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Relationships between brain-derived neurotrophic factor and immune function during dietary supplement treatment of elderly with Alzheimer's dementia

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1st Editorial decision

15-Jul-2019

Ref.: Ms. No. JCTRes-D-19-00008
Relationships between brain-derived neurotrophic factor and immune function during dietary supplement treatment of elderly with Alzheimer's dementia
Journal of Clinical and Translational Research

Dear Dr. Heger,

Reviewers have now commented on your paper. You will see that they are advising that you revise your manuscript. If you are prepared to undertake the work required, I would be pleased to reconsider my decision.

For your guidance, reviewers' comments are appended below.

If you decide to revise the work, please submit a list of changes or a rebuttal against each point which is being raised when you submit the revised manuscript.Also, please ensure that the track changes function is switched on when implementing the revisions. This enables the reviewers to rapidly verify all changes made.

Your revision is due by Aug 14, 2019.

To submit a revision, go to https://www.editorialmanager.com/jctres/ and log in as an Author. You will see a menu item call Submission Needing Revision. You will find your submission record there.

Yours sincerely

Michal Heger
Editor-in-Chief
Journal of Clinical and Translational Research

Reviewers' comments:

Reviewer #1: 1.The content of this manuscript lacks scientific rigor and integrity. The title is "Relationships between brain-derived neurotrophic factor and immune function during dietary supplement treatment of elderly with Alzheimer's dementia", while the authors did not give a precise explanation on how aloe polymannose multinutrient complex (APMC) treatment works on BDNF and biomarkers and the relativity. And this manuscript mentioned the Mini-Mental State Examination (MMSE) scale as enrollment clinical criteria. But after the treatment, BDNF and other blood immune biomarkers are not so related with clinical outcomes, like MMSE score or something. There is some blur and misunderstanding in connecting the title and the content of the article.
2.The relevant comparison of basic information between pre- and post-clinical should also be given.
3.Since the manuscript is concerning BDNF and immune biomarkers in AD dementia patients, healthy people as control groups should be included. If the content is about the drug functioning, the difference between patients with AD consumed 4 teaspoons/day of APMC and didn't consume APMC should be explained.
4.Table 1 is suggested to replace with three-line table. And meanwhile, the correlation analysis figure should be shown
5.In previous published articles, the different time points of baseline and 3, 6, 9 and 12 months follow-up were mentioned. While no descriptive values of the proBDNF and BDNF and cytokines, growth factors, T-cell and B-cell subsets, and complete blood count to reflect immune function at baseline and 3, 6, 9 and 12 months follow-up in this manuscript. Whether BDNF and biomarker levels of these time point are significant values at these time points？
6.The discussion part only gives the explanation on BDNF and immune system, the function of the drug during the procedure should be mentioned.

Reviewer #2: Interesting study evaluating the relationships among proBDNF and mature BDNF and immune functioning during aloe polymannose multinutrient complex (APMC) treatment in persons with
moderate to severe Alzheimer's dementia (AD). The authors are very aware of the limitations of the study and their conclusions are consistent with the objectives, mainly aiming to stablish relationships and associations, instead of correlations. However, the lack of collection and analysis of other variables, as described in the limitations section, reduces the power of the findings. Lack of control by disease severity, diet, exercise and comorbidities is a significant flow in the study. Some questions that would be interesting to be answered: Are patients in advanced stages of AD compliant and adherent with treatment? Are there variations related to level of physical activity? What is the general nutritional status of the patients? I'd suggest to include additional information to better characterize the study population.

Author's rebuttal

November 27, 2019
Michal Heger, Ph.D.
Editor-in-Chief

Journal of Clinical and Translational Research

Dear Dr. Heger:

We thank the Reviewers for their thorough evaluation of our manuscript. As per the requests of the Reviewers, we have endeavored to modify our paper to improve its quality and suitability for publication. We have addressed the following Reviewers' comments:

Reviewer #1
1. The content of this manuscript lacks scientific rigor and integrity. The title is "Relationships between brain-derived neurotrophic factor and immune function during dietary supplement treatment of elderly with Alzheimer's dementia", while the authors did not give a precise explanation on how aloe polymannose multinutrient complex (APMC) treatment works on BDNF and biomarkers and the relativity. And this manuscript mentioned the Mini-Mental State Examination (MMSE) scale as enrollment clinical criteria. But after the treatment, BDNF and other blood immune biomarkers are not so related with clinical outcomes, like MMSE score or something. There is some blur and misunderstanding in connecting the title and the content of the article.

