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OPEN PEER REVIEW

Immunologic Profile of Severe COVID-19 Patients in Alborz Province, Iran

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About this report

This open peer review (OPR) report shows the public comments made on this article during peer review, by the reviewers, the associate editor and the editor-in-chief, for every revision round, together with the authors' replies. Comments appear only with the consent of the people who wrote them. Reviewers who chose to sign their report are named; the others are shown as "Referee". Comments are edited for language only, and confidential remarks to the editors are not included.

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1 Original submission

3 comments · 15 Jan 2023 – 30 Jan 2023

Referee 1 Reviewer

REJECT 15 Jan 2023

1. Obviously, protein expression is more important than mRNA levels in assessing the immunologic profile of each patient, especially when the aim is to address intervention with anti-inflammatory drugs. It seems that the most important drawback of this study is not using ELISA or western blot techniques to measure protein expression levels.
2. The methodology and findings presented in the current article did not emphasize the necessity of using personalized medicine.
3. The population investigated in this study is not statistically large enough to validate the results and generalize them universally.
4. Table No. 1 is not referred to anywhere in the text; however, the table itself is not informative enough, and it is not clear whether the primers were designed in this study or obtained from other studies. Also, the size of the PCR products was unclear.
5. The method of analyzing RT-qPCR results and the formula used to calculate the relative expression levels of the genes have not been explained.
6. Although the English language used in the text has few grammatical flaws, it needs to be edited due to the use of very simple language and the presence of some errors. For example, on page 6, line 8, “On the cDNA samples, a Q-6000 (Qiagen, Germany) device was used” is not correct, and on page 12, line 16, “Data available regarding IFNs, pro- and anti-inflammatory cytokines…” is also incorrect.

Referee 2 Reviewer

MAJOR REVISION 20 Jan 2023

The manuscript about the status of immunological factors in COVID patients provides useful information but needs to be revised considering the following points:
1- Exclusion and inclusion criteria for sample collection must be added.
2- What was the immune status of the study population regarding chronic or recent acute infections?
3- Instead of housekeeping gene, it is better to use reference gene.
4- More explanation is needed in the caption of fig 3.
5- The authors concluded that “The results could suggest that as the disease progresses to a severe state, type 1 and type 3 interferon production could be replaced by excessive proinflammatory cytokine secretion (IL-1, IL-6 and IL-10)” while they have not made any classification in terms of disease progression in the examined samples. How did they
come to this conclusion?
6- In the discussion, it was stated that “These findings are consistent with our earlier observations, which showed a non-significant difference in IFN-a, IFN-b and higher IL-10 expression between severe COVID-19 and control group. These findings shows that reduced levels of IFN-I in the peripheral blood of severe patients”; the two parts of the sentence contradict each other. Also, as mentioned above, it is not correct to refer to severe disease without separating the samples in terms of disease severity.
In fact, the study cannot describe the cytokine and interferon status because these factors change as the disease progresses.
7- The text needs to be rechecked for grammatical errors.

Associate Editor

MAJOR REVISION 30 Jan 2023

Reviewer 1:
Comments to the authors:
The manuscript about the status of immunological factors in COVID patients provides useful information but needs to be revised considering the following points:
1- Exclusion and inclusion criteria for sample collection must be added.
2- What was the immune status of the study population regarding chronic or recent acute infections?
3- It is better to use a reference gene instead of a housekeeping gene.
4- More explanation is needed in the caption of fig 3.
5- The authors concluded that "The results could suggest that as the disease progresses to a severe state, type 1 and type 3 interferon production could be replaced by excessive proinflammatory cytokine secretion (IL-1, IL-6 and IL-10)" while they have not made any classification in terms of the progression of the disease in the examined samples. How did they
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6- In the discussion, it was stated that "These findings are consistent with our earlier observations, which showed a non-significant difference in IFN-a, IFN-b and higher IL-10 expression between severe COVID-19 and control group. These findings show that reduced levels of IFN-I in the peripheral blood of severe patients"; the two parts of the sentence contradict each other. Also, as mentioned above, it is not correct to refer to severe disease without separating the samples in terms of disease severity.
In fact, the study cannot describe the cytokine and interferon status because these factors change as the disease progresses.
7- The text needs to be rechecked for grammatical errors.

Reviewer 2:
Comments to the authors:

1. Obviously, protein expression is more important than mRNA levels in assessing the immunologic profile of each patient, especially when the aim is to address intervention with anti-inflammatory drugs. It seems that the most important drawback of this study is not using ELISA or western blot techniques to measure protein expression levels.
2. The methodology and findings presented in the current article did not emphasize the necessity of using personalized medicine.
3. The population investigated in this study is not statistically large enough to validate the results and generalize them universally.
4. Table No. 1 is not referred to anywhere in the text; however, the table itself is not informative enough, and it is not clear whether the primers were designed in this study or obtained from other studies. Also, the size of the PCR products was unclear.
5. The method of analyzing RT-qPCR results and the formula used to calculate the relative expression levels of the genes have not been explained.
6. Although the English language used in the text has few grammatical flaws, it needs to be edited due to the use of very simple language and the presence of some errors. For example, on page 6, line 8: "On the cDNA samples, a Q-6000 (Qiagen, Germany) device was used" is not correct, and on page 12, line 16: "Data available regarding IFNs, pro- and anti-inflammatory cytokines…" is also not correct.

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Revision 1

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

The next pages contain the authors' point-by-point reply to the comments of this revision round, as submitted with the revised manuscript. It is published as the authors wrote it.

