Author’s response to reviews

Title: Serum klotho: a potential predictor of cerebrovascular disease in hemodialysis patients

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Author’s response to reviews:

Dear Editors:

We would like to submit the revised manuscript “BNEP-D-17-00713”, which we wish to be considered for publication in “BMC Nephrology”.

Thanks very much for the comments from the reviewers. Our point-by-point responses to the comments are below.

Reviewer 1:

Major comments:

1. Methods: The authors describe as one of the inclusion criterion the presence of head CT or MRI within 1 year of enrollment. Is it possible that most of these patients have prevalent cerebrovascular disease? Please clarify if the outcome of interest is recurrent events, de novo events or both.
This is a prospective study. So we need to exclude the history of ischemic stroke or hemorrhagic stroke with head CT or MRI before the study. In fact, we often do head CT or MRI screening for hemodialysis patients with dizziness or headache, but some patients may have no evidence of clinical or subclinical stroke at the time. Based on it, the outcome of interest is de novo events.


Cerebrovascular diseases, including clinical stroke and silent brain infarction (SBI), were diagnosed by two neurology specialists and two imaging specialists. Clinical stroke were diagnosed through head CT or MRI scans and clinical symptoms which contain sudden onset of unilateral numbness, unilateral weakness, loss of ability to communicate, loss of ability to understand, loss of vision, and loss of half the visual field. SBI was diagnosed through brain MRI.

3. Methods: Please report the time relationship between the Klotho measurement and the cerebrovascular disease event (mean/median time between measurement and event).

It is 16.86±8.29(months). It was added into our revised paper. Thank you very much for your advice.

4. Methods: Please provide reference(s) regarding the Klotho assay used and the intra-assay or inter-assay CVs if available. Have the authors utilized this assay before? This is important as there is no established assay for serum Klotho in humans.

We haven’t utilized this assay before, but there are some articles (as below) used the same assay to measure serum klotho in humans. We refer to these articles.


5. Methods: Was the variable "diabetes" utilized in the analysis as a categorical, ordinal or continuous variable using A1C or serum glucose? Please clarify as Spearman correlation performs better for continuous/ordinal variables.

The previous study found that serum klotho level was significantly decreased in T2DM patients compared to controls. We want to study the relationship between serum klotho and DM history, so we choose “diabetes history” as a variable.

The data does not conform to the bivariate normal distribution, so we choose Spearman not Pearson.

6. Methods: Data pertaining to hypertension, prior stroke, diabetes, and tobacco smoking are relevant but not included in Table 1 or tested in the multivariable models. In particular, "diabetes" was not included in the multivariable models despite it was found to be significantly correlated with Klotho levels. Please clarify.

Thank you very much for your advice. The participants all have no prior stroke. We have added hypertension, diabetes, and tobacco smoking in Table 1. In the revised article, Cox regression analysis was used to assess the association between baseline variables and cerebrovascular disease in Table 2. We found the p values of diabetes, hypertension and tobacco smoking were bigger than 0.05 in univariable regression, so they were not included in the multivariable models. Because "diabetes" was found to be significantly correlated with Klotho levels, so in table 6, it was included in multivariable models.

7. Methods: Please report Model 3 for serum Klotho and cognitive impairment in the subgroup of patients with cerebral infarction.

Thank you very much for your advice. In the revised article, we added it in table 6.

8. Methods: Was dividing serum Klotho levels in tertiles explored (rather than dichotomizing levels by the mean)? Any specific reason for dichotomizing Klotho levels in the multivariable models?
In our pre-article, we used dichotomizing Klotho levels for grouping. It’s not rigorous. Thank you very much for your suggestion. So, in the revised paper, we firstly certified serum klotho was the independent risk factor of cerebrovascular disease; and then, we used ROC curves to study the value of serum klotho to predict cerebrovascular disease, and we also gave out the optimal cut-off value.

9. Results: Please provide some characteristics of the ESRD/HD such as etiology of ESRD and HD vintage (months or years). I suggest adding these parameters to Table 1 if data are available.

Thank you very much for your advice. Some patients don’t know the etiology of ESRD, but we can add some HD vintage (months) in table 1.

Minor comments:

10. Stats paragraph: Add median (IQR) to continuous data reporting.

Variables with non-normal distribution were expressed as medians and interquartile ranges (25th and 75th percentiles) or percentages for categorical variables. Variables with normal distribution were expressed as mean±SD.

11. Discussion: Please summarize second paragraph of the Discussion which is a little extensive. 请总结讨论的第二段，有点广泛。

Thank you very much for your advice. We have re-edited this paragraph.

12. References: I suggest the authors to add these 2 references to the manuscript (PMID:21115613 for Klotho pathobiology in vascular calcification; PMID:28115282 for Klotho candidacy as a biomarker in renal disease; and PMID 19419323 for Klotho variants and mortality in ESRD pts).

