Archives of Pediatric Infectious Diseases
Volume 12 · Issue 2 · e135959

OPEN PEER REVIEW

Co-detection of Human Herpes Viruses in Cerebrospinal Fluid Samples of Pediatric Patients with Multi-System Inflammatory Syndrome: A Single Center Experience in Isfahan, Iran

Hamid Rahimi Hajiabadi, Rana Saleh, Niloofar Javadi, Amin Dehghan, Atefeh Sadeghizadeh, Sharareh Moghim, Maryam Khalili, Zahra Pourmoghaddas

SUBMITTED	REVISED	ACCEPTED	DOI
23 Mar 2023	26 Feb 2024	27 Feb 2024	10.5812/apid-135959

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.

More about open peer review: brieflands.com/knowledgebase/opr

Contents

1 **Original submission** Referee 1, Marzieh Jamalidoust, Associate Editor 2
2 **Revision 1** Marzieh Jamalidoust, Referee 1, Associate Editor 5
Authors' reply to reviewers 6
Reviewer and editor comments 16
3 **Revision 2** 18
Authors' reply to reviewers 19
Reviewer and editor comments 23

1 Original submission

3 comments · 29 Mar 2023 – 18 May 2023

Referee 1 Reviewer

MAJOR REVISION 29 Mar 2023

Dear author
Thank you for your submission.
Totally:
It is necessary to note that a positive EBV PCR result in CSF is nonspecific and may not be associated with the patient's neurological symptoms. Please check "Clinical significance of Epstein-Barr virus polymerase chain reaction in cerebrospinal fluid. Clinical significance of Epstein-Barr virus PCR in cerebrospinal fluid encephalitis. 2022;2(1):1-8 Publication date (electronic) : 2021 November 17 doi : https://doi.org/10.47936/encephalitis.2021.00115". In this situation, it is recommended to use other diagnostic tools, such as laboratory findings and imaging, to confirm EBV-related disease.
As you know, encephalitis can occur as a component of MIS-C, so it is necessary to know your objective in showing such co-infection. What are your recommendations regarding treatment?

Abstract:
What does this sentence mean: "EBV and HSV were detected in three, two, and one patients." Which virus was the one patient positive for? Line 17-18
Background:
Please explain the pathogenesis of co-infection in patients with MIS-C encephalitis and the role of the COVID-19 virus.
What is the reference for this sentence? "However, no relationship between the occurrence of COVID-19-associated reactivation of viruses and neurological involvements is reported by previous studies."
If the above sentence is postulated, why was this study done?
Material and Method:
How did you calculate the sample size? This sample size is not sufficient for any statistical analysis.
Results:
In one case, the CSF was positive for SARS-CoV-2. As we know, the pathogenesis of MIS-C involves activation of the immune system by the COVID-19 virus, so the main cause of CNS involvement in these cases is related to their original disease. How can these findings be logically related, and how can the effects of different viruses be distinguished?
What is the severity of MIS-C in your cases, and how were these cases differentiated from severe COVID-19 infection?
Why were all cases treated with the same protocol? As you know, the treatment protocol changes based on severity and organ involvement. For example, Kawasaki cases are treated with aspirin, and severe cases are treated with prednisolone pulse therapy.
Please specify which CNS findings, such as imaging or other findings, are related to which virus. The sentences in this part are confusing. Line 116-120
Despite positive PCR findings in two cases with HSV, how could you decide not to start antiviral treatment? Did you test the CSF a second time? Did you consider the results to be false positives, contamination, or something else?
What was the difference between PCR+ and PCR- cases?
Discussion:
The main focus of the study is encephalitis in children with MIS-C, but there is no explanation of CSF or imaging findings in these cases. Please provide articles in this field and explain the clinical, laboratory, and

imaging findings related to the CNS in cases with different PCR results.
Please discuss the findings in the Results section and compare them with other authors' findings.
Conclusion:
How did you show spontaneous recovery in your cases? It is not mentioned in the Results section. Did the PCR+ cases become PCR-?
Best Regards

Marzieh Jamalidoust Reviewer

MAJOR REVISION 26 Apr 2023

Dear editor of "Archives of Pediatric Infectious Diseases"

Thank you for inviting me to review this article entitled " Reactivation of various viruses in the patients with the coincidence of multisystem inflammatory syndrome in children and encephalitis". Congratulations to the authors on carrying out this valuable project. I have some comments that I hope can improve the manuscript.
1- There are some writing errors in the manuscript, so please check and correct them.
2- Since reactivation is not a suitable term for some of the evaluated viruses, such as influenza, it is better to rewrite the title. Proposed title: "Viral infection pattern in pediatric MIS-C patients with encephalitis"
3- The sentences in lines 17-18 of the "Abstract" do not correctly reflect the number of cases of HSV and EBV infection.
4- In the objective of the manuscript, the term reactivation is used again, although it can only be used for members of the herpes family.
5- The sentences in lines 41-43 need to be reviewed and referenced.
6- The first and second sentences of lines 44-45 should be combined.
7- In the data collection section, you must describe the exact method of performing PCR for EBV, HSV, influenza, and other viruses.
8- In line 103, it is suggested that other underlying diseases be discussed.
9- In line 106, the p-value should be presented to indicate whether the two groups differed statistically significantly!
10- In Table 1, add a column titled p-value to describe the statistical difference by presenting this statistical index.
11- In the first sentence on line 111, add "respectively" to the end of the sentence.
12- In lines 116 and 123, "viral genome" should be used instead of "viral particle".
13- The discussion section should provide more information about the importance of the association between encephalitis and MIS-C.
14- What was the reason for performing the influenza PCR test on the CSF of patients with COVID-19 during the peak period of this disease if it was possible to perform the SARS-CoV-2 PCR test on those samples (CSF samples)?

Associate Editor

REJECT 18 May 2023

Dear authors,
Your manuscript has now been reviewed. Any reviewer comments on the suitability of your manuscript have been appended below. As a result, I regret to inform you that we cannot publish your manuscript in the Archives of Pediatric Infectious Diseases.
Thanks

Editor's comment

The authors could not confirm the causal association between co-detected viral pathogens and neurological findings in the context of MISC. So, the title is misleading. "Co-detection of human herpes viruses in cerebrospinal fluid samples of pediatric patients with the multi-system inflammatory syndrome infected by SARS-CoV-2" may be better wording for introducing the study subject.

What did you think about isolated CSF reactivation of HHVs (of course, if it actually occurred) without concurrent viremia in the context of a systemic disease like MISC? Why did you not check the serum HHV-qPCR simultaneously?

Without thorough diagnostic investigation (including EEG, serum qPCR assay, and concurrent virologic assays), any conclusion should be made cautiously.

The sample size is very small.

It is necessary to determine the SARS-CoV-2 CSF PCR status of tested children.

2

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 editor

Thank you very much for communicating the comments of reviewers to me which are invaluable to guide revision of the manuscript. We also wish to acknowledge the reviewers for the time and effort that they have put into assessing our manuscript as well as for their careful and constructive revision. We have now revised the manuscript in light of reviewer's comments and tried to fully accommodate the concerns of reviewers resulting in an improved manuscript. I hope our revision has improved the manuscript to a level of their satisfaction. I am grateful for your consideration, and I hope that you will find the current version of the manuscript suitable for publication in **Archives of Pediatric Infectious Diseases**. I look forward to hearing from you and to respond to any further questions and comments you may have.

Best regards

Dr. Zahra Pourmoghaddas

Reviewer 1:

1- There are some writing errors in the manuscript, so please check and correct them.

Response: grammatical errors were corrected.

2- Since reactivation is not a suitable term for some of the evaluated viruses such as Influenza, it is better to rewrite the title. proposed title: "Viral infection pattern in the pediatric MIS-C patients coincide with encephalitis"

Response: was corrected.

3- The sentences mentioned in lines 17-18 of the "Abstract" do not correctly reflect the number of cases of the HSV and EBV viruses.

Response: was corrected.

4- In the objective of the manuscript, the term reactivation is used again when it can only be used for members of the herpes family.

Response: was corrected.

5- The sentences in lines 41-43 need to be reviewed and referenced.

Response: was corrected.

6- The first and second sentences of lines 44-45 should be combined.

Response: was performed.

7- In the data collection section, you must describe the exact method of performing PCR for EBV, HSV, influenza, and other viruses.

Response: Dear reviewer, Our study utilizes the method mentioned in the following reference.

Khalili M, Hajiabadi HR, Akbari M, Saleh R, Esfahani BN, Moghim S. Assessment of a multiplex RT-PCR for Simultaneous, Rapid Screening of Common Viral Infections of Central Nervous System: A Prospective Study for Enteroviruses and Herpesviruses. Postępy Higieny i Medycyny Doświadczalnej. 2022 Jan 1;76(1):91-6.

8- In line 103, it is suggested to talk about other underlying diseases.

Response: was corrected. None of them had any underlying neurological or systemic disease.

9- In the line of 106, by presenting the p-value, it should be determined whether the two groups had a statistically significant difference or not!

Response: Thanks for your comment. We added p-value; however, there is no significant difference between groups.

10- In Table 1, add a column under the title p-value to describe the statistical difference by representing this statistical index.

Response: Thanks for your comment. We added p-value.

11- In the first sentence on line 111, add "respectively" to the end of the sentence.

Response: was performed.

12- In lines 116 and 123, "viral genome" should be used instead of "viral particle".

Response: was performed.

13- The discussion part should provide more information about the importance of the relationship between the occurrence of encephalitis in patients with MIS-c.

Response: was performed.

14- What was the reason for performing the influenza PCR test on the CSF of patients with covid-19 during the peak period of this disease, if it was possible to perform the SARS-CoV-2 PCR test on the said samples (CSF Samples)!

Response: Due to the fact that encephalitis patients were examined in this study, it is necessary to check for influenza in the CSF.

Reviewer 2:

1- in line 92 in data collection section, you said “follow up visits were performed to evaluate …”, on the other hand, in line 52, you stated that “The present study is a descriptive cross-sectional study”, if you were followed the patients, so your study was not cross-sectional, please explain about this.

Response: was added (analytical cross-sectional study)

2- you used of similar sentences in line 102 and line 106, in line 102, you stated that “The median age of patients was 44 months” and in line 106 you expressed that “Forty-four-month-old was the median age of this group”, please delete one of them.

Response: was performed.

3- why you reported the median age of patients? Do you have a specific reason for not reporting average?

Response: We only intended to provide complete information about the patient.

4- In line 121, you stated that” Table 2 compares laboratory data between the PCR+ and PCR- groups”, you didn’t use any statistical test in your study?

Response: the results of statistical analyze was added in Table 1.

5- in line 126, you reported the median age as an indicator of concentration, why didn’t you use the average?

Response: Because our data did not have normal distribution, we use median instead of mean.

6- in line 133, you concluded that “the quality of life in these patients was normal after six months”, did you analyze data from PedsQL? Did you use questionnaire? Please explain about this result.

Response: we used PedsQL questionnaire for analyze quality of life in the patients. The analysis of PedsQL showed no significant difference between the two groups in terms of assessing the quality of life of patients.

7- were patient follow up data reported only descriptively? You didn’t analyze these data?

Response: the results of statistical analyze was added in Table 1.

Reviewer 3:

The number of samples is not enough to draw conclusions.

Response: Dear reviewer, in the present study, we investigated patients with multisystem inflammatory syndrome in children (MIS-C) and encephalitis in Isfahan city. The frequency of patients with these characteristics is very rare.

Reviewer 4:

Totally:

1- It is necessary to remind that positive EBV PCR in CSF is nonspecific and may not be associated with patient’s neurological symptoms. Plz check “Clinical significance of Epstein-Barr virus polymerase chain reaction in cerebrospinal fluid. Clinical significance of Epstein-Barr virus PCR in cerebrospinal fluid encephalitis. 2022;2(1):1-8 Publication date (electronic): 2021 November 17 doi: https://doi.org/10.47936/encephalitis.2021.00115 In this condition it is recommended to use other diagnostic tools such laboratory findings and imaging to confirm EBV related disease

Response: EBV reactivation does not cause neurological symptoms. The CNS involvement in these patients has been attributed to MIC-S.

2- As you know encephalitis due to MIS-C involvement can occur and it is one of its components so it is necessary to know what is your objective to show such co-infection.? Recommendation on treatment field?

Response: In this study, it was necessary to collect CSF samples to rule out other causes. The PCR test was performed retrospectively, and viral particles were confirmed in the patients. Considering the lack of administration of antiviral medication and a favorable disease prognosis, we speculate that the viruses have undergone reactivation.

Abstract:

3- What does this sentence mean “EBV and HSV were detected in three, two, and one patients” One patient was positive with what? Line 17-18.

Response: was corrected.

Background:

4- Plz give explanation about the pathogenesis of co-infection in patients with MIS-C encephalitis and what is the rule of COVID-19 virus

Response: was performed.

5- What is the reference of this sentence?” However, no relationship between the occurrence of COVID-19-associated reactivation of viruses and neurological involvements is reported by previous studies.”

Response: the references of this sentence was added.

6- If the above sentence is postulated, why this study was done?

Response: Dear reviewer, there are a limited information in this issue, and further studies are required.

Material and Method:

7- How did you calculate the sample size; this sample size is not enough for any statistical analysis?

Response: Dear reviewer, in the present study, we investigated patients with multisystem inflammatory syndrome in children (MIS-C) and encephalitis in Isfahan city. The frequency of patients with these characteristics is very rare.

Results:

8- In one case sars-cov2 was positive in CSF. As we know MIS-C pathogenesis is activation of immune system by COVID19 virus, so the important cause of CNS involvement in these cases are related to their original disease, how these findings can be related logically and how the effect of different viruses are separated?

Response: In this study, it was necessary to collect CSF samples to rule out other causes. The PCR test was performed retrospectively, and viral particles were confirmed in the patients. Considering the lack of administration of antiviral medication and a favorable disease prognosis, we speculate that the viruses have undergone reactivation.

9- What is the severity of MIS-C in your cases and how they were differentiated with sever COVID19 infection?

Response: The severity of illness in cases under investigation were moderate and required hospitalization, but none of them were admitted to the ICU and were not in shock state.

10- Why all cases were treated with the same protocol? As you know treatment protocol is changed based on severity and Organ involvement for example Kawasaki cases are treated with aspirin and sever cases are treated with prednisolone pulse therapy.

Response: All cases were moderate treated with the same protocol.

Khalili M, Hajiabadi HR, Akbari M, Saleh R, Esfahani BN, Moghim S. Assessment of a multiplex RT-PCR for Simultaneous, Rapid Screening of Common Viral Infections of Central Nervous System: A Prospective Study for Enteroviruses and Herpesviruses. Postępy Higieny i Medycyny Doświadczalnej. 2022 Jan 1;76(1):91-6.

11- Please specify which finding in CNS such as imaging or other findings are related to which virus? The sentences in this part are confusing. Line 116-120

Response: was mentioned in Table 1.

12- In spite of PCR positive finding in two cases with HSV how could you decide not to start antivirus treatment, did you check the CSF for the second time? Did you assume the result as false positive or contamination or……?

Response: The samples were meticulously collected using a sterile method to ensure they were contamination-free. PCR testing was conducted on each sample using two methods, and both tests yielded positive results. Following an examination for MIS-C criteria, these individuals displayed symptoms of encephalitis, prompting the collection of CSF samples for PCR and culture analysis. The PCR results were obtained two weeks later, and upon diagnosis of MIS-C, they were promptly treated. After a 6-month follow-up, all patients were without complications, despite not receiving antiviral drugs

13- What was the difference between PCR+ and PCR- cases?

Response: the patients with viral genome detected by PCR (PCR+) and the patients without viral genome detected by PCR (PCR-).

Discussion:

14- The main focus of study is encephalitis in children with MISC but there is no explanation about CSF or imaging findings in these cases, please give articles in this field and explain clinical and laboratory and imaging findings of CNS in cases with different PCR results.

Response: The mentioned data has been provided. in Table 2. We added some imaging findings in the manuscript. We did not find any publication on this regard.

15- Please discuss on the findings of result part and compare it with other authors findings.

Response: was corrected.

Conclusion:

16- How did you show the spontaneous recovery in your cases? It is not mentioned in result part. Did the PCR+ cases changed to PCR-?

Response: was corrected.

Associate Editor 1:

1- The authors could not confirm the causal association between co-detected viral pathogens and neurological findings in the context of MISC. So, the title is misleading. "Co-detection of human herpes viruses in cerebrospinal fluid samples of pediatric patients with the multi-system inflammatory syndrome infected by SARS-CoV-2" may be better words for introducing the study subject.

Response: was corrected.

2- What did you think about isolated CSF reactivation of HHVs (of course if actually occurred) without concurrent viremia in the context of systemic disease like MISC? Why did not check the serum HHV-qPCR simultaneously?

Response: In the protocol used in this study, simultaneous examination of serum and CSF is not necessary.

3- Without thorough diagnostic investigation (including EEG, serum qPCR assay, and concurrent virologic assays), any conclusion should be made cautiously.

Response: the conclusion was corrected.

4- The sample size is very small.

Response: Dear editor, in the present study, we investigated patients with multisystem inflammatory syndrome in children (MIS-C) and encephalitis in Isfahan city. The frequency of patients with these characteristics is very rare.

5- It is necessary to determine the SARS-CoV-2 CSF PCR status of tested children.

Response: was added to the results section.

2 Revision 1

3 comments · 25 Dec 2023 – 2 Jan 2024

Marzieh Jamalidoust Reviewer

ACCEPT 25 Dec 2023

Dear editor of “Archives of Pediatric Infectious Diseases”
Thank you and the authors. Corrections were made in response to all questions asked; only the answer to question 14 (reviewer 1) was not convincing, due to the very low probability of influenza causing encephalitis and the very low prevalence of influenza during the COVID-19 pandemic in Iran and other parts of the world.

Referee 1 Reviewer

MINOR REVISION 31 Dec 2023

Dear Author
Thank you for the correction.
There are a few notes:
Method:
1- What are the characteristics of the hospital and its referral and admission rates?
What were the exclusion and inclusion criteria?
Discussion:
1- Please explain the reason for not treating patients with positive PCR results for HSV in their CSF.
2- Please provide a more extensive literature review of your other study results and discuss them accordingly.
3- Please explain the advantages of your study.
Best regards

Associate Editor

MINOR REVISION 2 Jan 2024

Dear authors
We are pleased to inform you that your manuscript has passed through the review stage and is ready for revision. The manuscript's overall presentation improved after amendments; however, the manuscript still needs revision.

Editor comments:
First of all, the English language of the submitted version is weak in the revised parts.
Second, the conclusion interprets CSF HHVs co-detection as an HHVs reactivation phenomenon without strong evidence (even without an available pre_MISC HHVs status to confirm any reactivation). So, as mentioned in the previous round, your findings did not support your aim regarding reactivation, and you should simply report raw results and avoid inaccurate interpretation.

The title should be corrected. "Co-detection of human herpes viruses in cerebrospinal fluid samples of pediatric patients with the multi-system inflammatory syndrome, a single center experience, Isfahan, Iran"

Your response to the following Editor's comment is unacceptable:
"What did you think about isolated CSF reactivation of HHVs (of course if actually occurred) without concurrent viremia in the context of systemic disease like MISC? Why did not check the serum HHV-qPCR simultaneously?" Is there a piece of evidence to support "isolated CSF reactivation of HHVs"? The

discussion should be improved.
Besides, inadequate diagnostic workup should be mentioned as a study limitation in the discussion section.

Finally, follow the respected reviewers' comments point-by-point.
Yours

3

Revision 2

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 editor

Thank you very much for communicating the comments of reviewers to me which are invaluable to guide revision of the manuscript. We also wish to acknowledge the reviewers for the time and effort that they have put into assessing our manuscript as well as for their careful and constructive revision. We have now revised the manuscript in light of reviewer's comments and tried to fully accommodate the concerns of reviewers resulting in an improved manuscript. I hope our revision has improved the manuscript to a level of their satisfaction. I am grateful for your consideration, and I hope that you will find the current version of the manuscript suitable for publication in **Archives of Pediatric Infectious Diseases**. I look forward to hearing from you and to respond to any further questions and comments you may have.

Best regards

Reviewer 1:

Dear author, thanks for applying my comments, please consider below comments:

1. Comment 1: in table 1, it's better that replace p-value>0.999 instead of 1 in last column of table 1.

Response: Was performed.

2. Comment 2: please add the percentage values in table 1.

Response: Was performed.

Reviewer 2:

I read the revised manuscript, I have no more comments to add.

Response: Thank you for your attention.

Reviewer 3:

Dear editor of "Archives of Pediatric Infectious Diseases", Thank you and the authors. Corrections were made in all asked questions, only for question 14 (reviewer 1), the answer was not convincing due to the very low probability of Influenza causing encephalitis and very low prevalence of influenza during the COVID-19 pandemic in Iran and the other parts of the world.

Response: Patients presenting respiratory symptoms and encephalitis may not necessarily have COVID-19. Consequently, both viruses were tested for in both the nasopharyngeal and cerebrospinal fluid samples. As you are aware, the coexistence of COVID-19 and influenza has been reported in studies.

Reviewer 4:

Thank you for correction, there are a few notes:

1. Method: what are the characteristic of hospital, referral and admission rate?

Response: The pediatric referral hospital admits 1000 patients per month

2. Method: What was the exclusion and inclusion criteria?

Response:

inclusion criteria: Completing the encephalitis and (MIS-C) criteria form and parental consent for entry into the study.

Exclusion criteria: Parental dissatisfaction and reluctance to complete two criteria forms.

3. Discussion: Pls explain the reason of no treatment in patients with positive PCR of HSV in their CSF.

Response: Since the study was conducted retrospectively, initially only cerebrospinal fluid analysis and culture were available. Given the normal results of these tests and patients completing the MIS-C criteria form, they exclusively underwent MIS-C treatment and were not administered antiviral therapy. Performing the PCR test was done retrospectively and solely as a diagnostic test, which was conducted after receiving MIS-C treatment

3. Discussion: Pls give more literature review of your other study results and discuss them accordingly.

Response: We added some other studies in Discussion section.

3. Discussion: Pls explain the advantages of your study.

Response: The advantage of our study is essentially introducing patients with Reactivation. These individuals exhibited distinct prognoses and required different treatment approaches compared to patients experiencing primary infection and virus-induced encephalitis

Associate Editor 1:

1. First of all, the English language of the submitted version is weak in the revised parts.

Response: The manuscript is being submitted to the Kowsar Institute for English language editing purposes."

2. Second, the conclusion is generalized CSF HHVs co-detection to HHVs reactivation phenomenon without strong evidence (even without available pre_MISC HHVs status to confirm any reactivation). So, as mentioned in the previous round your findings did not support your aim regarding reactivation and authors should simply report raw results and avoid inaccurate interpretation.

Response: We proposed a probability, and undoubtedly, more research is required for a definitive outcome."

3. The title should be corrected. "Co-detection of human herpes viruses in cerebrospinal fluid samples of pediatric patients with the multi-system inflammatory syndrome, a single center experience, Isfahan, Iran"

Response: was corrected

4. Your response to the following Editor's comment is unacceptable:

"What did you think about isolated CSF reactivation of HHVs (of course if actually occurred)?

without concurrent viremia in the context of systemic disease like MISC? Why did not check the serum HHV-qPCR simultaneously?" Is there a piece of evidence to support "isolated CSF reactivation of HHVs"? Discussion should be improved.

Response: Due to study limitations and financial budget, we were unable to check the blood of these patients. Furthermore, there is no need for blood PCR testing to diagnose encephalitis caused by HSV and VZV.

5. Besides, inadequate diagnostic workup should be mentioned as a study limitation in the discussion section.

Response: was added

3 Revision 2

0 comments

No public comments in this round.