We agree with the Reviewer that our article is considered a lower level of scientific rigor, e.g., a lack of control group and the investigation of cross-sectional relationships. However, while the level of scientific rigor might not be optimal or of the highest standard, the relationships between BDNF and the other biomarkers are noteworthy and generally uncommon in the literature, particularly in a sample of subjects with moderate to severe Alzheimer's. Compared to the overall body of science related to the links between BDNF and other biomarkers in response to dietary supplement intervention, our findings are unique and worthwhile to report. We respectfully and completely disagree with the Reviewer that the title of our article is

disconnected from the study and the article's findings. Our intention was to correlate BDNF with other biomarkers of immune functioning within the context of dietary supplementation, which is exactly what we did, and our article's title reflects that. The Reviewer says that we did not precisely explain how APMC treatment works on BDNF, but we encourage the Reviewer to understand that a very clear understanding of this mechanism **does not exist at this time**, which is exactly why we did this study and analysis. Our findings reveal trends for certain biomarkers at baseline and at follow-up, and then we explain what is relevant and what is not. We certainly did not claim that our study shows definitive proof of a clear understanding or relationship between APMC and BDNF and other biomarkers, which is why we called for future studies to be conducted to address this matter. The Reviewer mentions the MMSE and says that we did not correlate BDNF with it, but the MMSE was only intended to be part of our inclusion criteria. The MMSE was used to help document and determine impairment in our study sample and to control for their clinical status, not to use the MMSE as an outcome variable and its change before and after treatment. We encourage the Reviewer to read our previous paper, which addresses the relationships between BDNF and clinical measures, such as the MMSE and others, c.f., Martin, A., Stillman, J., Miguez, M.J., McDaniel, H.R., Konefal, J., Woolger, J.M., & Lewis, J. E. (2017). The effect of dietary supplementation on brain-derived neurotrophic factor and cognitive functioning in Alzheimer's dementia. *Journal of Clinical and Translational Research, 3(3),* 1-6.

2. The relevant comparison of basic information between pre- and post-clinical should also be given.

We do not know what data or variables the Reviewer has implied by "basic information." Please clarify. As mentioned, we have already examined pre- and post-clinical cognitive functioning in our prior BDNF publication and in our first article from this study, c.f., Lewis, J. E., McDaniel, H. R., Agronin, M., Loewenstein, D., Riveros, J., Mestre, R., Martinez, M., Colina, N., Abreu, D., Konefal, J., Woolger, J. M., & Ali, K. H. (2013). The effect of an aloe polymannose multinutrient complex on cognitive and immune functioning in Alzheimer's disease. *The Journal of Alzheimer's Disease, 33,* 393-406.doi: 10.3233/JAD-2012-121381. PMID: 22976077.

3.Since the manuscript is concerning BDNF and immune biomarkers in AD dementia patients, healthy people as control groups should be included. If the content is about the drug functioning, the difference between patients with AD consumed 4 teaspoons/day of APMC and didn't consume APMC should be explained.

While we appreciate the Reviewer's suggestion to include a control group to enhance the rigor of the study, this was not the purpose of this study, as we did not have the capacity or funding to run a randomized clinical trial. We suggested that future studies should include more rigorous designs with the appropriate levels of financial support. Also, we encourage the Reviewer to understand that all subjects in our study and in this article consumed 4 teaspoons/day of APMC, which is a dietary supplement, not a drug. We had no control or comparison group.

4. Table 1 is suggested to replace with three-line table. And meanwhile, the correlation analysis figure should be shown.

We have adjusted Table 1 to the three-line format. We do not believe it would be effective to display the correlations in a figure because we have too many different significant correlations depending on whether the correlation is at baseline or follow-up and whether it is for BDNF, proBDNF, or the BDNF/proBDNF ratio. We prefer listing the correlations in the text as they currently are.

