

Format for ANSWERING REVIEWERS

June 18, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 9135-revised.doc).

Title: Oxidative Stress and Labile Plasmatic Iron in Anemic Patients Following Blood Therapy

Author: Mar íia Sabo Fernandes, Tatiana Tamborena Rissi, Luisa Zuravski, Juliana Mezzomo, Carmen Regla Vargas, Vanderlei Folmer, Felix Alexandre Antunes Soares, Vanusa Manfredini, Mushtaq Ahmed, Robson Luiz Puntel

Name of Journal: *World Journal of Experimental Medicine*

ESPS Manuscript NO: 9135

We would like to thank the reviewers for the careful reading of our manuscript and for the comments and corrections suggested. We have incorporated all the criticisms raised by the reviewers in the revised manuscript. Below, we document the responses to the reviewers' queries. Moreover, it is confirmed that all references and typesetting are rectified.

Reviewers' comments:

- **Reviewer 43980:**

We have carefully evaluated this new manuscript.

MAJOR COMMENTS:

1) The methods describing how the various materials are determined are not correctly referenced.

We would like to thanks the reviewer for the relevant appointment. In the revised manuscript we carefully revised the entirely text and the methods are, now, correctly referenced. Please, see these on pages 5-9.

2) The results section is succinct, yet comprehensive. Further attention to the final paragraph of the results should be made as it is somewhat difficult to follow and the summary sentences are unclear.

We would like to thanks the reviewer for the criticism raised. In the revised manuscript, we carefully revised the entirely text in order to avoid unclear sentences/ paragraphs. Indeed, our manuscript was carefully edited by a native speaker English. Please, see the revised version of the last paragraph of the results on page 10.

3) The discussion is well written and points out the experiment's interesting findings and the implications for medical practice.

We would like to thanks the reviewer for his appreciated comments.

MINOR COMMENTS:

Page 2, line 3: It should read "however, in excess it can be a major cause..."

In the revised version of the manuscript, the abstract was re-write and this sentence was



deleted. Please, see the Abstract from the revised version on page 2.

Page 2, line 5: Add an apostrophe to enzymes prior to activity to make it possessive.

In the revised version of the manuscript, the abstract was re-write and this fragment was deleted. Please, see the Abstract from the revised version on page 2.

Page 3, line 3: Change “capacity” to “ability to”

Ok, in revised manuscript we used “ability to” as suggested by the reviewer. Please, see this on page 4, line 3.

Page 3, line 7: Change “permeate” to “permeating”

Ok, in revised manuscript we used “permeating” as suggested by the reviewer. Please, see this on page 4, line 7.

Page 3, line 17: This sentence is not worded well, suggest rewriting. Particularly “it was pointed” is unclear in the sentence.

Ok, in revised manuscript we re-write this sentence as suggested by the reviewer. Please, see this on page 4, first sentence from the 2^o paragraph.

Page 3, line 22: Change “increases” to “increase”

Ok, in revised manuscript we used “increase” as suggested by the reviewer. Please, see this on page 4, line 21.

Page 4, line 17: Change “no transfused” to “not transfused”

Ok, in revised manuscript we used “not transfused” as suggested by the reviewer. Please, see this on page 5, lines 16-17.

Page 4, line 17-18: The last part of this sentence is not worded well, suggest rewriting. Also would add “levels” between “ferritin” and “in” (line 17).

Ok, in revised manuscript the sentence was re-write, as suggested by the reviewer. Please, see this on page 5, lines 17-18.

Page 5, line 3: Add “well as” between “as” and “healthy”

Ok, in revised manuscript we re-write this sentence was deleted. Please, see this on page 6, lines 2-3.

Page 5, line 5: Change “do not presented” to “did not present”

Ok, in revised manuscript we used “did not present” as suggested by the reviewer. Please, see this on page 6, line 5.

Page 5, line 6-11: These sentences are not worded well and are unclear, suggest rewriting.

Ok, in revised manuscript we re-write these sentences as suggested by the reviewer. Please, see this on page 6, lines 7-9.

Page 5, line 13: Delete “up” between “five” and “to”

Ok, in revised manuscript “up” was deleted, as suggested by the reviewer. Please, see this

on page 6, lines 13.

Page 6, line 9: When including a reference for how a material was determined the name from the reference must be included in the sentence.* For example, “Content ferritin was determined as described by Bernard and Lauwerys [20].”

Ok, in revised manuscript we revised all citations as suggested by the reviewer. Please, see this on pages 5-9.

Page 6, line 10: Add apostrophe to “particles” before “agglutination” (in line 11) to make it possessive

Ok, as suggested by the reviewer, in revised manuscript an apostrophe was added. Please, see this on page 7, lines 10.

Page 6, line 15: When including a reference for how a material was determined the name from the reference must be included in the sentence. See *asterisk above for example.

Ok, as pointed below, in revised manuscript we revised all citations as suggested by the reviewer. Please, see this on pages 5-9.

Page 6, line 16: Add “to” between “added” and “0.2 mL”

Ok, as suggested by the reviewer, in revised manuscript “to” was added. Please, see this on page 7, lines 14.

Page 6, line 17-19: This sentence is not worded well, suggest rewriting.

Ok, in revised manuscript we re-write this sentence, as suggested by the reviewer. Please, see this on page 7, lines 15-18.

Page 6, line 21: Should the sentence be written in future tense? It says “will be done”. Suspect should be in past tense.

We would like to thanks the reviewer by this pertinent comment. Now, in revised manuscript we re-write the sentence as suggested by the reviewer. Please, see this on page 7, lines 19-21.

Page 7, line 8: When including a reference for how a material was determined the name from the reference must be included in the sentence. See *asterisk above for example.

Ok, as pointed below, in revised manuscript we revised all citations as suggested by the reviewer. Please, see this on pages 5-9.

Page 7, line 18: When including a reference for how a material was determined the name from the reference must be included in the sentence. See *asterisk above for example.

Ok, as pointed below, in revised manuscript we revised all citations as suggested by the reviewer. Please, see this on pages 5-9.

Page 7, line 22: Add “by a” between “measured” and “spectrophotometer”. Change “during 2 min” to “for a duration of 2 min”.

Ok, as suggested by the reviewer, in revised manuscript both changes were made. Please, see both on page 8, lines 21-23.

Page 8, line 3: When including a reference for how a material was determined the name from the reference must be included in the sentence. See *asterisk above for example.

Ok, as pointed below, in revised manuscript we revised all citations as suggested by the reviewer. Please, see this on pages 5-9.

Page 8, line 23: Change “on Table I” to “in Table I”

Ok, in revised manuscript we used “in Table I” as suggested by the reviewer. Please, see this on page 9, lines 20-21.

Page 8, line 25: Change “none” to “no”

Ok, in revised manuscript we used “no” as suggested by the reviewer. Please, see this on page 9, line 22.

Page 8, line 26: Change “was” to “were” as discussing LPI and ferritin

Ok, in revised manuscript we used “were” as suggested by the reviewer. Please, see this on page 9, line 23.

Page 8, line 27: Change “high” to “higher”

Ok, in revised manuscript we used “higher” as suggested by the reviewer. Please, see this on page 9, line 24.

Page 8, line 31: Change “significantly” to “significant”

Ok, in revised manuscript we used “significant” as suggested by the reviewer. Please, see this on page 9, line 28.

Page 9, line 4-6: This sentence is not worded well, suggest rewriting.

Ok, in revised manuscript we re-write this sentence, as suggested by the reviewer. Please, see this on page 10, lines 5-8.

Page 9, line 7: Change “the all” to “all the”. Reverse order of words.

Ok, in revised manuscript we used “all the” as suggested by the reviewer. Please, see this on page 10, line 9.

Page 9, line 15: Change “controls” to “control”

Ok, in revised manuscript we used “control” as suggested by the reviewer. Please, see this on page 10, line 17.

Page 9, line 15-25: This paragraph is difficult to read and follow, particularly the last couple sentences in lines 22-25. Suggest rewriting.

Ok, in revised manuscript we re-write this paragraph, as suggested by the reviewer. Please, see this on page 10, lines 19-27.

Page 9, line 31-32: This sentence is not worded well, suggest rewriting.

Ok, in revised manuscript we re-write this sentence, as suggested by the reviewer. Please, see this on page 10 (last phrase) and page 11 (first phrase – lines 1-3).

Page 9, line 32: Change “is” to “are” after “data” as data is a plural word

Ok, in revised manuscript we used “are” as suggested by the reviewer. Please, see this on page 10, last line.

Page 10, line 1: Change “that” to “those”

Ok, in revised manuscript we used “those” as suggested by the reviewer. Please, see this on page 11, line 6.

Page 10, line 11: Deleted “called”

Ok, in revised manuscript “called” was deleted, as suggested by the reviewer. Please, see this on page 11, lines 15-16.

Page 10, line 14: Add ‘s’ to “lead” and change “cultures” to “cultured”

Ok, as suggested by the reviewer, in revised manuscript both changes were made. Please, see both on page 11, line 19.

Page 10, line 20: Add “factor” following “responsible”

Ok, as suggested by the reviewer, in revised manuscript “factor” was added. Please, see this on page 11, lines 26.

Page 10, line 24: Add ‘s’ to “transfusion” to make plural.

Ok, in revised manuscript we used “transfusions” as suggested by the reviewer. Please, see this on page 11, line 29.

Page 10, line 29: Add ‘s’ to “transfusion” to make plural.

Ok, in revised manuscript we used “transfusions” as suggested by the reviewer. Please, see this on page 11, line 34.

Page 10, line 30-32: This sentence is not word.

Ok, in revised manuscript we re-write this sentence. Please, see this on page 12, lines 1-3.

We would like to thanks the reviewer for all the suggestions outlined. The revised version of the manuscript was carefully edited by a native speaker English. Anyway, we regret any errors that persist.

- Reviewer 482024:

The theme of this paper is a noteworthy one in that it is known that oxidative stress is a problem in premature infants who are subjected to a multitude of blood transfusions, but it is unknown what, if any, this scenario would have in adults. The authors used adequate methods to address their research question, but by using plasma they are not examining the tissue where oxidative damage occurs (e.g., arterial lining, liver, etc). Existing literature linking the observed levels of the various biomarkers to health outcomes such as heart attacks and stroke would strengthen the paper.

We would like to thanks the reviewer for the pertinent comment. In the revised format of the manuscript we included a brief comment regarding the point raised by reviewer. Please, see this sentence on page 11, lines 3-5.

The fact that ferritin levels increase in the group where there is a greater oxidative stress environment is not congruent with the hypothesis.

We would like to thank the reviewer for the criticism raised. Here we would like to mention that ferritin levels were found to be increased (26% greater) in the group with > 10 transfusions when compared to control subjects. On the other hand, in the same groups (> 10 transfusions) the LPI was found to be ~230% higher than control subjects. Based on this, we believed that, despite the increase in ferritin, this fact is in accordance with our hypothesis. In other words, repeated blood transfusions lead to iron overload (catalytically active iron), which culminates with oxidative stress status; despite increase in ferritin levels. However, despite the reviewer comment, we do not include this fragment on manuscript.

The authors also must address how transfusions can cause stress apart from the iron levels.

We would like to thank the reviewer for the pertinent comment. In the revised format of the manuscript we included a brief discussion based on presented results that could, at least in part, explain how transfusions could cause stress apart from the iron levels. Importantly, our speculation regarding this point was based, exclusively, in the results obtained in this study without use literature data to extrapolate it. Please, find the following fragment, regarding this point, on the discussion section (Page 11, lines 20-27).

“However, we found some changes in the oxidative stress parameters even in the absence of significant iron accumulation, suggesting that alterations in oxidative stress markers could precede iron accumulation in patients following blood therapy. Accordingly, it seems logical that the differences in other parameters, in the hemoglobin and ferritin levels for example, could potentially contribute to the different oxidative state among patients. So, it is hard to affirm that iron alone is the main responsible factor for these differences. Anyway, this point is extremely relevant and deserves further attention in future investigations.”

- Reviewer 35901:

The authors demonstrated the involvement of oxidative stress in patients following therapy with repeated blood transfusions. Additionally they found that the changes in the oxidative stress markers are tightly correlated with labile plasmatic iron, ferritin and the number of transfusions. This study was well-organized and well-investigated. I have no claim in the present manuscript.

We would like to thank the reviewer for his appreciated comments.

We wish to thank all the reviewers for their thoughtful comments and criticisms. We believe they have led to refinement of our hypotheses, and led to a far better understanding on the toxicity associated with iron overload. We hope that you will find this manuscript suitable for publication in its revised format.

Thank you again for publishing our manuscript in the *World Journal of Experimental Medicine*.

Sincerely yours,



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