Author's response to reviews

Title: Circulating levels of lycopene and its possible influence on the oxidative damage and cardiovascular risk in chronic hemodialysis patients

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Author's response to reviews: see over
Dear Prof. Dra. Christna Chap, Executive Editor – BMC Nephrology

We would like to thank the Reviewers for their comments and especially for their consideration concerning this manuscript. All points were very well commented and critically analyzed in a very constructive manner. We have made the changes in order to cover all the points raised. The changes are indicated below with our comments, whenever they were necessary. I am certain that these suggestions were essential to improve our work. Moreover, the points suggested for you as “Ethical approval” and “Competing interests” were added. In relation to English language copyedit, we send the manuscript to “Professional Services Editing” and also to English teacher. In this form, we hope that the language mistakes were resolved. I like to explain that the title was modified following reviewer suggestion. Moreover, following the suggestions of reviewers we performed additional statistic tests inclusive with regression models. With this, we found others interesting results as the influence of GPx activity on lipid damage in this patients and an association of lycopene levels with LDL-c. Thus, more important aspects were added and discussed.

Manuscript:

Title: Exogenous antioxidants and its relationship with lipid profile and oxidative stress biomarkers in haemodialysis patients

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**REVIEWER 1 – BELDA DURSUN**

We greatly appreciated all of your comments, and gladly attended all of them. They were very important for the quality improvement of our paper. We would like to apologize for not having noticed these points before.

**Commentary 1:** In the abstract part, aim should be described more clearly. Also the authors mention in the abstract that they analyzed kidney function markers, however, there are no kidney function parameters demonstrated in the manuscript.

**Answer 1:** We found that really did not meet goals the central idea of the work. So as suggested goals were reviewed (page 2, lines 29-31) and we hope it has been clearer. Also we included in the Table 1 data about renal function, such as serum urea and creatinine levels.

**Commentary 2:** Throughout the whole manuscript there are a number of misspelt words (for example plasma carotenoids levels on page 1, polycistic kidney on page 7, haemodylalysis on page 12, etc) and misstructured sentences that need to be corrected.

**Answer 2:** The terms misspellings were corrected in the text as requested. Although there are words and misspellings, the text in question was extensively corrected by a reviewer of the english language in Brazil and was also corrected by the correction system suggested by the english language by “Professional Editing Services”.

**Commentary 3:** The study subjects do not seem to be matched by age and gender. The authors should mention if they compared the groups by statistical methods. Also they should provide more data to describe the groups, including
cardiovascular risk markers such as their renal functions, blood pressure levels, body mass index etc.

**Answer 3:** In according with the suggestion we performed statistical analysis considering the age and gender (Tables 1-4). The age was significantly higher in HD patients than in healthy individuals. We additioned this information in the result sections (page 7, lines 152-153). In relation to gender we plotted in all the tables the values of each parameter subgrouped by gender within the groups. In addition, in multivariate analyses, age and gender were included as continuous and categorical covariable, respectively.

More data, as concentrations of albumin, iron, ferritin, urea, creatinine, hemoglobin and hematocrit were added in the Table 1. Also the reference values for these variables were additioned. Unfortunately, others informations such body mass index were not obtain.

**Commentary 4:** In the reference 39 quoted by the authors, total cholesterol/HDL was suggested to be a better measure of risk for coronary artery disease, it would be nice to learn if the authors have made any analysis to see any correlations with that parameter itself as well.

**Answer 4:** The affirmation that total cholesterol/HDL is better measure for cardiovascular risk did not possible in our work, since we conducted a single blood collection. To demonstrate the TC/HDL cholesterol in the group of patients studied would require a long-term monitoring, similar to that made by Kinosian et al (quoted in reference 39) who accompanied the patients for 8 to 10 years. Furthermore, no significant difference was found in TC/HDL index when we compared haemodialysis (HD) patients who died within two years with the HD patients that did not died during the study. This absence of statistic difference in this index and in other parameters may be due to low number of the patients in the HD group who died. This latter supposition we additioned in the result section (page 8, line 191 – 193).