Thank you very much for your advice. These two articles were very important study. We had added these 2 references to the manuscript.

charat thongprayoon (Reviewer 2):

1. Is this a prospective study? What is the study period? Did you screen all hemodialysis patients at the study hospital? What is the time frame between serum klotho measurement and CT or MRI test? Was it before or after klotho measurement? Were MRI or CT done for the study purpose or other purposes?

Yes, this is a prospective study. The study period is 24 months. Yes, we screened all hemodialysis patients at the study hospital. CT or MRI test was after klotho measurement. If the participants have stroke symptoms, head CT or MRI were scanned immediately. The others without stroke symptoms during the following time, head MRI were carried out in the last month of the study.

2. "A total of 185 patients were screened, and 88 patients consented to participate in our study and completed all questionnaires, laboratory tests and had head CT or MRI scan records" What was the reason of excluding 97 patients?

The hemodialysis patients with stroke history (n=20) or without head CT or MRI in the past one year to certify who had no stroke or silent brain infarction were excluded (n= 15). Patients were also excluded if they had an active psychiatric disorder (n=2), Alzheimer's disease (n=18), or if they had significant visual or hearing impairment (n=24). 18 patients refused to participate in the study.

3. What was the definition of cerebrovascular disease? Did it include any ischemic, hemorrhagic or intracranial bleeding? How was it ascertained and by who? I think that the investigators excluded patients without CT or MRI. Can these patients without CT or MRI be considered not having cerebrovascular disease?

Cerebrovascular diseases, including clinical stroke and silent brain infarction (SBI), were diagnosed by two neurology specialists and two imaging specialists. Clinical stroke were diagnosed through head CT or MRI scans and clinical symptoms which contain sudden onset of unilateral numbness, unilateral weakness, loss of ability to communicate, loss of ability to understand, loss of vision, and loss of half the visual field. SBI was diagnosed through MRI.

All the participants had CT or MRI scans before and during the study to guarantee that the outcomes can certify the relationship between serum klotho and de novo cerebrovascular disease.
Some patients without CT or MRI may have cerebrovascular disease, but it not affects our study. After all, we can't force all patients to participate in our research. Of course, only 88 samples are our limitations.

4. What regression model did you perform the assess the association between klotho and cerebrovascular disease or klotho and cognitive function in patients with cerebrovascular disease?

Cox regression analysis was used to assess the association between klotho and cerebrovascular disease in Table 2. In table 6, Logistic regression analyses the association between klotho and cognitive impairment of hemodialysis patients with cerebrovascular disease or cerebral infarction.

5. Table 1, did you have information regarding cause of ESRD, comorbidities, smoking, and medication use?

Thank you very much for your advice. Some patients don’t know the etiology of ESRD, but we can add some HD vintage (months), Hypertension and Tobacco smoking history in table 1. The information of comorbidities such as anemia and calcium and phosphorus metabolism disorder (Hb, Ca, P and PTH) was also in Table 1.

5. What was the rationale of using cut-off 119 pg/ml

In our pre-article, we used dichotomizing Klotho levels for grouping. It’s not rigorous. Thank you very much for your suggestion. So, in the revised paper, we firstly certified serum klotho was the independent risk factor of cerebrovascular disease; and then, we used ROC curves to study the value of serum klotho to predict cerebrovascular disease, and we also gave out the optimal cut-off value.

6. In table 2, Table 2 OR for Kltho < 119 and ≥119 were reciprocal value. I think it is less confusing to report only one value.

Thank you very much for your advice. In our pre-article, we used dichotomizing Klotho levels for grouping. It’s not rigorous. Thank you very much for your suggestion. So, in the revised paper, we firstly certified serum klotho was the independent risk factor of cerebrovascular
disease; and then, we used ROC curves to study the value of serum klotho to predict cerebrovascular disease, and we also gave out the optimal cut-off value.

8. In Table 6, what is the definition of cognitive impairment

Cognitive impairment was defined as a score at least 1.5 SD below normative values for age in 2 or more cognitive domains. We refer to the study as below.


9. What is the limitation of this study. 这项研究的局限性是什么？

Only 88 samples are our limitation. Large samples of prospective studies are needed to certify our results. Further studies are needed to explore the reason for the serum klotho decline in hemodialysis patients. Prospective studies will be needed to determine whether treatment of klotho deficiency may be a promising strategy to decrease the burden of comorbidity in hemodialysis patients.

I hope this paper is suitable for “BMC Nephrology”.

We deeply appreciate your consideration of our manuscript, and we look forward to receiving your decision. If you have any queries, please don’t hesitate to contact me at the address below.

Thank you and best regards.

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