Author’s response to reviews

Title: Clinical features and dysfunctions of iron metabolism in Parkinson disease patients with hyper echogenicity in substantia nigra: a cross-sectional study

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Author’s response to reviews:

Dear editor in-chief of BMC Neurology:
Please find the enclosed manuscript entitled “Clinical features and dysfunctions of iron metabolism in Parkinson disease patients with hyper echogenicity in substantia nigra: a cross-sectional study”, by Shu-Yang YU et al., to be submitted as an original article to BMC Neurology for the consideration of publication. The following are the answers for the questions raised by the reviewers.

Reviewer reports:

(Reviewer 1): The article entitled "Clinical features and dysfunctions of iron metabolism in Parkinson disease patients with hyper echogenicity in substantia nigra: a cross-sectional study" by Yu et al examined the relationship between hyper-echogenicity of substantia nigra and motor/non-motor symptoms of patients with PD. This study is a cross-sectional study design. The authors also compared the levels of iron and related proteins in serum and cerebrospinal fluid (CSF) between hyper-echogenicity group and normal echogenicity group. The total enrolled patient number was 374 in the study. The authors observed that PD patients with hyper echogenicity in substantia nigra were older, at more advanced disease stage, had severer motor symptoms, cognitive impairment and autonomic dysfunction than patients with normal echogenicity. The protein levels of iron metabolism in CSF were also different between hyper-echogenicity group and normal echogenicity group.

The manuscript is clearly written. However, I have some concerns for this study listed as below.

1. Although the grading of hyper-echogenicity was based on the criteria proposed by Bartova P et al (Ultrasound Med 2010, 29(1):37-42.), I would suggest the authors to illustrate the echo photos of each grade to make readers clearer.

   Thanks for your suggestion and we have added and illustrated echo photos of each grade of hyper-echogenicity in SN in the revised version. (Please see Figure 1)

2. Is there any correlation between iron levels of CSF or serum and motor symptoms severity?

   There are no significant relationships between iron level in CSF or serum and motor (H-Y stage) and non-motor symptoms, including cognitive impairment (total score of MoCa and score of memory in MoCa), and autonomic dysfunction (total score of SCOPA-AUT, and scores of...
urinary symptoms and pupil adjustment symptom in SCOPA-AUT) (P>0.05). (Please see page 9, line 213-217)

3. Is there any correlation of the levels of iron metabolism proteins between CSF and serum?

Yes. The levels of iron and related proteins in CSF were significantly correlated with that in serum: r = 0.182 and P = 0.030 for iron, r = 0.538 and P = 0.001 for ferritin, r = 0.292 and P = 0.001 for light chain ferritin, and r = 0.444 and P = 0.001 for transferrin. (Please see page 8, line 207-210)

4. The major limitation is there were no age-matched controls in the study. I would suggest the author to list this point as one of the study limitations in the discussion.

Yes. It is very difficult to obtain the CSF sample from the elderly PD patients, let alone the age-matched healthy control. Whatever reasons we have, lack of age-matched healthy control group is a limitation of this study anyway. The results from this investigation limit to explain the potential relationship between SN echogenicity and iron metabolism within PD population. Age-matched healthy control is needed in order to compare the difference in terms of iron metabolism between these two groups.

We have listed this point as one of the study limitations in the part of discussion (Please see page 12, line284-288): “This study lacked of age-matched healthy control group, because it was very difficult to obtain the CSF sample from the age-matched healthy control. This is a limitation of this study and the results from this investigation limit to explain the potential relationship between SN echogenicity and iron metabolism within PD population. Age-matched healthy control group is needed in the future study.”

(Reviewer 2): For the whole manuscript: English-editing is required.

Introduction:

1. In the first paragraph, a citation is required for this statement: Berg et al found that in the premotor period, treatment before the significant neural degeneration might protect neurons against rapid deterioration, thereby delaying the disease progression.

Thank you so much for your valuable suggestion and we have added this citation in the revised version of manuscript: “Berg et al found that in the premotor period, treatment before the
significant neural degeneration might protect neurons against rapid deterioration, thereby delaying the disease progression[2]." (Please see page 3, line 86-88)

2. In the second paragraph, the following statement "Transcranial ultrasound (TCS), a novel and non-invasive neuroimaging technique" should be mended. TCS is not novel at all.

Thanks and we have removed “novel” from this sentence in the revised version of manuscript: “Transcranial ultrasound (TCS), a non-invasive neuroimaging technique, is a useful tool for providing the evidences for the early diagnosis and differential diagnosis of PD.” (Please see page 4, line 93-94)

Methods:

Is this study a retrospective study? Had patients signed the informed consent or the informed consent were waived? Had the study approved by local review board?

This study is a cross-sectional study. This study was approved by Beijing Tiantan Hospital review board. Written informed consents were obtained from all participants in this study. All methods were performed in accordance with the relevant guidelines and regulations. (Please see page 4, line 111-113)

1. In the "Detection of echogenicity and hyperechoic area of SN by TCS" section, the authors make the following statement: One experienced ultrasound practitioner who had no idea about clinical diagnosis was responsible for detecting SN echogenicity. Since the present study did not include control, the practitioner must know all subjects were PD patients. The statement should be mended.

