Author’s response to reviews

Title: Detection of varicella-zoster virus from cerebrospinal fluid using advanced fragment analysis in a child with encephalitis: A case report

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Author’s response to reviews:

Dear Editor,

Thank you very much for your email on March 23rd regarding the decision of our manuscript (INFD-D-19-00142R1), entitled "Detection of varicella-zoster virus from cerebrospinal fluid using advanced fragment analysis in a child with encephalitis: A case report". Two reviewers offered us valuable suggestions and comments, which are helpful for us to revise our manuscript. We revised the manuscript by a point-by-point response to reviewers’ comments as following:

Response to the comments of Dr. Benhur Şirvan Çetin (Reviewer 1):

To the specific comment 1: “Was that VZV infection in their patient primary? We know that primary infection with the VZV usually manifests with rashes (chickenpox), but reactivation of VZV can present with different clinical manifestations. Reactivation of VZV from the geniculate ganglion, the nucleus of the sensory root of the facial nerve, can cause peripheral facial weakness as well as rash around the ear, known as Ramsay Hunt syndrome. So, it would be herpes zoster with CNS complication. The patient's VZV serological status may help us at this point. In the case section and discussion, those points should be mentioned.”

Thank you very much for your comments. In this case, the child indeed initially presented with left facial paralysis, but the patient’s parents denied that he has a history of chickenpox infection before. Combined with the serological results, it is possible that the boy had latent VZV infection
in geniculate ganglion in the past, although he didn’t have chickenpox. According to the comment, we have discussed this part in the revised manuscript (Case presentation section, line 1-9, page 8).

Response to the comments of Dr. Daniel Depledge (Reviewer 2):

To the specific comment 1: “The style of writing is a little unusual and is littered with small mistakes in grammar etc. While this is not a major issue, some editing would be prudent prior to publication.”

We felt very sorry for such error. According to this suggestion, we have carefully checked the grammar mistakes and made some corrections in the revised manuscript.

To the specific comment 2: “While the signal indicating the presence of VZV is clear and strong - it is noticeable that there is a small bump around 200nt which could suggest some level of HSV-1 being present. While it is still most likely that VZV is causing encephalitis here, the authors should either note the possibility that HSV may be contributing or else explain why they are not considering it. If it is below a specific threshold then the authors need to explain how this is established.”

Thank you very much for your meticulous and comprehensive revision for our manuscript. In figure 2A, there is indeed a small bump around 200nt, which is an interference peak rather than HSV-1. There are three reasons for this judgment: Firstly, the location of this peak is not within the gray area, and the fragment size is slightly larger than the fragment size of HSV-1 (197nt). Secondly, the height of this peak is less than the threshold of 500. The threshold value is calculated from the average peak heights of 20 low dose quality control products. The minimum detection limit for VZV is 25 copies per microliter. Thirdly, the interference peaks are usually blunt and broad, while the true pathogen peaks are sharp and narrow.

To the specific comment 3: “The data in figure 2B are representative of 1 / 3 experiments with similar results observed in the others. I don't see any reason why the data from the other experiments cannot simply be overlaid on these plots?”

Thank you very much for your comments. Since the VZV RT-qPCR experiment is a qualitative experiment, I only selected representative curves for demonstration in this case report. Of course, I will pay attention to this problem in future research.

To the specific comment 4: “The authors should include the primer sequences used for the VZV RT-qPCR approach.”

Thank you very much for your comments. Actually, in this experiment, we used a commercial VZV-DNA detection kit (Sansure Biotech, China), whose primer sequence was protected and not
published in the manual, so we had no way to obtain the sequence information of the primer. We felt sorry about this.

Again, we authors thank all reviewers and their suggestions and comments. We hope the revised manuscript correctly address the comments from the reviewers. Please contact us if there are any additional questions.

We are appreciating you if you would contact with us by e-mail.

Sincerely yours,

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