Jundishapur Journal of Microbiology
Volume 17 · Issue 2 · e145397

OPEN PEER REVIEW

Evaluation of Integrated SARS-CoV-2 Genome Presence in PBMC, Oropharyngeal, and Nasopharyngeal Samples of COVID-19 Patients

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SUBMITTED	REVISED	ACCEPTED	DOI
27 Jan 2024	16 Feb 2024	17 Feb 2024	10.5812/jjm-145397

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

2 comments · 30 Jan 2024 – 2 Feb 2024

Referee 1 Reviewer

MINOR REVISION 30 Jan 2024

In general, the authors evaluated the presence of an integrated SARS-CoV-2 genomic DNA form in PBMC, oropharyngeal and nasopharyngeal samples from COVID-19 patients.

These are my comments:

From an editorial point of view, the article's flow and progression were reasonable; however, I encountered several typographical errors and grammar and writing issues throughout the text. Please double-check the whole text for revision.

- The title reflects the manuscript content.

- The abstract is adequate, describing the message of the experiments.

- Background: Adequately described.

- M&M: Reproducible.

- Results are described in a proper way.

- Discussion: It is nicely organized and presented, justified by the data, and compared with other similar literature.

- References are up to date; just double-check for consistency and the accuracy of journal names.

Overall comments:
Despite having no novelty, the scientific content of the manuscript is interesting and worth reporting for the health care system, enabling researchers to compare the results from different parts of the world and debate the contradictory results. Just double-check the whole text for writing errors and apply corrections with critical consideration.
It has merit for publication after minor modification.

Referee 2 Reviewer

MAJOR REVISION 2 Feb 2024

Thanks for inviting me to review the manuscript entitled “Evaluation of the Presence of Integrated SARS-CoV-2 Genome in PBMC, Oropharyngeal and Nasopharyngeal Samples of COVID-19 Patients”. This topic is interesting, but there are a couple of major issues that need to be addressed:
• Abbreviations such as COVID-19 and SARS-CoV-2 in the abstract and throughout the manuscript should be written out in full when first mentioned.
• The main concern about this study relates to the patients. How were the patients selected? What about the patients who were hospitalized, recovered, and then discharged? What is the control group? Which variant of the virus were the patients infected with?
• What was the vaccination status of all the patients? Which types of vaccine did the patients receive?
• Inclusion and exclusion criteria are missing.
• Why did the respected authors examine only three genes? How were these 3 genes chosen to check for the presence or absence of the viral genome? Some studies have been able to find traces of the viral genome after deep sequencing. Maybe the reason for not finding the genome is the examination of three genes.
• What was the positive control? How was the accuracy of the primers confirmed?

- Limitations and strengths of the study should be mentioned in the discussion section.
- Why did the respected authors not mention Moustafa et al.'s study (doi: 10.1089/omi.2021.0068)?
- Table 5: It would be better to add an additional column for references.

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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.

ACTION: ID 145397 Needs Major Revision; rev [1]

Manuscript ID: 145397

Dear colleague,

I am writing to inform you that, I have submitted a revision of my **manuscript ID: 145397** entitled **"Evaluation of the Presence of Integrated SARS-CoV-2 Genome in PBMC, Oropharyngeal and Nasopharyngeal Samples of COVID-19 Patients"**. I've been answering to each of the reviewer comments point-by-point. I hope you accept my responses.

Yours sincerely,

Dr. Farah Bokharaei-Salim

Reviewers' Comments:

Reviewer 1:

In general, the authors evaluated the presence of integrated SARS-CoV-2 genomic DNA form in PBMC, oropharyngeal and nasopharyngeal samples of COVID-19 Patients.

These are my comments:

From the editorial point of view, the article flow and path was reasonable, however, I encountered several typo mistakes and grammar and writing issues throughout the text. Please double-check the whole text for revision.

