Author's response to reviews

Title: Saliva levels of Abeta1-42 as potential biomarker of Alzheimer's disease: a pilot study

Authors:

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Version: 3 Date: 2 November 2010

Author's response to reviews: see over
Dear Editor,

In reference to the manuscript *Saliva levels of Abeta1-42 as potential biomarker of Alzheimer's disease: a pilot study*, we have taken into account all the editor and reviewer comments and suggestions, and have produced a new version of the manuscript. We hope these modifications make the manuscript easier to follow and more suitable for publication in this journal.

We hope you consider our manuscript suitable for final publication in this journal.

Sincerely,

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Reviewer's report
Title: Saliva levels of Abeta1-42 as potential biomarker of Alzheimer's disease: a pilot study

Version: 1 Date: 30 September 2010
Reviewer: Anil K. Nair

Reviewer's report:
Authors: Felix F Bermejo-Pareja, Desiree D Antequera, Teo T Vargas, Jose Antonio JA Molina and Eva E Carro
Title: Saliva levels of Abeta1-42 as potential biomarker of Alzheimer's disease: a pilot study
Journal: BMC Neurology
Reviewer: Dr Anil K. Nair, Chief of Neurology, Quincy Medical Center / Boston University (nair.anil@gmail.com or an@bu.edu)

Summary:
The authors compared salivary Abeta (ELISA) in 76 AD, 51 PD subjects to 56 elderly controls. They did not find a significant difference due to possibly inadequate sample size. Only the mild AD subset showed significant difference in Ab42 levels from controls.

Minor Essential Revisions
1. Results, Page 8
"Overall, saliva Aβ42 levels revealed a non significant tendency to increase with age," suggest changing to "Overall saliva Aβ42 levels were not significantly higher with age".

As suggested by the reviewer, the sentence has been changed and incorporated in the revised version of the manuscript (Results section, page 9).

2. I cannot understand the logical reason for using only 2 subgroups 60-65 vs 66-70. What is special about these age ranges that the authors decided to analyze the data. I would suggest deleting that statement: "However, comparison of AD patients aged 60-65 with those aged 66-70 showed highly significant elevation in saliva Aβ42 levels (1.64 ± 0.44 pg/ml versus 6.46 ± 3.43 pg/ml, p=0.016)". (Page 8 and 9)

Due to we had seen a tendency to increase with age, we decided to analyzed saliva Aβ42 levels for age ranges, accordingly with other study about plasma Aβ42 levels (Ertekin-Taner et al, Neurology, 2008;70:596-606). In the present study we included 60-65, 66-70, 71-75, 76-80, 81-85 age ranges, and Aβ42 levels were compared between all groups and the increase is only statistically significant between 60-65 and 66-70 age range. However, and as the reviewer suggest, the sentence: "However, comparison of AD patients aged 60-65 with those aged 66-70 showed highly significant elevation in saliva Aβ42 levels (1.64 ± 0.44 pg/ml versus 6.46 ± 3.43 pg/ml, p=0.016)" may be delete.

3. Table 3: Please add numbers on mean ab42, ab40 and number for entire AD group (in order to show not significantly different and make the table comparable to PD and control subjects.)

As indicated by the reviewer, numbers on mean ab42, ab40 and number for entire AD
group have been incorporated in the revised version of the manuscript (Table 3).

4. This will also change the conclusion to reflect the summary. I would suggest: "There was no significant differences in Ab42 levels between AD, PD and controls overall. However, our findings show that saliva concentration of A#42 differs between MILD AD and nondemented control subjects, and that this is a specific characteristic of AD, being absent in PD."

As suggested by the reviewer, the sentence: "There was no significant differences in Ab42 levels between AD, PD and controls overall. However, our findings show that saliva concentration of A#42 differs between MILD AD and nondemented control subjects, and that this is a specific characteristic of AD, being absent in PD" has been incorporated in the revised version of the manuscript (Conclusion section, page 15).

**Discretionary Revisions:**

1. Abstract: Please correct grammar in statement: "In addition, there were not differences in salivary concentration of A#42 between patients with PD and healthy controls."

As indicated by the reviewer, grammar in statement: "In addition, there were not differences in salivary concentration of A#42 between patients with PD and healthy controls" has been corrected in the revised version of the manuscript (Abstract section, page 2).

2. Page 4: Suggest deleting "potentially dangerous" for describing LP. The LP side effects are minimal, 5% headaches with new needles, also is much more widely accepted with nearly 70% of our patients opting to have one.

As suggested by the reviewer, "potentially dangerous" has been deleted in the revised version of the manuscript (Introduction section, page 4).

3. Eliminate the outliers (4 in mild AD and 1 in moderate AD subjects) and re-run the analysis.

As suggested by the reviewer, we may eliminate the outliers (4 in mild AD and 1 in moderate AD subjects) and re-run the analysis, and then data change. However, we consider that these outliers may represent a normal range of saliva Aβ42 levels, as take place in other biological samples, including CSF, where Aβ42 levels come from 25 to 880 pg/ml in AD patients, and from 25 to 155 pg/ml in control non-demented subjects (Mehta et al, Arch Neurol, 200;57:100-105).

4. Increase the sample size.

We absolutely agree with the reviewer. We are going to increase the sample size, but we are also planning to include other dementias and mild cognitive impairment. As a
consequence, this objective will involve future strategies, experimental techniques, collaborations and measurements from a new manuscript.

Section Editor comments:

This is an important study demonstrating that Ab42 is detectable in saliva. This has been attempted in the past by others but unsuccessfully. Thus the findings themselves are important. The findings are sound and justified. The methods are reasonable and appropriate. Concerns are minor
- Table 1 and 2 should be collapsed. Table 1 needs to be expanded to have more data about the groups (onset age, duration, education, MMSE, etc)

As indicated by the Section Editor, Table 1 has been expanded and partially collapse with Table 2. This change has been described in the revised version of the manuscript (Methods section, page 5). Additional information from Table 2 has been also incorporated in the revised version of the manuscript (Results section, page 10).

- Figure 1a and table 3 present the same data. Either they present as a figure or tabulated. The figure makes it difficult to see significant differences.

As indicated by the Section Editor, Figure 1a and table 3 present the same data, and the figure makes it difficult to see significant differences. We agree with it, then we have decided to delete in the revised version of the manuscript.

- Since they have MMSE data, it would be interesting to correlate to salivary Abeta.

We agree with Section Editor, and we did it, as we show in the Discussion section, page 12, “we showed no significant relationship between saliva Aβ42 levels and MMSE scores”.

Editorial comments:

Experimental research that is reported in the manuscript must have been performed with the approval of an appropriate ethics committee. Research carried out on humans must be in compliance with the Helsinki Declaration (http://www.wma.net/en/30publications/10policies/b3/index.html), and any experimental research on animals must follow internationally recognized guidelines. A statement to this effect must appear in the Methods section of the manuscript, including the name of the body which gave approval, with a reference number where appropriate.

As indicated by the Editorial, a sentence about Ethic Comitee approval has been incorporated in the revised version of the manuscript (Methods section, page 5).

Please include an Authors' contributions section before the Acknowledgements and Reference list.

As indicated by the Editorial, we have already included an Authors' contributions section before the Acknowledgements and Reference list, page 15.