Transcranial sonography of the substantia nigra and its correlation with DAT-SPECT in the diagnosis of Parkinson’s disease

Dun-hui Li, Lin-yuan Zhang, Yun-yun Hu, Xu-feng Jiang, Hai-yan Zhou, Qiong Yang, Wen-yan Kang, Jun Liu, Sheng-Di Chen

A T I C L E  I N F O

Article info
Article history:
Received 21 January 2015
Received in revised form 17 April 2015
Accepted 31 May 2015

Keywords:
Parkinson’s disease
Transcranial sonography
Echogenicity
SPECT

A B S T R A C T

Introductions: Transcranial sonography (TCS) of the substantia nigra is a new and promising method to diagnose Parkinson’s disease (PD) but its effectiveness is controversial. Methods: All 55 PD patients involved in the study underwent single photon emission computed tomography (SPECT) imaging using the labeled dopamine transporter radiotracer 99mTc-TRODAT-1 to assess nigrostriatal dopaminergic function. The echogenicity of the substantia nigra was measured by TCS in all patients who received DAT-SPECT scanning. Finally, statistical analysis was carried out to determine the diagnostic accuracy of TCS as well as its correlation with 99mTc-TRODAT-1 SPECT, its positive predictive value (PPV), and negative predictive value (NPV).

Results: Contralateral striatal 99mTc-TRODAT-1 uptake was significantly reduced compared to ipsilateral striatal uptake, and had a negative correlation with UPDRS-III (r = -0.334, p = 0.013), disease duration (r = -0.393, p = 0.003) and H–Y stage (r = -0.330, p = 0.014). After TCS measurement, the contralateral SN echogenic area was similar to the ipsilateral SN echogenic area (27.77 ± 13.19 vs 25.98 ± 11.94 mm², p = 0.402, n = 24). No correlation was identified between TCS and UPDRS-III (r = 0.383, p = 0.065), disease duration (r = 0.371, p = 0.075) or H–Y stage (r = 0.259, p = 0.222). The sensitivity and specificity of SN TCS for the diagnosis of PD were calculated as 64.70% and 60% according to DAT-SPECT, respectively, while the positive predictive value and negative predictive value was calculated as 91.67% and 20%, respectively.

Conclusions: Compared to DAT-SPECT, TCS is a non-radioactive and convenient procedure to perform. In our investigation, TCS had no correlation with DAT-SPECT. However, the high positive predictive value of TCS highlights its possible utility as a routine diagnostic test.

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1. Introduction

Parkinson’s disease (PD) is the second most common neurodegenerative disease after Alzheimer’s disease. Currently, the diagnosis of PD is based primarily on clinical manifestation and physician’s expertise. Due to the absence of an accurate, economic and practical method for early diagnosis, a large proportion of early stage PD patients cannot be identified until extensive damage has occurred [1]. In order to solve this problem, we sought to investigate an accurate and economic method that could be used in the early diagnosis of PD.

Single photon emission computed tomography (SPECT) is an excellent technique to improve clinical PD diagnosis and monitor disease progression [2]. This is achieved using the dopamine transporter (DAT) specific radiotracer, 99mTc-TRODAT-1, developed by Kung et al., in 1997 [3], in which the function of presynaptic dopaminergic neurons can be evaluated safely, longitudinally and repeatedly by SPECT in PD patients [4]. Recent studies have revealed that 99mTc-TRODAT-1 SPECT imaging may serve as a diagnostic marker for PD and represent an adequate approach to evaluating the status of PD patients [5].

Alternatively, transcranial sonography (TCS) of the substantia nigra...
nigra is a new, promising, non-radioactive method to diagnose PD [6]. Accumulating evidence suggests that substantia nigral hyper-echogenicity is present in more than 90% of Caucasian PD patients, versus only 9% in healthy controls [7]. The underlying mechanism contributing to SN hyper-echogenicity may be caused by the deposition of iron in the midbrain and several studies demonstrated a correlation between SN hyper-echogenicity and tissue iron content in animals as well as in a postmortem study [8,9]. Therefore, the aim of this study is to examine the diagnostic utility of TCS in determining its correlation with \(^{99m}\text{Tc}\)-TRODAT-1 SPECT imaging.