5. In previous published articles, the different time points of baseline and 3, 6, 9 and 12 months follow-up were mentioned. While no descriptive values of the proBDNF and BDNF and cytokines, growth factors, T-cell and B-cell subsets, and complete blood count to reflect immune function at baseline and 3, 6, 9 and 12 months follow-up in this manuscript. Whether BDNF and biomarker levels of these time point are significant values at these time points?

We are not entirely clear what the Reviewer has suggested that we should do. However, all descriptive information for the study has been reported in our two previously published articles. For simplicity, we chose not to include the descriptive information for all of these variables, and we refer the reader to our two previous articles in the third sentence of the Results section. If the Editor prefers that we include this descriptive information in the current paper, then we can do that assuming we do not have any copyright conflicts. In addition, all of the changes in the clinical variables and biomarkers were assessed pre- and post-intervention in the first published article, so those analyses do not need to be repeated in the current paper.

6. The discussion part only gives the explanation on BDNF and immune system, the function of the drug during the procedure should be mentioned.

With all due respect to the Reviewer, we believe he is confusing the intention of the study. Drugs are very different from dietary supplements. A drug is typically one chemical that is evaluated and meant for one mechanism of action for one disease or symptom of disease. In contrast, APMC is a dietary supplement with multiple ingredients containing thousands of nutrients, phytonutrients, compounds, elements, co-factors, metabolites, among others, so we cannot possibly elucidate one function from this treatment. The purpose of our study was to see how BDNF and markers of immune functioning were related prior to and in response to dietary supplementation, which is the main focus of the Discussion section of the paper. Mechanistic properties of all of the ingredients of APMC on BDNF and immune system biomarkers is far beyond the scope of this paper.

Reviewer #2

Interesting study evaluating the relationships among proBDNF and mature BDNF and immune functioning during aloe polymannose multinutrient complex (APMC) treatment in persons with moderate to severe Alzheimer's dementia (AD). The authors are very aware of the limitations of the study and their conclusions are consistent with the objectives, mainly aiming to stablish relationships and associations, instead of correlations. However, the lack of collection and analysis of other variables, as described in the limitations section, reduces the power of the findings. Lack of control by disease severity, diet, exercise and comorbidities is a significant flow in the study. Some questions that would be interesting to be answered: Are patients in advanced stages of AD compliant and adherent with treatment? Are there

variations related to level of physical activity? What is the general nutritional status of the patients? I'd suggest to include additional information to better characterize the study population.

We appreciate the Reviewer's thoughtful assessment of our paper that highlights some of the limits inherent in conducting nutritional/dietary supplement clinical trials in a very difficult population with funding limitations. While the Reviewer mentions a certain lack of control in our study, we would like to point out that our second inclusion criterion was: "a clinical 4 diagnosis by the study psychiatrist of probable moderate-to-severe AD for at least one year." We also had other particular age, clinical, and medical criteria that were utilized to recruit subjects for the study, so we respectfully suggest that we actually had a quite homogenous sample for this study.

As far as trying to control or stratify for diet (nutritional status) and exercise, we view that as next to impossible to comprehensively account for in a population as difficult as people with moderate-to-severe AD, who were intimately dependent on continuous caregiving. In the Limitations sub-section of the Discussion section, we pointed out that our findings may have been influenced by variables unaccounted for, such as "overall diet, physical activity level, caregiver support, and polypharmacy." We have added a statement to suggest that future studies should include these variables to the greatest extent possible. It would have been interesting to assess dietary habits of the subjects, but for subjects who are dependent on overburdened caregivers, such a requirement of study participation may have caused problems with adherence to the protocol. The same issue would have applied for physical activity/fitness level, as the caregiver would have had to record all of that information. More ongoing data collection for the caregiver could have created additional stress and burden on this aspect of the subject dyad.

Regarding the Reviewer's question about adherence to the treatment/protocol, a formal compliance measure was not utilized for this analysis. However, we are certain that the caregiver/subject was compliant with the study for one primary reason: the desperation of the caregiver to find anything to help the patient. Nearly every caregiver in the study reported that this study was a welcome opportunity to help the patient because we were assessing patients with advanced disease severity, which normally renders those people ineligible for research studies. As all of these patients had previously taken (or were currently taking) one or more of the five FDA-approved drugs for dementia with no success, these caregivers were desperate for anything to offer an opportunity to help. Thus, they were more than happy to try APMC and follow the protocol, particularly given that it is an all-natural product without any expected side effects or adverse interactions. In addition, all of our participants were either coming to the daily daycare program at the center or living on the center's campus, so they had direct and frequent contact with the study staff, who were vigilant about telling the caregiver about the importance of compliance to the protocol. Nonetheless, we have included a statement in the Limitations sub-section of the Discussion to address the issue of compliance.