However, in the section results, the multivariate regression model shows that both GPx activity (continuous variable) and gender (categorical variable) were associated with index of the TC/HDL cholesterol (pages 9 and 10, lines 222-226). Thus, our results demonstrate a significant association between an
endogenous antioxidants and TC/HDL index, considering this index is important in studies involving lipid profile.

**Commentary 5:** The title of the article does not reflect the whole idea of the manuscript; because the manuscript does not seem to be prepared to examine the effects of lycopene itself on cardiovascular risk, but a number of carotenoids, tocopherols and oxidative stress markers as well were included, therefore title should be changed appropriately.

**Answer 5:** Actually the title of the article did not reflect the overall idea of the manuscript, because along with lycopene other carotenoids and tocopherol also was analyzed with biochemical markers and oxidative stress biomarkers in hemodialysis patients. So as a suggestion the title of the text was changed and hopefully now it demonstrates the central idea of the text.

**Commentary 6:** It would be interesting to see if lycopene came up a predictor of cardiovascular risk in a regression analysis. Also the authors do not mention if there were any correlation between antioxidant enzyme levels and lycopene in HD patients.

**Answer 6:** In regression analysis we observed that LDL cholesterol was associated with lycopene levels. The majority of the lipid profile such as HDL; total cholesterol/HDL-c index and LDL-c/HDL-c indexes were associated with the GPx antioxidant. Moreover, there was found a positive correlation between lycopene levels and GPx activity through of correlation test (page 9, lines 217-218).

**Commentary 7:** In the conclusion part of the manuscript, there should be a better definition of the final results obtained from this paper.

**Answer 7:** Following you suggestion we conducted a review of the conclusion of the article and obtained a better definition of the final results (pages 13 and 14, lines 318-327).
We greatly appreciated all of your comments, and gladly attended all of them. They were very important for the quality improvement of our paper. We would like to apologize for not having noticed these points before.

Commentary 1: The authors mention that among the 29 HD patients included in the study, only 6 were receiving a vitamin D analog, 17 an erythropoiesis stimulating agent and 11 iron. Since hyperparathyroidism and anemia can impact CV function, my concern is that many of these patients were not optimally treated for CV risk. Please explain the treatment choices.  

Answer 1: The commentary is relevant, and we added information regarding in this text (page 7, line 162-167). These patients receive free treatment of the government, as such laboratory tests, drugs and even the hemodialysis process. Being a public hospital, the biochemical monitoring for cardiovascular disease was restricted. The anemia was controlled and prevented with hematological analysis and the use of erythropoietin and iron supplements, respectively. The abnormal range of the hematocrit and hemoglobin presented in the Table 1 support this therapeutic option.

The lipid profile as a risk factor for cardiovascular disease was also monthly monitoring. The treatment with statins was performed when necessary (page 7, lines 162-167). Importantly, the drug treatment was prescribed by a nephrologist’s doctor and we do not have power of decision on the patient’s treatment.

Furthermore, we believe that our findings, lipid profile higher than the controls, the increased LDL-c/HDL-c in HD patients in comparison to control group, the exacerbation of oxidative stress, and the protective effect of lycopene on the latter can guide health managers in directing public policy in order that they be extended in the sense of the monitoring of biochemical HD patients treated by public services.

Commentary 2: There is no measure of CV function in these patients. It would seem advantageous to have that data.
Answer 2: Despite knowing that these data are important for further evaluation, unfortunately we not verified the measure of CV function.

Commentary 3: Nutritional intake may have impacted various levels of vitamins and/or lipids. Please provide the following information:
- How many patients received a multivitamin
- How many were receiving a lipid-lowering agent
- How many were receiving nutritional supplementation
- Serum Albumin and normalized protein catabolic rates values at the time of the other analyses.

Answer 3: We also known that nutritional intake can influence in the levels of some vitamins and lipids. All patients included in this study had a follow-up of nutritionists to prepare meals. This work also served to demonstrate that these patients have a reduction in some important carotenoids when compared to healthy subjects. These reductions may be happening due to nutritional metabolism problems. Additionally we believe that these found can be useful for nutritionists to guide the diet of these patients.