2. Patients and methods

2.1. Participants

Fifty-five patients with a clinical diagnosis of PD, according to the United Kingdom Parkinson’s Disease Society Brain Bank criteria [11], were included in this study. All patients underwent thorough physical examinations by two different senior neurologists, and their neuropsychological assessments were performed using the Unified Parkinson’s Disease Rating Scale (UPDRS), Mini-mental state examination (MMSE), 17-item Hamilton Rating Scale for Depression (HAM-D-17), SCOPA-AUT, olfactory function and REM Sleep Behavior Disorder scales. In addition, UPDRS motor scores were evaluated in the ‘on’ stage. Patients with gaze palsy, pyramidal signs, cerebellar deficits, and history of cerebrovascular disease, psychiatric disorder, any possible cause of secondary Parkinsonism, atypical Parkinsonism, neuroleptic intake history, tricyclic antidepressant drug history, or serotonin uptake inhibitor history were excluded. In addition, 12 healthy controls were recruited from the Shanghai Wuliqiao community. Cerebral magnetic resonance imaging (MRI) was normal for all participants involved. This study was approved by the Ethics Committee of Ruijin Hospital affiliated to Shanghai Jiao Tong University School of Medicine. Written informed consents were acquired from all participants in this study.

2.2. Experimental procedure

2.2.1. \(^{99m}\text{Tc}\)-TRODAT-1 SPECT

\(^{99m}\text{Tc}\)-TRODAT-1 SPECT was performed as described in our previous study [12]. Briefly, 2 h after intravenous injection of \(^{99m}\text{Tc}\)-TRODAT-1, prepared from a pre-formulated kit provided by Jiangsu Nuclear Medicine Institute (Wuxi city, China), \(^{99m}\text{Tc}\)-TRODAT-1 uptake was assessed by SPECT imaging. Images were acquired via a double-headed gamma camera (Siemens, Symbia T6), with a 140 ± 14 keV energy window. Acquisition time for the projection was 30s, with a 1.45 zoom and 3 mm slice thickness at the level of the basal ganglia. Regions of interest (ROIs) for \(^{99m}\text{Tc}\)-TRODAT-1 uptake were established by MRI (1.5T, Siemens, Germany). Of note, if the average \(^{99m}\text{Tc}\)-TRODAT-1 uptake was less than 1.21 for the right side or 1.31 for the left side, striatal uptake (a combination of putamen and caudate uptake) of the alternative side was considered pathological.

2.2.2. Transcranial sonography

TCS examination was performed using a color-coded phase array ultrasound system (MyLab90, ESAOTE, Italy) with a 2.5 MHz phased array transducer. The examination was performed as described in our previous study [13]. In order to obtain a standardized view, patients were instructed to lay supine and the transcranial ultrasound probe was placed on the temple, parallel to the orbito-mental line. Special attention was given to the mesencephalic brainstem, with normal brainstem anatomy visualized as a low echogenic butterfly-shape. Qualitatively, hyper-echogenicity was identified as an abnormally increased intensity of the ultrasound signal, and if identified, the image was frozen immediately and the surrounding area manually demarcated using the trackball, resulting in automatic calculation of the hyper-echogenic area. An area of echogenicity ≥19 mm² was considered normal, while an area of echogenicity ≥20 mm² was defined as hyper-echogenic. All TCS investigations were performed by one experienced sonographer, blinded to clinical symptoms and \(^{99m}\text{Tc}\)-TRODAT-1 SPECT data. 16 (29.09%) patients were excluded due to insufficient temporal bone window.

2.3. Statistical analyses

Statistical analysis was carried out using SPSS version 21. For normally distributed data, the Pearson’s correlation coefficient was chosen for correlation analysis, and the Student’s paired and unpaired t-test was chosen for comparison of paired or unpaired data, respectively. For data that is not normally distributed, the Spearman’s correlation coefficient was used for correlation analysis, and the Mann–Whitney U test was chosen for unpaired data.

