Author's response to reviews

Title: Evaluation of subcortical grey matter abnormalities in patients with MRI-negative cortical epilepsy determined through structural and tensor magnetic resonance imaging

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Author's response to reviews: see over
Dear Dr. Aidan Neligan and Reviewers:

We have great appreciation for you and the reviewers for the careful critiques of the manuscript (MS: 1443335159117423 in old titled Clinical correlation of subcortical gray matter alterations of MRI-negative neocortical epilepsy).

We majorly revise the manuscript to address each of the reviewers’ suggestions. We have itemized and indicated how we addressed every comment. Based on these revisions, we think the manuscript is significantly strengthened and would be of great interest to readers of journal of Biomedical Research International.

All of the authors have read and contributed scientifically to this manuscript. All authors have read and approved submission of the revised manuscript and the manuscript has not been published and is not being considered for publication elsewhere in whole or part in any language except as an abstract.

Sincerely,

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The Editor's comment:

Overall we feel that your paper is of interest and should be considered for publication assuming you can adequately respond to the reviewers' comments below (within a period of 3 months). In particular the methodology requires further statistical clarification and the input from a statistician is advised. Similarly the paper requires significant grammatical revision.

Our response:
Thank you very much for the comment. After we review our methodology and statistical clarification carefully, we decide to report our findings step by step. Initially we found the grey matter density changes in subcortical regions by voxel-based morphometry. Then we further segmented subcortical structures, calculated the normalized volumes of these subcortical nuclei, and then compared with the normal controls. Thereafter, we correlated two clinical variables with volume and diffusion of these structures that had significant differences. In the revised manuscript, we remove the confused section of “individual and group analyses of vertex-based morphometry”. Finally, the English writing was corrected by American Journal Experts.
Point-by-point responses

Referee 1: the concerns of review 1#

Concern 1
The title of the article is perhaps misleading as the bulk of the results and discussion relates to the absolute changes in the grey matter structures relative to healthy controls. Only minimal attention is given to the correlation with the two clinical variables assessed (age of onset, duration) which is presented in a supplementary table. The title and background should be revised accordingly.

Our response:
The article title was modified to “Evaluation of subcortical grey matter abnormalities in patients with MRI-negative cortical epilepsy determined through structural and tensor magnetic resonance imaging”.

Concern 2
The background and methods are generally clear. However I have some concerns regarding the statistical analysis:

a) The two clinical variables explored (age of onset and disease duration) are not independent, so was any correction performed for this in the individual analyses?

b) The Student’s t-test for volumes performed was one-sided which assumes a reduction in the volume of these structures whereas the possibility that there could be an increase should not be discounted when such a population has not been studied before especially such a heterogeneous population. I note that a two-sided test was used for the diffusion parameters.

c) The clinical correlations assessed are 7 structures x 2 sides x 3 imaging parameters x 2 clinical parameters = 84 comparisons in total. There does not appear to be correction for the multiple comparisons performed so it is hard to interpret these results. Without appropriate correction it may be better to remove this and concentrate on the absolute differences.

Our responses:
2(a)
With refer to many articles, the age at seizure onset and duration of epilepsy is correlated with the changes of brain structures independently. These articles are cited in the manuscript.
2(b) Thank you for the correction and comment. The section has been modified to “patients with normalized subcortical volumes were compared with controls by using a two-sample two-tailed \( t \)-test. A significant difference was accepted if the \( p \) value was less than 0.05”.

2(c) After we review our data, we decide to report our findings step by step. Initially we found the density changes in subcortical regions by voxel-based morphometry. Then we further calculated the volumes of subcortical nuclei and compared with the normal controls. Thereafter, we correlated two clinical variables with the structures that had significant differences. In the revised manuscript, we remove the confused section of “individual and group analyses of vertex-based morphometry”.

Concern 3
The discussion is sound although in the second paragraph states that the reduction in subcortical grey matter is universal among patients with frontal, lateral temporal, parietal or occipital lobe seizures. The data do not prove this and instead show that as a whole, a group involving patients from these groups shows a reduction in volume of these structures. It may be that some subgroups do not show changes which could only be answered by considering the subgroups separately with much larger numbers.

Our response:
Thank you for the correction and comment. Our patient numbers were not enough for further classification. In the revised manuscript, our patients were the only one group for comparison. The same characteristics of our patients were neocortical epilepsy and normal brain MRI. Our patients shared a seizure semiology indicating secondary generalization and postictal confusion.

Concern 4
I am not clear what the "antecedent and progressive factors" referred to in the Abstract, Conclusions are and need clarification of similar references in the conclusion stating "Chronic neocortical epilepsy is antecedent to TLE and IGEs..."
**Our response:**

In our revised manuscript, to accord our findings we rewrote the conclusion: “Subcortical GM involvement in the pathogenesis of chronic neocortical epilepsy is supported by our DTI-derived and T1-weighted MRI-derived evidence. However, a longitudinal study is needed to determine whether neurodegeneration observed in subcortical regions in neocortical epilepsy patients is accelerated beyond the effects of normal aging. Comorbid interictal and postictal psychomotor symptoms also require further investigation in light of coexisting subcortical structural changes”.

Concern 5
Abstract, Background - this states the aim and does not frame the background for performing this study

**Our response:**
Thank you very much for the comment. We have rewritten the abstract and background.

Concern 6
Abstract, Methods - refers to ADC here and throughout the paper but given this measure derived from DTI would it not be better termed MD (mean diffusivity)? This is what FDT in FSL generates

**Our response:**
Thank you very much for the comment. The section has been modified.

Concern 7
Abstract, Results - amgydale is a typo for amygdala here and elsewhere in Paper

**Our response:**
Thank you very much for the comment. The section has been modified.

Concern 8
Methods, Subjects - state years as units for age of patients and give gender breakdown for patients (only given for controls)

**Our response:**
Thank you very much for the comment. The section has been modified.

Concern 9
Methods, MRI acquisition - the FOV is 22x22cm, not 22cm^2. Please clarify the voxel size and slice thickness for DTI as the voxels appear very anisotropic

Our response:
Thank you very much for the comment. The section has been modified.

Concern 10
Results, second sentence - what does "seizures in the forehead" mean - frontal lobe seizures?

Our response:
Thank you very much for the comment. The section has been modified. It means frontal lobe seizures.

Concern 11
Results, changes in subcortical structures - give statistics, p-values for non-significant difference in brain volume; "interested regions" should be "regions of interest"

Our response:
Thank you very much for the comment. The section has been modified.

Concern 12
Discussion, 4th paragraph - what is an "altered linearity" in the subcortical structures - is this referring to altered diffusion parameters?

Our response:
Thank you very much for the comment. The section has been modified.

Concern 13
Discussion - “Tendency of the left hemisphere to predominant observed in our study" does not make sense

Our response:
Thank you very much for the comment. The section has been removed.
Concern 14
Table 1 - multiple patients are listed with an "undetermined" seizure focus - therefore on what basis is the diagnosis of a focal rather than generalised seizure made?

Our response:
We termed “undetermined” focus if the leading EEG activity arose from bilateral frontal regions simultaneously or diffuse epileptiform discharging with asymmetric body posturing at seizure onset.

Concern 15
Table 2 - the results for FA and ADC (MD) need to be given to 3dp since changes are small e.g. from 0.21 to 0.20

Our response:
Thank you very much for the comment. The section has been modified.
Referee 2: the concerns of review 1#

Concern 1
The introduction is poorly structured and does not give a succinct rationale for this study. In particular the authors do not provide any explanation why the 7 sub-cortical areas chosen for analysis were selected. Similarly the clinical correlation with the selected areas is not explained.

Our response:
Thank you very much for the comment. After we review our methodology and statistical clarification carefully, we decide to report our findings step by step. Initially we found the grey matter density changes in subcortical regions by voxel-based morphometry. Then we further segmented subcortical structures, calculated the normalized volumes of these subcortical nuclei, and then compared with the normal controls. Therefore, the manuscript is majorly revised including the introduction section. The algorithm FIRST could be used to separately segment seven subcortical regions: hippocampus, caudate nucleus, putamem, pallidum, nucleus accumbens, thalamus, and amygdale. In general, the clinical variables of epilepsy research were the age of onset and disease duration.

Concern 2
The statistical analysis needs a separate section and the methodology needs to be clearly outlined.

Our response:
Thank you very much for the comment. The results section has been modified.

Concern 3
The results section is poorly presented and needs to be more coherently summarised. Similarly the statistical p-values should be included.

Our response:
Thank you very much for the comment. The results section has been modified.

Concern 4
My main concern is with the discussion as the clinical implications or indeed the pathogenesis of the identified abnormalities is not clearly. Similarly the authors compare previous imaging findings in different epileptic syndromes
simultaneously and I think these would be better discussed in sequence.

**Our response:**
In the revised manuscript, we described our patient group clearly. Our patients were the only one group for comparison. The same characteristics of our patients were neocortical epilepsy and normal brain MRI. Our patients shared a seizure semiology indicating secondary generalization and postictal confusion.
In the discussion section, we cited the human and animal studies to support our findings.

Concern 5
The grammatical structure of the paper is very poor and needs considerable revision.

**Our response:**
Thank you very much for the comment. The English writing was corrected by American Journal Experts.