Dear Prof. Mahmoudabadi,

It is with immense pleasure that I resubmit to you a fully revised version of our manuscript entitled, "**An Immunologic Profile of COVID-19 Patients: Personalized medicine may be addressed in treating COVID-19 patients with anti-inflammatory drugs."**, for the prestigious journal "***Jundishapur Journal Of Microbiology"*.** We are grateful for granting us the opportunity to revise and resubmit this review article and wish to thank the esteemed reviewers for their insightful comments. The authors have done their best to rectify the issues raised by you and the reviewers to provide a point-by-point response to your comments. All changes are accessible through the track changes function in Microsoft Word. The manuscript has been revised by an English native speaker.

We hope that the revisions done by the authors and our accompanying responses will be sufficient to make our manuscript suitable for publication. We do appreciate in advance your kind consideration and shall look forward to hearing from you at your earliest convenience.

Best Regards,

Response to reviewers:

#1

Dear Reviewer,

Thank you for your kind and insightful comments. We have revised the manuscript to improve clarity and readability and to address the points raised by your comments.

Detailed response to your comments is as below:

1. Exclusion and inclusion criteria for sample collection mast be added?

Thank you for your kind input. The inclusion and exclusion criteria have been provided in the methods section.

2. What was the immune status of study population regarding of having chronic infection or recent acute infection?

Thank you. Based on our inclusion criteria, we did not include patients with chronic infection or recent acute infection. Immunosuppressed patients were excluded from our study.

3. Instead of housekeeping gene is better to use reference gene

Thank you. Changes amended.

4. A more explanation is needed for caption of fig 3.

Thank you. Changes amended.

5. The authors concluded that "The results could suggest that as the disease progresses to a severe state, type 1 and type 3 interferon production could be replaced by excessive proinflammatory cytokine secretion (IL-1, IL-6 and IL-10)" while they have not made any classification in terms of the progress of the disease in the examined samples. How did they come to this conclusion?

Thank you for this keen remark. We revised the above sentence according to our findings.

6. In discussion, it was stated that "These findings are consistent with our earlier observations, which showed a non-significant difference in IFN-a, IFN-b and higher IL-10 expression between severe COVID-19 and control group. These findings shows that reduced levels of IFN-I in the peripheral blood of severe patients " the two parts of the sentence contradict each other. And also, as mentioned above, it is not correct to refer to a severe disease without separating the samples in terms of the severity of the disease.

In fact, the study can not describe the cytokine and interferon status because these factors change by the disease progress.

Thank you. The whole section was revisited based on our findings.

7. The text needs to recheck for grammatical errors.

Thank you. A native English speaker revised the whole script.

We hope that by revising the manuscript, we have been able to provide a satisfactory response to the esteemed reviewer's comments. Please let us know if further edits are needed.

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#2

Dear Reviewer,

Thank you for dedicating your time to review this manuscript and your kind and insightful comments. We have amended the manuscript text to address the points raised by you during your comprehensive review.

Please refer to the section below for detailed edits and point by point response to your comments:

1. Obviously, protein expression is more important than mRNA levels in assessing immunologic profile of each patient, specially, when the aim is to address intervention with anti-inflammatory drugs. It seems that the most important drawback of this study is not using ELISA or western blot techniques to measure protein expression levels.

Thank you for these insightful comments. We totally agree with what you mentioned. These results are the preliminary findings of our future projects which we will measure the cytokine levels using ELISA assays alongside RNA expression levels. To address your important comment, we will describe this limitation of our study in the discussion section. Although it should mentioned that the previous research has supported the positive correlation between RNA expression and protein expression levels (1).

2. The methodology and findings presented in the current article did not emphasize the necessity of using personalized medicine.

Thank you for your comment. We removed the personalized medicine section from the title of our manuscript.

3. The population investigated in this study is not statistically enough to validate the results and generalize them into a universal reality.

Thank you. Prior to our sampling, a statistician was contacted regarding the optimal sample size needed for this study. The sample size for each group was calculated with G*POWER software (Power= 0.95, Effect Size= 3.12). However, the results should be confirmed in a large cohort. We addressed this issue in the discussion part.

4. Table No. 1 is not referred to anywhere in the text however, the table itself is not informative enough and it is not clear whether the primers were designed in this study or obtained from other studies. Also the size of PCR products was unclear.

Thank you. We removed Table 1 from the manuscript.

5. The method of analyzing RT-qPCR results and the formula used to calculate the relative expression levels of the genes have not been explained.

Thank you. We explained the details of analyzing RT-qPCR *results and the formula used to calculate the relative expression levels of the genes in section "RNA extraction, cDNA synthesis, and qRT-PCR".*

6. Although the English language used in the text has few grammatical flaws, it needs to be edited due to the use of very simple language and the presence of some errors. For example, page 6 line 8: "On the cDNA samples, a Q-6000 (Qiagen, Germany) device was used" is not correct or in page 12 line 16: "Data available regarding IFNs, pro- and anti-inflammatory cytokines..." is not also correct.

Thank you. A native English speaker revised the whole script.

We hope that the revised manuscript is able to address all of the points raised during the review by the esteemed reviewer. Please let us know if further edits are needed.

1. Guo Y, Xiao P, Lei S, Deng F, Xiao GG, Liu Y, et al. How is mRNA expression predictive for protein expression? A correlation study on human circulating monocytes. Acta biochimica et biophysica Sinica. 2008;40(5):426-36.

2 Revision 1

0 comments

No public comments in this round.