3. Results

3.1. \(^{99m}\text{Tc}\)-TRODAT-1 SPECT scan of PD patients

Right and left striatal \(^{99m}\text{Tc}\)-TRODAT-1 uptake in the 12 healthy controls was 3.56 ± 1.17 and 3.31 ± 1.00, respectively, while those values among 55 PD patients were 0.98 ± 0.45 and 1.04 ± 0.51, respectively. The demographic data of the 55 PD patients is presented in Table 1. Among the 55 patients who underwent \(^{99m}\text{Tc}\)-TRODAT-1 DAT-SPECT imaging, we found that the contralateral putamen and striatal (contralateral to the clinically more affected side of the patient) \(^{99m}\text{Tc}\)-TRODAT-1 uptake (n = 55, 0.58 ± 0.38, 0.88 ± 0.47) was reduced significantly compared to the putamen and striatal (0.72 ± 0.31, 1.15 ± 0.45) ipsilateral to the clinically more affected side of the patient (p < 0.001, p < 0.001) (Fig. 1A and B). Furthermore, we found the contralateral putamen and striatal \(^{99m}\text{Tc}\)-TRODAT-1 uptake correlated negatively with UPDRS-III (r = −0.334, r = 0.013), disease duration (r = −0.393, p = 0.003) and H-Y stage (r = −0.330, p = 0.014) (Fig. 1C and D).

Non-motor symptoms, including depression and hyposmia, have been reported to result from dopamine deficiency in the non-motor portion of the striatum [14]. Consequently, we investigated

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Demographic and clinical characteristics of controls and PD patients.</th>
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<tbody>
<tr>
<td>Healthy controls</td>
<td>PD Patients</td>
</tr>
<tr>
<td>Number</td>
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</tr>
<tr>
<td>Age (years)</td>
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</tr>
<tr>
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<tr>
<td>Disease duration (years)</td>
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</tr>
<tr>
<td>UPDRS-III</td>
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</tr>
<tr>
<td>H-Y stage</td>
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</tr>
<tr>
<td>SS-16</td>
<td>N/A</td>
</tr>
<tr>
<td>HAMD-17</td>
<td>N/A</td>
</tr>
<tr>
<td>R-Striatal (^{99m}\text{Tc})-TRODAT-1 uptake</td>
<td>3.56 ± 1.17</td>
</tr>
<tr>
<td>L-Striatal (^{99m}\text{Tc})-TRODAT-1 uptake</td>
<td>3.31 ± 1.00</td>
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UPDRS: Unified Parkinson’s disease rating scale; H-Y: Hoehn and Yahr; SS-16: 16-item order identification test; HAMD-17: 17-item Hamilton rating scale for depression.

* Means significant age difference between PD patients and healthy controls, p = 0.004.
the correlation between $^{99m}$Tc-TRODAT-1 uptake and non-motor symptoms of PD. However, we found that neither the putamen nor striatal $^{99m}$Tc-TRODAT-1 uptake correlated with depression or olfaction (Fig. 1E and F).

3.2. Transcranial sonography measurement in PD patients

TCS was conducted on all 55 patients, of which 16 (29.09%) participants had an insufficient temporal bone window. Of the remaining 39 patients, 24 (61.54%) demonstrated SN hyper-echogenicity. The mean area of total SN echogenicity (contralateral and ipsilateral) among the recruited patients was $53.70 \pm 22.95 \text{ mm}^2$. TCS image from one PD patient is shown in Fig. 2A. No significant difference was found between the extent of contralateral SN echogenicity and ipsilateral SN echogenicity ($n = 24$, $p = 0.402$) (Fig. 2B). Moreover, contralateral SN echogenicity did not correlate with UPDRS-III ($r = 0.383$, $p = 0.065$), disease duration ($r = 0.371$, $p = 0.075$) or H–Y stage ($r = 0.259$, $p = 0.222$) (Fig. 2C). Furthermore, contralateral SN echogenicity did not correlate with depression score ($r = -0.092$, $p = 0.699$) nor olfactory test results ($r = 0.011$, $p = 0.960$) (Fig. 2C).

