table 2 and Fig. 3.

**Discussion**

This study evaluates the ability of QSM method to quantify the susceptibility changes in the basal ganglia and brain stem of patients with neurological WD. Our data presents that increased susceptibility changes in the brains of patients with WD compared to the control group could be detected with QSM method prior to any alterations evident on T₁- and T₂-weighted MR images.
The pathologic effect of increased extracellular copper is primarily oxidative stress and cell demolishment that particularly affects the brain stem and basal ganglia. Ceruloplasmin, in addition to being the main copper carrying plasma protein, catalyzes the conversion of Fe(II) into Fe(III) and plays a crucial role in iron transportation across cell membrane. Ceruloplasmin deficiency may cause brain iron deposition as well as copper (II) and facilitate neuronal degeneration. Since both copper (II) and iron are paramagnetic, the susceptibility changes observed in the basal ganglia and brain stem in the patients with WD might be attributable to iron and copper deposition.

Quantitation of minerals such as iron and copper deposited in the human brain is a subject of radiological studies. Copper and copper (I) compounds are diamagnetic like water while copper (II) compounds are paramagnetic. Paramagnetic minerals in tissue alter the phase of local MR signal. The phase shift depends on echo time and perturbation of magnetic field due to susceptibility effects. Susceptibility Weighted Imaging (SWI) relies on phase information to yield a contrast based on local susceptibility differences in the tissue. Depending on the susceptibility differences, SWI has shown to reveal deep gray nuclei and cerebral cortical mineralization in patients with WD. Furthermore, SWI technique may indicate mineral deposition in caudate nuclei, globus pallidus, putamen, thalamus, substantia nigra, and red nucleus based on the phase values in WD. With the introduction of QSM technique, it has been possible to map magnetic susceptibility of brain tissue in vivo. QSM is a post-processing technique that inverts the phase data to magnetic susceptibility maps. Unlike SWI, QSM enables quantitative measurement of tissue susceptibility rather than its effect on the phase data. QSM is a relative technique. The susceptibility value on a region of interest needs to be evaluated to a reference region that was CSF in our analysis. Increased susceptibility value relative to the reference region generally indicates paramagnetic minerals such as iron and copper (II) compounds in the tissues. Magnetic susceptibility of an object is theoretically independent of the static magnetic field. The susceptibility values obtained at 1.5T and 7T must be theoretically same. However, phase contrast increases at high magnetic field strengths. Thus, QSM benefits from the high field strength, resulting in better susceptibility contrast at high tesla. This study demonstrates that QSM images obtained from 1.5T data can be sensitive to the susceptibility changes in the diseased brain.

Previously, putamen has been reported to be the most frequently involved region in the basal ganglia revealing hyperintensity on T2-weighted MR images followed by the other components of the corpus striatum and thalamus. Since our study group consisted of predominantly pediatric patients, all the
patients except two had no signal alterations in the basal ganglia on T₁- and T₂-weighted MR images possibly because of the short duration of the disease. One patient had T₂ prolongation involving bilateral basal ganglia while another one exhibited hyperintensity in bilateral globus pallidus on T₁-weighted MR image. However, QSM images were capable of showing increased susceptibility differences of basal ganglia and thalami in the patients compared to the control group even before the manifestation of signal alterations on T₁- and T₂-weighted images. The susceptibility changes of right entire and left anterior globus pallidus, right entire and left posterior putamen, right thalamus were significantly different between the patients and controls. The distribution of increased susceptibility changes in the basal ganglia of patients with WD is concordant with a recent report of Fritzsch et al. The susceptibility measures of the left anterior putamen, left thalamus and left posterior globus pallidus were not significantly different between the patients and the controls. This could be related to artificial effect due to the artifact on QSM maps.

T₁-weighted MR images may reveal the characteristic ‘double panda sign’ in WD that points the involved sites of the brain stem: ‘Giant panda’ is seen in the midbrain and constituted by the hyperintensity of the tegmentum, preservation of signal intensity of the bilateral red nuclei, the lateral part of the substantia nigra and hypointensity of superior colliculus. ‘Panda cub’ is formed by the hyperintensity of dorsal pons. In the current study, solely one patient had aforementioned typical involvement of the midbrain on T₁-weighted MR image. We, for the first time in the literature, compared the susceptibility differences of the brain stem of the patients with WD and healthy controls. The measured susceptibility values of substantia nigra and dorsal pons in the patients exhibited significant increase except the left side of the substantia nigra.

The study has some limitations. Our study group was relatively small since we only focused on the neurologically impaired patients with WD. Therefore, further studies with larger patient groups are needed to test and validate our preliminary results. The scope of the new study to be conducted may be comparing the susceptibility changes of patients with neurologic and hepatic WD at a magnetic strength of 1.5 Tesla. The susceptibility changes might be an early indicator for WD.

In conclusion, QSM method may exhibit increased susceptibility differences of basal ganglia and brain stem in patients with neurologic WD prior to the detection of any signal alteration on T₁- and T₂-weighted MR images at a magnetic field strength of 1.5 Tesla. The susceptibility changes might be an early indicator for WD.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

References