Reply: Thank you for your consideration in improving the level of this article. I revised the entire text (grammar and writing issues throughout the text) and highlighted the changed parts in red.

1. The title reflects the manuscript content.

Reply: Thank you

2. Abstract is adequate, describing the message of the experiments.

Reply: Thank you

3. Background: is adequately described.

Reply: Thank you

4. M&M: is reproducible.

Reply: Thank you

5. Results are described in proper way.

Reply: Thank you

6. Discussion: It is nicely organized and presented on the data justified, compared with other similar literatures.

Reply: Thank you

7. References are up to date, just double-check for the consistency and journals names accuracy.

Reply: I double checked all the references.

Overall comments:

Despite having no novelty, the scientific content of the manuscript is interesting and worth reporting for the health care system enabling researchers to compare the results from different parts of the world and debate the contradictory results. Just double-check the whole text for the writing errors and apply corrections with critical consideration.

It has merit for publication after minor modification.

Reply: Thank you for analyzing this manuscript in detail. I corrected the problems you mentioned.

Reviewer 2:

Thanks for inviting me to review the manuscript entitled "**Evaluation of the Presence of Integrated SARS-CoV-2 Genome in PBMC, Oropharyngeal and Nasopharyngeal Samples of COVID-19 Patients**". This topic is interesting but there are a couple of major issues that need to be addressed:

Reply: Thank you for your consideration in improving the level of this article.

1. The abbreviation such as COVID-19, SARS-CoV-2, and etc. in the abstract and throughout the manuscript should be fully written when mentioned for the first time.

Reply: I have done as you suggested and colored the changed parts in red.

Line 9 and 10:

(**Background:** The long-term severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA detection in individuals who successfully underwent coronavirus disease (COVID-19) is an unexplained phenomenon that deserves further investigation.)

Line 29 and 30:

As of November 27, 2022, one of the dangerous respiratory diseases called coronavirus disease (COVID-19) that stemmed from severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) resulted in more than 645 million approved cases of the disease and more than 6.6 million mortalities.

2. The main concern about this study entails the patients. How were the patients selected? What about the patients who were hospitalized, recovered and then discharged? What is the control group? Which variant of the virus were the patients infected with?

Reply:

A. The studied patients were actually those who tested positive for SARS-CoV-2 infection. These people were either treated on an outpatient basis (the first group) or their condition worsened and they were admitted to the hospital (the second group).

In line 73 to 79 we described our studied groups:

B. Of course, it should be mentioned that people were included in the study who agreed to come to the researcher every 15 days for the molecular detection test of SARS-CoV-2 infection (in oropharyngeal and nasopharyngeal samples of patients) and also to give blood to separate peripheral blood mononuclear cells (for detection of SARS-CoV-2 integrated genome in PBMC sample).

This was done in order to see, firstly, whether the virus genome is present in the peripheral blood mononuclear cells, and secondly, if the virus is cleared from the nasopharynx and oropharynx, the virus is still present in the peripheral blood mononuclear cells or not.

C. Regarding the hospitalized patients, they agreed that the researcher would visit them every 15 days and take the required samples, or after being discharged from the hospital, the researcher would visit their homes every 15 days and take samples from them.

D. The negative control group (healthy group) in terms of SARS-CoV-2 infection (third group), were relatives and acquaintances of the researcher, as well as professors and students of the virology department of Iran University of Medical Sciences who had agreed to cooperate with the researcher.

E. As mentioned in the last part of the discussion about the limitations of the plan, all the people with Covid-19 disease were infected with the delta variant of SARS-CoV-2.

It is written in lines 196 to 199:

The small sample size and examination of SARS-CoV-2 integration in only cases infected with the delta variant are the limitations of this study. These may affect our findings. Therefore, our interpretations of these findings should be considered cautiously.

3. What was the vaccination status of all the patients? Which types of vaccine did the patients use?

Reply: Unfortunately, we do not have complete information about the vaccination of the studied subjects.

4. Inclusion and exclusion criteria are missing.

Reply: Inclusion and exclusion criteria are:

This part was in the manuscript and I just added the word " inclusion criteria " and " exclusion criteria " and made it red:

It is written in lines 80 to 85:

As mentioned earlier, in the present survey, the presence of SARS-CoV-2 genome in the PBMCs, Oropharyngeal as well as Nasopharyngeal samples of cases suffering from COVID-19 disease were evaluated (inclusion criteria). It is noteworthy that none of the individuals with COVID-19 disease and healthy participants who entered to this research as controls (exclusion criteria) had co-infection with human cytomegalovirus (HCMV), mycobacterium tuberculosis, hepatitis B virus (HBV) and hepatitis C virus (HCV), human immunodeficiency virus (HIV).

5. Why did the respected authors examine only three genes? How have these 3 genes been chosen to check the presence or absence of the vial genome? Some studies have been able to

find traces of the viral genome after deep sequencing. Maybe the reason for not finding the genome is the examination of three genes.

Reply: These three genes have been used in many references to identify this virus. Since I have been using this method for many years to identify occult HCV and HBV infection, and it is a very powerful method, I chose these three genes to detect this virus sequences in PBMC samples. It is worth mentioning that if I could trace this virus genome, I would sequence it in the next step. Of course, the full genome sequencing method for this virus, which has a very long length, is very expensive and beyond the financial capacity of this project.

6. What was the positive control? How was the accuracy of the primers confirmed?

Reply: This study was conducted in one of the centers for the molecular diagnosis of SARS-CoV-2 infection of the Vice-Chancellor of Health, Iran University of Medical Sciences, which is approved by the Ministry of Health and Medical Education of Iran. It should be noted that molecular diagnosis kits approved by the Ministry of Health are used in this laboratory. The positive samples of this laboratory were used as positive control samples in terms of infection with SARS-CoV-2.

The sequence of primers and probes used in this research is taken from authentic articles, and the method of doing the work, etc., has been carefully set up in the laboratory. All the results of the tests conducted in this research have been checked with the kits approved by the Ministry of Home Affairs that have been sent to the laboratory.

7. Limitations and strengths of the study should be mentioned in the discussion section.

Reply: In the final part of the discussion, the limitations of this research are mentioned, as follows:

It is written in lines 196 to 199:

The small sample size and examination of SARS-CoV-2 integration in only cases infected with the delta variant are the limitations of this study. These may affect our findings. Therefore, our interpretations of these findings should be considered cautiously.

8. Why did the respected authors not mention Moustafa et al.'s study (doi: 10.1089/omi.2021.0068)?

Reply: Thanks for your suggestion, a part of this article has been added to the manuscript:

This part was added to the article in lines 41 to 43:

It has been reported in a research that they were able to find the RNA of SARS-CoV-2 in peripheral blood mononuclear cell (PBMC) samples in different studies by analyzing the available sequence information in two samples from two independent studies (7).

The address of the desired article was added to the references section in lines 231 to 232:

7. Moustafa A, Khalel RS, Aziz RK. Traces of SARS-CoV-2 RNA in Peripheral Blood Cells of Patients with COVID-19. OMICS. 2021;25(8):475-83.

9. Table 5: Additional column for references is better to be added.

Reply: Dear colleague, this table is a report of the laboratory results obtained from the samples of the three studied groups and does not need to be referenced **(Line 321).**

Associate Editor's Comments:

Associate Editor 1:

Dear Dr Zarei

The manuscript entitled "Evaluation of the Presence of Integrated SARS-CoV-2 Genome in PBMC, Oropharyngeal and Nasopharyngeal Samples of COVID-19 Patients" needs MAJOR revision. Authors have to answer all question raised by reviewers point by point.

Reply: As you suggested, the reviewers' comments were answered point by point.

2 Revision 1

0 comments

No public comments in this round.