Further studies were conducted to determine the differences between patients that had pathological striatal $^{99m}$Tc-TRODAT-1 uptake with normal SN echogenicity or SN hyperechogenicity. No significant differences were found between the two groups in age, sex, age of disease onset, H–Y stage, disease duration, UPDRS-III score, olfactory test score or depression score (data not shown).
3.3. Correlation between $^{99m}$Tc-TRODAT-1 SPECT and transcranial sonography

Although SN echogenicity did not correlate with disease duration and H–Y stage, we compared TCS to SPECT imaging. We found that contralateral striatal $^{99m}$Tc-TRODAT-1 uptake and contralateral SN echogenicity had no significant correlation ($r = -0.046$, $p = 0.830$) (Fig. 3A), neither did ipsilateral SN echogenicity ($r = 0.106$, $p = 0.622$). Furthermore, both striatal $^{99m}$Tc-TRODAT-1 uptake and SN echogenicity were measured, with no significant correlation ($r = 0.119$, $p = 0.580$) identified.

Finally, we calculated the correlation between contralateral putamen and contralateral caudate $^{99m}$Tc-TRODAT-1 uptake with contralateral SN echogenicity, respectively. Again, no correlation was identified between contralateral putamen uptake and contralateral SN echogenicity ($r = -0.080$, $p = 0.712$) (Fig. 3B) or contralateral caudate uptake and contralateral SN echogenicity ($r = 0.087$, $p = 0.686$).

3.4. Accuracy and predictive value of SN-TCS in clinically diagnosed PD patients

Although we did not identify a correlation between DAT-SPECT and SN-TCS, we evaluated the accuracy and predictive value of SN-TCS. Of the 55 patients, 47 (85.45%) had reduced $^{99m}$Tc-TRODAT-1 uptake. Among those 47 patients, 22 had SN hyperechogenicity and 12 had normal echogenicity. Moreover, 3 of the 5 patients with normal $^{99m}$Tc-TRODAT-1 uptake had normal SN echogenicity. Therefore, using $^{99m}$Tc-TRODAT-1 SPECT as the gold standard, the sensitivity of SN hyperechogenicity for diagnosing PD was calculated as 64.70% (22/(22 + 12)), and the specificity was calculated as 60% (3/(3 + 2)).

Of the 24 patients with SN hyperechogenicity, 22 patients had reduced striatal $^{99m}$Tc-TRODAT-1 uptake detected by SPECT. Therefore, the PPV of a positive TCS, with $^{99m}$Tc-TRODAT-1 uptake as the gold standard, was calculated as 91.67% (22/(22 + 2)), while the NPV of a negative TCS was calculated as 20% (3/(3 + 12)).

4. Discussion

In this study, both contralateral putamen and striatal $^{99m}$Tc-TRODAT-1 uptake was found to be reduced more significantly than the ipsilateral putamen and striatal, which were inversely correlated with the motor component of UPDRS, disease duration, and H–Y stage. However, when TCS was compared to $^{99m}$Tc-TRODAT-1 SPECT imaging, minute differences were found between the extent of contralateral SN hyperechogenicity and ipsilateral echogenicity. Furthermore, no correlation was found between contralateral SN echogenicity and ipsilateral echogenicity. Moreover, no correlation was found between the extent of SN hyperechogenicity and striatal $^{99m}$Tc-TRODAT-1 uptake, which was consistent with Edson et al. [15]. In contrast, Bartova P et al. [16] found a negative correlation between SN echogenicity measured by TCS and striatal binding ratio by $^{123}$I-FP-
from dopamine deficiency in the non-motor portion of the striatum or gray matter loss within the limbic circuit [17]. And, other mechanisms may be involved in the complicated pathogenesis of depression in PD patients, or other non-motor symptoms. In our study, we just tried to implement an exploratory trial to investigate the correlation between DAT-SPECT, TCS and depression and hyposmia, but with negative results. We will initiate further investigations to identify these mechanisms.

In all 55 participants that received TCS, 16 patients were excluded due to an insufficient temporal bone window, a finding that is much higher than in a previous study [15]. Recently, several Asian studies [21,22] reported a higher temporal insufficiency rate, which serves as an important reminder of the differences between Asian and Caucasian temporal bone structure. In the remaining 39 patients, 61.54% were found to have hyperechogenicity, a figure which is consistent with our previous study [13], but very different from the Caucasian population. The reason that other than midbrain iron deposition, inflammation, ischemic processes or environmental pollution may be contributing to the difference observed among mainland China in the recent years may account for this problem.

