Author’s response to reviews

Title: Analysis of Apoptosis Related Genes in Nurses Exposed to Anti-Neoplastic Drugs
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Author’s response to reviews:

Dr. Simone Brogi,
BMC Pharmacology and Toxicology
Re: PHAT-D-19-00189R1
Analysis of Apoptosis Related Genes in Nurses Exposed to Anti-Neoplastic Drugs
Maral Ramazani; Razieh Pourahmad Jaktaji; Seyed Farshad Hosseini Shirazi; Maria Tavakoli-Ardakani; Ahmad Salimi; Jalal Pourahmad
BMC Pharmacology and Toxicology

Dear Dr. Brogi,

Thank you very much for your comments on our submitted manuscript. We carefully read your comments and tried to comply with your requests as much as we could. We made major revisions as requested and you can see the revised version (corrections shown by yellow bands). We also provided thoughtful answers to some questions. We hope it is now satisfactory. In the end, I would like to thank you and the all respected reviewers for the precious time and scientific comments.

Detailed corrections are listed below point by point:

With kind regards,

Jalal Pourahmad, Ph.D
Corresponding author
Reviewer reports:

Marcelo Arbo, Ph.D. (Reviewer 1): Please include all comments for the authors in this box rather than uploading your report as an attachment. Please only upload as attachments annotated versions of manuscripts, graphs, supporting materials or other aspects of your report which cannot be included in a text format.

Please overwrite this text when adding your comments to the authors.

The aim of this study was to monitor cellular toxicity parameters and gene expression in nurses who work in chemotherapy wards and compare it to nurses who work in other wards.

1) The results are interesting however the manuscript needs a review in some aspects. Tables need edition to become more clear to the reader.

Our answer: As requested by the respected reviewer we edited the Tables to become more clear to the reader. Please see the revised version (all the corrections appear in yellow bands).

2) The english language is difficult to understand in some parts, such as:

"According to the Figure 2 A cell viability in isolated lymphocytes of oncology nurses significantly was reduced as well as control nurses (p&lt;0.001)."

"When florescence of rhodamine 123 is much means loss of mitochondrial membrane potential also is much."

This reviewer was not able to understand these sentences.

Our answer: As requested by the respected reviewer we corrected these two paragraphs as;

" According to figure 2, the lymphocyte viability of oncology nurses was significantly lower than those of control nurses (p&lt;0.001)."

“ When the fluorescence intensity related to Rhodamine 123 release from mitochondria into the cytosol, is high, it means that the loss of mitochondrial membrane potential is also high.”

Please see the revised version (all the corrections appear in yellow bands).

3) Results of Annexin V-PI assay present only cells in apoptosis, how was the percentage of cells marked with PI or annexin V only, or both annexin V and PI?

Our answer: In figure 3(Page 6), Quadrant 1 includes necrosed cells (Annexin V-/PI+), Quadrant 2 includes cells under secondary necrosis or late apoptosis (Annexin V+/PI+), Quadrant 3
includes live cells (Annexin V-/PI-) and Quadrant 4 includes cells under early apoptosis (Annexin V+/PI-).

4) Results presented in table in table 3 should be statistically analyzed, using qui-square test for example.

Our answer: As requested by the respected reviewer we statistically analyzed table 3 using qui-square test, and then we transferred the information of table 3 into figure 1 in the revised version.

Please see the revised version (all the corrections appear in yellow bands).

5) The lack of exposure biomarker, such as the values of antineoplastic drugs in the blood of the nurses is a weak point of this manuscript.

Our answer: As explained in page 9, line 27, We couldn’t detect all chemotherapy drugs concentration in the blood of nurses because the number of drugs that are in use in chemotherapy wards of our country Iran are more than 40 different drugs and measurement of their exact concentration in the blood sample is not possible at the same time. Besides, these drugs have synergistic and antagonistic effects on each other. Therefore, the presence or absence of a single drug in blood at the time of measurement is not a reason for the absence or present of adverse effects. Please see the revised version (I demonstrate this paragraph in yellow bands).

MajaAntunović (Reviewer 2): The article covers very interesting topic while the authors used very wide methodology assessment to evaluate apoptotic effects of anti-neoplastic drugs. My major concern is that the manuscript is extremely poorly written and needs to be largely reworked (grammatical errors, awkward sentence construction and poor sentences in result analysis).

My suggestions are as follows:

6) The manuscript should be revised by an English-speaking person before submitting it for a detailed revision.

Our answer: As requested by the respected reviewer we revised manuscript's English language with an appropriate vocabulary and grammatical check software. We also asked an English-speaking colleague in Canada to check the revised version.

7) The methodology and consequently result analysis, should be ordered as follows:

Sample collection
Isolation of lymphocytes

MTT assay

Annexin V/Propidium Iodide staining

Caspase-3 activity assay

ROS detection

Lipid Peroxidation assessment

Lysosomal damage assay

MMP assay

RT-qPCR analysis

Statistical analysis

Our answer: As requested by the respected reviewer we changed the order of subtitles in methodology and consequently result analysis accordingly. Please see the revised version (all the corrections appear in yellow bands).