- A) None of the patients were taking multivitamin.
- B) Only four of these were using lipid-lowering drugs such as statins, which did not influence the statistical analysis.
- C) None of these were receiving nutritional supplementation.
- D) Levels of serum albumin are inserted into the Table 1.

Commentary 4: The authors mention that during two years after the collection of the blood samples 9 (24.1%) patients died, 6 of whom died of CVD. Were the levels of carotenoids and/or anti-oxidants different in this sub-group? The results of that analysis, even if not statistically different b/o small sample size, may be interesting.

Answer 4: We are in agreement with this commentary, and we performed the suggested statistic test. Mann Whitney test did not reveal significant statistical difference among the parameters analyzed in this study when the subgroups were compared. The subgroups include HD patients that died during this study and HD patients that did not die during this study. This information was additioned in the result section (page 8, lines 191-193).
Commentary 5: I assume that the data presented in Figures 1-3 pertains only to HD patients. Is that true? If so, given the “n” values of 49, it would seem that some lab values were measured more than once in certain patients. Please elaborate.

Answer 5: We realized new statistic test inclusive with regression models. We found influence of lycopene levels on LDL-c. Thus, we replace the figures and we added more tables. Moreover, the results of test correlations were written in the text in result sections. All these results were discussed in the adequate section.

Commentary 6: Did the authors measure CRP?

Answer 6: We did not measured C-reactive protein (CRP), however after your question we understand as important analysis to further studies in this line.

Commentary 7: It would be helpful to provide the “normal” values in the general population for carotenoids and tocopherols levels. Please indicate how many HD patients had low levels for each of those values.

Answer 7: There isn’t a general consensus for the reference values of the carotenoids. There are a few isolated publications, such as Olmedilla et al, 2001 (Br J Nutr), who obtained values for five different European countries, and these showed much difference between them, ranging from country to country according to local habits of each. Also, in Brazil there isn’t still a work in the literature by establishing reference values for these tests. Thus, we chose to use the control group as reference for this study. In relation to tocopherol (with values established) reference values were introduced in the text, the discussion section.

Commentary 8: Please provide data on hemoglobin, serum calcium, serum phosphorous and PTH. Abnormalities in these indices could have contributed to oxidative stress.

Answer 8: The hemoglobin values were additioned in Table 1. Although the PTH, calcium and phosphorus analysis are very important, because they
have a higher cost they are performed at a lower frequency and therefore at the time of sampling they were not performed. We agree that these data could be useful, however they were not realized in this study.

Commentary 9: Were any patients receiving immunosuppressive medications and/or aspirin during the study period? If so, their data should be excluded or separately analyzed. Similarly, patients with a fever or those using a central venous catheter (even if uninfected by culture b/o risk for inflammation with a catheter) should be excluded.
Answer 9: None of the HD patients received immunosuppressive or salicylic acetyl acid drugs.

Commentary 10: Did dialysis vintage relate to any of the parameters measured.
Answer 10: All sample collect were realized before the haemodialysis section.

Commentary 11: Was there an impact of gender?
Answer 11: After regression model, we observed that there was an influence of gender only in the TC/HDL-c index (Table 5). Following your suggestion, we added in almost all tables values for men and women. Although to some analysis there was significant difference between gender, in the multivariate analysis only was found to TC/HDL-c index.

Commentary 12: There are numerous errors in grammar and segments where it is difficult to discern the message. The entire manuscript should be thoroughly reviewed. One example is the first paragraph of the “Background”. The sentence that begins with “Moreover, in CRF patient” needs complete refining. It is rambling and the message is very confusing.
Answer 12: The language and grammar errors were corrected again by professionals (professional services editing) and a English teacher.
Commentary 13: The authors state in the “Discussion” that HD leads to dyslipidemia. Actually, the prevalence of dyslipidemia is reduced in HD compared to CKD. Please consider.

Answer 13: We considered this aspect and delete this of the discussion. In this line, we like to thank yours important contributions in our manuscript.

With best regards,

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