With regard to gender, a recent study found that male subjects may have a larger echogenic area than females and that older individuals may also have a larger echogenic area [23]. Additional studies found that participants with SN hyperechogenicity were more vulnerable to olfactory dysfunction [24] and depression [25], as well as severe extrapyramidal symptoms [23]. To that end, in order to further analyze the differences between patients who had reduced $^{99}$mTc-TRODAT-1 uptake with hyperechogenicity and the ones with normal SN echogenic signal, we compared age, sex, H–Y stage, disease duration, UPDRS-III, olfactory test scores and depression scores between the subgroups. However, no positive results were discovered. Furthermore, one study based on a Taiwanese population revealed that compared to early onset PD patients, an enlarged SN echogenic area was commonly seen in late onset PD patients [26].

The present study used $^{99}$mTc-TRODAT-1 SPECT as the diagnostic standard instead of pathological examination for the clinical diagnosis of PD. 8 of 55 PD patients who received $^{99}$mTc-TRODAT-1 SPECT were shown to have normal $^{99}$mTc-TRODAT-1 uptake (i.e. “Scans without evidence of dopaminergic deficit” (SWEDDs)). Explanations for SWEDDs could be Parkinson’s disease, late-onset dystonia, Secondary Parkinsonism, Essential tremor, or Psychogenic conditions. Actually, about 15% early PD were shown normal brain scans, due to the theoretical possibility in the early phase of PD or “false negative” originated from quantitative analysis of SPECT scans [27]. In addition, some of SWEDDs may be misdiagnosed, but others continue to fulfill PD diagnostic criteria. Moreover, Silveira-Moriyama has demonstrated that SWEDDs with lower olfaction score were high probability of PD [28]. In our study, olfaction score (SS-16) of the 8 SWEDDs was 6.625 ± 1.30, which is meaning high probability of PD. Simultaneously, SWEDDs do not respond to L-DOPA or dopaminergic agonist [29]. However, in our study the 8 SWEDDs well responded to dopaminergic treatment or agonist, which indicated that it’s reasonable to identify them as PD patients. Thus, we found that the sensitivity of TCS was 64.70% according to DAT-SPECT, a value that is different from the value reported from a Brazilian study [15]. However, among the 24 patients with SN hyperechogenicity, 22 patients demonstrated reduced striatal $^{99}$mTc-TRODAT-1 uptake. Therefore, the positive predictive value was high, calculated as 91.67%, a value consistent with previous study [30]. Consequently, the abnormal SN echogenicity may be used as an indicator of dopaminergic neuron dysfunction and aid in the diagnosis of early PD.

The present study is the first to assess the clinical utility of SN-
TCS and its correlation with 99mTc-TRODAT-1 SPECT imaging in a Chinese population. Nevertheless, several limitations are found in this study. Firstly, the rate of insufficient temporal bone window has yet to be explored. Secondly, because healthy participants are reluctant to receive unnecessary radiation, we recruited only 12 healthy controls. Thirdly, a cohort of 55 PD patients is not enough, and a larger cohort of patients should be utilized in further studies.

In conclusion, transcranial sonography of the substantia nigra is a non-invasive, non-radioactive, convenient and low cost procedure. Despite the fact that our results revealed no correlation between SN hyperechogenicity measured by TCS and presynaptic dopaminergic function detected by DAT-SPECT, indicating a possible independent mechanism between the two components, the high positive predictive value indicates that SN-TCS might be useful as a routine diagnostic test.

Acknowledgments

This work was supported by grants from the National Program of Basic Research (2010CB945200, 2011CB504104) of China, National Natural Science Fund (81071024, 81171202, 30870879, 8128007 and 81471287), Shanghai Shuguang Program (11SG20), the Fifth National Undergraduate Student Innovating Program (2011015), National Key Basic Research Program of China (No.2011CB504104) and Natural Science Fund of China (81430022; 81371407). We acknowledge all the patients and healthy subjects for their participation.

References