8) Please do not to repeat same headings for methodology and results. Capitation for results should offer short description of your results, often combining more methods.

Our answer: As requested by the respected reviewer, we corrected Capitation for results and offered short description of our results without combining more methods.

9) Gene abbreviations should be written in italic.

Our answer: As requested by the respected reviewer, we wrote gene abbreviations in italic. Please see the revised version (all the corrections appear in yellow bands).

Natália Brucker (Reviewer 3): The aim of this study was to evaluate apoptosis related genes in nurses who work in chemotherapy wards and compare it to nurses who work in other wards.

Some topics to be improved:
10) There is no description of the objectives in the abstract.

Our answer: As requested by the respected reviewer, we included our research objectives in the abstract. Please see the revised version (all the corrections appear in yellow bands).

11) Methods: A brief procedure section could be added to the methods section to describe the sequence of events in the study and for the subjects. Due to the small sample size, it is necessary to clarify and describe: How was the sample size determined? - How were the subjects recruited?

Our answer: As requested by the respected reviewer, we added a brief procedure to the methods section to describe the sequence of events in the study and for the subjects. Regarding the sample size; all nurses that work in chemotherapy wards of Shahid Beheshti University hospitals were chosen by entering and exit criteria applied by physician test. The sample size was determined based on the power and standard deviation of variables in previous studies by power and sample size program software (50 nurses for each group). We included this explanation in the Materials and Method of revised version under Sample collection sub heading. Please see the revised version (all the corrections appear in yellow bands).

12) The total amount of blood sample (ml) must be reported. It is necessary details about anticoagulant.

Our answer: As requested by the respected reviewer, we added a brief procedure to the methods section to describe the sequence of events in the study and for the subjects. Blood samples were collected (about 15 ml) in tubes containing EDTA-K2 anticoagulant agent. We included this explanation in the Materials and Method of revised version under Sample collection sub heading. Please see the revised version (all the corrections appear in yellow bands).

13) Were the patients fasting? How long occupational exposure per week? It is necessary inform how many years of occupational exposure to these drugs in years or moths.

Our answer: Yes, as mentioned all the nurses given blood samples to us were fasting. As indicated in the Materials and Methods of revised version under the sub heading “Sample collection” The minimum working time in the chemotherapy ward was 6 months and 45 hours per week, and in Results section of revised version under the sub heading “Demographic data” The average age of oncology nurses was 29.27 years and in the control group were 28.69 years. Control nurses never work in the oncology ward and an average of times of exposure to oncology nurses was 27.43 months.

Please see the revised version (all the corrections appear in yellow bands).

14) Statistical analysis: The authors provided generic languages of the statistical methods used as if they were extracted directly from a statistical textbook.
Our answer: As requested by the respected reviewer, The unpaired T-test was used to compare the results between two nurses' groups using GraphPad Prism 7 software, with P<0.05 being significant. For RT-qPCR, relative quantification was made using the Pfaffl method and another analysis was performed by the Two-way ANOVA test and Bonferroni post-test. Based on pfaffle analysis, a ratio above 2 is considered as overexpression and below 0.5 as low expression. Please see the revised version (all the corrections appear in yellow bands).

15) Results section, Please give more the demographic information of groups in a table 2.

Our answer: As requested by the respected reviewer, we added another data” Alcohol drinking” and “Pregnancy numbers” into table 2. Please see the revised version (all the corrections appear in yellow bands).

16) Table 3 could be improved.

Our answer: As requested by the respected reviewer we statistically analyzed table 3 using chi-square test, and then we transferred the information of table 3 into figure 1 in the revised version. Please see the revised version (all the corrections appear in yellow bands).

17) The discussion of the apoptosis-related genes overexpression could be more developed. It could be the novelty of this paper.

Our answer: As requested by the respected reviewer we developed The discussion of the apoptosis-related genes overexpression. Please see the revised version (all the corrections appear in yellow bands).

18) The quality of the figures should be improved.

Our answer: As requested by the respected reviewer we improved the quality of the figures.

19) The authors could cite the limitations of this study

As explained in the answer of question 5 of Reviewer 1, the only limitation of this study was the lack of exposure biomarker, such as the values of antineoplastic drugs in the blood of the nurses. BUT this limitation does not make any weakness to the value of this paper because as explained in page 9, line 27, We couldn’t detect all chemotherapy drugs concentration in the blood of nurses because the number of drugs that are in use in chemotherapy wards of our country Iran are more than 40 different drugs and measurement of their exact concentration in the blood sample is not possible at the same time. Besides, these drugs have synergistic and antagonistic effects on each other. Therefore, the presence or absence of a single drug in blood at the time of
measurement is not a reason for the absence or present of adverse effects. Please see the revised version (I demonstrate this paragraph in yellow bands).

20) Editing of English language is required.
Our answer: As requested by the respected reviewer we revised manuscript’s English language with an appropriate vocabulary and grammatical check software. We also asked an English-speaking colleague in Canada to check the revised version.

21) Moreover, the references could be updated.
Our answer: As requested by the respected reviewer we updated the References section by adding more recently published papers. Please see the revised version (all the corrections appear in yellow bands).