We have mended this statement as “One experienced ultrasound practitioner who was responsible for detecting SN echogenicity” according to your suggestion. (Please see page 5, line 127-128)

2. The authors mentioned that "In some patients, there was only one temporal window available for detection of echogenicity of SN". For those patients, whether the assessed side contralateral to prominent involved side of limbs is important.

Routinely, ultrasound practitioner detects echogenicity of two side SN from one temporal bone window. Due to the differences in the thickness and permeability of the two side temporal bone windows, the results of SN echogenicity from each side temporal bone window may be slightly different. Thus, generally, the higher grade of SN echogenicity is reported by ultrasound
practitioner and this result is used for the research and data analyses. In the case of one window being available, only the result from one temporal bone window is available for clinical research.

3. How many percentage of patients were excluded due to poor bilateral temporal windows should be mentioned.

According to your suggestion, we have mentioned that: “Total 12 patients (9.0%) with both sides of temporal window unavailable were excluded and 122 cases were recruited in the investigation”. (Please see page 5, line 137-138)

Results

1. The authors need to address the reasons of alteration of study case number (in methods: 374 PD patients were recruited, in table 1, 122 subjects were analyzed, in table 2, total 119 patients were analyzed)

374 PD patients had data about SN echogenicity, among them,122 cases finished all rating scales for clinical symptoms of PD, and 119 cases had CSF and blood samples for the detection. We have revised this part: “Total 374 PD patients were consecutively recruited from the Department of Geriatrics and Neurology, Beijing Tiantan Hospital, Capital Medical University. Demographics variables, including gender, age, age of onset and disease duration of were recorded. Among them,122 cases finished all rating scales for clinical symptoms of PD, and 119 cases had CSF and blood samples for the detection of iron and related proteins. In 122 PD patients, 79 cases (64.8%) were male and 43 cases (35.2%) were female; patients’ age were 34~84 years with an average of 60.0±10.5 years; disease durations were from 6 months to 33 years with an average of 2.0 (1.0~5.0) years”. (Please see page 5, line 116-123)

2. While comparing the NMS symptoms between two groups, the authors should adjust several factors, especially age and disease duration. Or otherwise, the results were very biased.

Disease duration was not significantly different between the PDSN+ and PDSN- group (Please see Table 1). In order to exclude the influence of age to SN echogenicity, multiple linear regression analysis was made between SN echogenicity and related factors, including age, age of onset, scores of memory, urinary system symptoms and pupil adjusting symptoms (Table 4). Results revealed that memory and urinary system symptoms were associated with SN echogenicity independently. (Please see page 8, line 197-201). However, age was not associated with SN echogenicity.
3. For the table, it would be nice to make all the presentation universal (some parameter presented as mean+SD but some were median and quartile, which introduced confusion)

In the part of “Data Analyses”, we made an explanation for that presentation: “Continuous variables were presented as mean ± standard deviations and compared by 2-tailed T test if they were normally distributed, and presented as median (quartile) and compared by nonparametric test if they were not normally distributed.” (Please see page 7, line 169-172)

4. In table 1, the results of H/Y stage and UPDRS were exactly the same? It is the case or typo?

It is a typo and we have mended the typo in the revised manuscript. (Please see Table 1).

Discussion

1. The present study identified the association between age, gender, disease duration with PDSN+, however, in the discussion, some of the statements indicated a causal relationship which was not solid.

We have revised this part in the new version of manuscript as follow. (Please see page 9, line 221-235.

Although the reason for the gender difference was rarely explored, estrogens were found to be involved [11, 12]. Estrogens make female individuals less prone to iron accumulation due to the biologically reduced iron levels[16]. In PD, SN hyper echogenicity was associated with elevated unbound iron [13-15], which may explain the reason why females had lower SN echogenicity.

Brain iron accumulates increasingly during aging process, which explained older PD patients with later disease onset had higher SN echogenicity in the current study.

SN hyperechogenicity was not related to disease duration in this study. Another study also failed to observe a significant change in hyperechoic area in SN even with an interval of 8 years between examinations[17].

In this study, SN hyperechogenicity was associated with H-Y stage, which demonstrated that SN hyperechogenicity was associated with the progression of PD. With increasing iron deposits in SN, more and more neurons degenerate in SN through multiple mechanisms, such as oxidative stress, neuroinflammation etc, which establishes the close correlation between SN hyperechogenicity and H-Y stage.
2. The association between NMS with PNSD+ need to assessed by adjusting other factors.

Multiple linear regression analysis was made between SN echogenicity and related factors, including age, age of onset, scores of memory, urinary symptoms and pupil adjusting symptoms (Table 4). Results revealed that memory and urinary symptoms were associated with SN echogenicity independently. We have revised this part in the new version of manuscript. (Please see page 8, line 197-202)

Sincerely yours,

Wei ZHANG, on behalf of the authors