Please let us know if you have any additional questions or clarifications, and we look forward to the next review of our paper.

Best regards,
John E. Lewis, Ph.D.
Associate Professor

2nd Editorial decision

30-Nov-2019

Ref.: Ms. No. JCTRes-D-19-00008R1
Relationships between brain-derived neurotrophic factor and immune function during dietary supplement treatment of elderly with Alzheimer's dementia
Journal of Clinical and Translational Research

Dear author(s),

The editor in chief has evaluated your revised submission and his comments are appended below. Based on his comments and evaluation by the editorial board, your work was FOUND SUITABLE FOR PUBLICATION AFTER MINOR REVISION.

If you decide to revise the work, please itemize the reviewers' comments and provide a point-by-point response to every comment. An exemplary rebuttal letter can be found on at http://www.jctres.com/en/author-guidelines/ under "Manuscript preparation." Also, please use the track changes function in the original document so that the reviewers can easily verify your responses.

Your revision is due by Dec 30, 2019.

To submit a revision, go to https://www.editorialmanager.com/jctres/ and log in as an Author. You will see a menu item call Submission Needing Revision. You will find your submission record there.

Yours sincerely,

Michal Heger
Editor-in-Chief
Journal of Clinical and Translational Research

Reviewers' comments:

It is imperative that readers are not forced to paddle back and forth between this manuscript and previously published papers in order to fully understand the data. Therefore, the editorial board requests that the authors incorporate the descriptive information related to sociodemographic characteristics as well as the biomarker data from the previously published studies (refs 21 and 25). The cohort data as well as the biomarker data do not fall under copyright as long as the authors explicitly state that the data had been previously published and the cohort previously described. This information should be added to the first paragraph of the Results section entitled "Sociodemographics and Descriptives for all Biomarkers."

November 30, 2019
Michal Heger, Ph.D.

Editor-in-Chief

Journal of Clinical and Translational Research

Dear Dr. Heger:
We thank the Reviewer for the additional evaluation of our manuscript. We have addressed the Reviewer's comments:

Reviewer
It is imperative that readers are not forced to paddle back and forth between this manuscript and previously published papers in order to fully understand the data. Therefore, the editorial board requests that the authors incorporate the descriptive information related to sociodemographic characteristics as well as the biomarker data from the previously published studies (refs 21 and 25). The cohort data as well as the biomarker data do not fall under copyright as long as the authors explicitly state that the data had been previously published and the cohort previously described. This information should be added to the first paragraph of the Results section entitled "Sociodemographics and Descriptives for all Biomarkers."

We agree with the Reviewer that it is cumbersome to have a reader of the new article flip back and forth between the previously published papers to get the descriptive information for all of the demographics and biomarkers. Thus, we have included Table 1 for the demographics and Tables 2-4 for all of the biomarkers at baseline and 12-months follow-up. The previous Table 1 is now Table 5. We noted that these descriptive data were previously published as required in the first paragraph of the Results section.

Please let us know if you have any additional questions or clarifications, and we look forward to the next review of our paper.

Best regards,
John E. Lewis, Ph.D.
Associate Professor

3rd editorial decision

01-Dec-2019

Ref.: Ms. No. JCTRes-D-19-00008R2
Relationships between brain-derived neurotrophic factor and immune function during dietary supplement treatment of elderly with Alzheimer's dementia
Journal of Clinical and Translational Research

Dear authors,

I am pleased to inform you that your manuscript has been accepted for publication in the Journal of Clinical and Translational Research.

You will receive the proofs of your article shortly, which we kindly ask you to thoroughly review for any errors.

Thank you for submitting your work to JCTR.

Kindest regards,

Michal Heger
Editor-in-Chief
Journal of Clinical and Translational Research

Comments from the editors and reviewers: