Author’s response to reviews

Title: Functional capacity of Natural Killer cells in HTLV-1 associated myelopathy/tropical spastic paraparesis (HAM/TSP) patients

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Author’s response to reviews:

Dr. Rudra Channappanavar

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Salvador, 11 April 2019.

Dear Dr. Channappanavar,

We would like to thank you for the opportunity to revise our paper for resubmission. We would like to express our appreciation for the relevant comments and criticism provided by the reviewers. It is our opinion that their contributions have resulted in an improved manuscript and we have responded to the reviewers’ suggestions as follows:
Technical Comments:

Query 1) The main concern are: 1) The NK cells are a small portion of PBMCs and in such study it is better to use sorted cells for the studies. Please explain the methodology used. 2) PBMCs were stored in liquid nitrogen, at the time of revival many cells die. The question is whether there were enough NK cells survive to have a reliable study! Particularly in a study that deal with live and functional cells. The resolution of the Flows graphs need to be improved to appreciate the number and the intensity of fluorescence.

Reply: We agree with the referee’s comment that since NK cells constitute a very small proportion of PBMCs, it would have been ideal to use sorted cells. However, the purification of NK cells from PBMCs requires a large amount of PBMCs from many patients, which was not possible in the present study. In addition, several types of phenotypic and functional NK studies are carried out directly with the use of whole PBMCs or peripheral blood, which offers the advantage of maintaining similarity to physiological conditions (Petitdemange C, et al. PLoS neglected tropical diseases. 2016) (Brandt CS, et al. The Journal of experimental medicine. 2009). We have included a statement in the discussion section citing this concern as a limitation of the study (Lines 281-284).

Although frozen cells were used to perform functional analyses, the recovery of viable cells after thawing was greater than 85%. Considering the quality of these cells after thawing, together with the reproducibility of our results obtained from different samples, we feel our results are reasonably reliable.

As per the reviewer’s request, the resolution of the flow graphs has been improved.

Query 2) Please specify the inclusion and exclusion criteria in these studies. This manuscript would benefit from a detailed flow chart of patient inclusion/exclusion.

Reply: We have rephrased the inclusion/exclusion criteria for clarification, as follows:

For this cross-sectional study, HTLV-1-infected individuals were selected by convenience sampling at the Integrated and Multidisciplinary HTLV Center, (Salvador, Bahia-Brazil). All participants were sequentially included at the time of their previously scheduled appointments. Inclusion criteria were individuals of both genders, 18 to 65 years of age, with an available neurological evaluation used to differentiate asymptomatic from HAM/TSP individuals. Myelopathic symptoms, serological findings and/or the detection of HTLV-1 DNA, as well as the exclusion of other disorders, were all used as indicators in the diagnosis of HAM/TSP. Asymptomatic individuals (AS) were included if their neurologic examinations were normal and they reported no clinical complaints. Co-infected patients with HIV, HBV and/or HCV were excluded. HTLV-1 infection was diagnosed using ELISA (Cambridge Biotech Corp., Worcester,
MA) and confirmed by Western Blot analysis (HTLV blot 2.4, Genelab, Singapore). Eighteen laboratory staff volunteered as non-infected controls.

Query 3) The abstract and results sections need to be well organized. There are some unnecessary statements which simply can changed to the more informative sentences.

Reply: We have modified the abstract and results section to address this concern.

Query 4) Although, statistical analysis are very precise, some of the graphs do not make sense as the deviations are too high (Figs 2 and 3). Furthermore, in abstract and in the results the type of study and the type of samples were not specified. Please address these concerns.

Reply: We agree with the reviewer’s criticism and we recognize the occurrence of significant deviation in the frequencies of NK cells expressing inhibitory/activating receptors and cytotoxic markers. In spite of this, when evaluating individuals with differences in disease length, age, sex, etc. high biological variability among groups is an expected finding.

We have included the type of the study and samples used in the abstract and results sections.

Query 5) A clinical and demographic table will make the study more strong and necessary to put something like duration of the diseases, the spasticity and Urinary incontinence scores, underlying signs and symptoms and the correlations of the clinical and molecular finding should be included to see if NK cells finding had relations to the clinical outcomes.

Reply: We have added the clinical features of the recruited patients in Table 1. However, due to the limited number of studied individuals and great variation in the expression markers studied, no correlations between clinical outcomes and NK cells could be obtained.

We would like to inform the reviewers and editor, upon careful reanalysis, we have excluded three patients from the HAM/TSP group and changed the status of two to asymptomatic following the newly performed NK phenotypical analysis experiments. After our reanalysis of clinical records to complete Table 1, we identified misclassification in these individuals regarding their clinical status, and identified one patient who was coinfecte with HCV. Afterwards, we reanalyzed all the phenotypical profiles and as our statistical results remained similar, we only modified the frequencies. We sincerely apologize for this lack of oversight.

Query 6) The Biomedical Ethical approval should be included.
Reply: We have included a subsection of ethical consideration in methods section, as follows:

Ethical considerations. The present research protocol was approved by the Institutional Research Board (IRB) of the Bahiana School of Medicine and Public Health (EBMSP) in Salvador, Bahia, Brazil (protocol no. 187/2011). All procedures were performed in accordance with the principles established in the Declaration of Helsinki and its subsequent revisions.

Minor revisions

Abstract

Query 1) The aim in the abstract is not clear and the sentence is not in right form.

Reply: We have rephrased this to clarify the objective. “This study aimed to investigate the phenotypic profile and the functional capacity of NK cells in the context of HTLV-1 infection.”

Query 2) Please check methods section for accuracy and brevity.

Reply: We have revised the methods section and improved accuracy and brevity as suggested.

Query 3) Please include the full words for IQR abbreviation in the abstract.

Reply: We have included “Interquartile Range” prior to using this abbreviation in the text.

Other sections

Query 4) Line 149 instead of similar; is better to use "statistically matched".

Reply: We have replaced indistinguishable with statistically matched.

Query 5) The proviral load should be compared with the other endemic area like Japan, Africa and Asia (Iran) in discussion.

Reply:

We have included the following statement in the discussion section to address this issue (line 273-277).
“A clear association between HAM/TSP diagnosis and high HTLV-1 proviral load has been observed in several studies conducted in Japan, Martinique, Brazil, United Kingdom and Iran (7, 8, 39-41). Herein HTLV-1 proviral load was also consistently higher in HAM/TSP patients compared with AS individuals, however no correlations were found between HTLV-1 proviral load and any of the markers evaluated.”

Query 6) The result section should be reorganized in subsections.

Reply: We have incorporated subsections in the results section.

Query 7) Line 239 is ambiguous.

Reply: We have deleted this statement.

Query 8) Please include the limitations of the study.

Reply: We have included limitations of the study in the discussion section.

Query 9) Conclusion needs a careful revision for its accuracy

Reply: We have modified the conclusion in the abstract and in the discussion sections accordingly.

Query 10) Here are some incomplete sentences without matching verbs. Please check the manuscript and edit accordingly.

Reply: We have submitted the entire text for correction/editing by a native English speaker.

Sincerely yours,

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