

Response to the Reviewer's Comments

Reviewer #1

We appreciate your comments. Based on your comments, we checked our overall manuscript. As a result, our manuscript is improved the scientific quality and enriched. Thank you for using your precious time for us.

Below is the response to your comments.

Specific Comments to Authors:

Manuscript ID 74063 describes the effectiveness of UC-MSCs in the treatment of syringomyelia by Chiari malformations. In this case report study, the authors have used MSCs initially as a pain relief therapy but they observed the treatment of syringomyelia. Generally, the manuscript is clear and well written. The case is well explained and the authors are honest that the observed effects were not expected. Although this manuscript is very interesting the following points should be clarified before its potential publication. 1) In the introduction section, several references are missing. E.g. page 5 middle paragraph. 2) I have some concerns regarding the term uncultured and the number of MSCs. The authors mentioned that the administered MSCs were uncultured, yet, they have injected 5 times and each time 3M/ml (10 ml in total per injection) which is 30 million cells per injection. This is just impossible to have that many cells in P0 directly from the isolation step. 3) What was the weight of the patients and how did the authors evaluate how many cells to inject? 4) The fifth injection of MSCs happened later, did they use the same donor as the first four injections? If it as the first donor, then it makes it even more impossible that the cells were uncultured. 5) It is not clear for me when the authors write, the 110 ml of solution were injected first and after a 1-hour break the same amount was injected. So 2 times 30 million cells? Even the table does not help me to understand this point. 6) Why did the authors use UC source of MSCs? Why not bone marrow or adipose-derived MSCs? Several articles demonstrate that the source of MSCs can have different regenerative and immunological impacts. E.g. PMID:33597011 and 34567420 Please discuss this point.

Response

1) We added references to the introduction part.

- 2) We developed the method for isolation MSCs from the umbilical cord based on the method used in Ref. 19. Using this method, hundreds of millions of MSCs can be obtained from 30 cm umbilical cord.
- 3) The patient's weight was about 60 kg. We transplanted 1×10^6 MSCs per kg.
- 4) Unfortunately, the MSCs used in the fifth injection were obtained from a different umbilical cord than the MSCs used before. Because this was not the planned treatment.
- 5) Before and after break time, the same amount of MSCs was transplanted. The manuscript including table have been revised.
- 6) The reason we used MSCs that were not cultured was to avoid cell aging and mutation; and to use the most primitive MSCs for treatment. To do this, we had to select tissue from which we could obtain enough MSCs. The umbilical cord is the optimal tissue for this treatment. It is also known that the mesenchymal stem cells obtained from the umbilical cord have almost no immune rejection. In our previous papers, we described why we used uncultured umbilical cord MSCs (Ref. 20-22). In this manuscript, the content was excluded as it was judged inappropriate because this result was not result of planned case.

Reviewer #2

We appreciate your comments. Your comments have increased the scientific and grammatical quality of our manuscript. Thank you for using your precious time for us.

Below is the response to your comments.

Specific Comments to Authors:

In detail, the method of study and paper is well done and interesting. But, I have some comments to improve the paper which are listed as follows: -There are some scientifically/ grammatically errors in the paper. Please control the text in that manner. -The "abstract" should be modified and written scientifically. -The keywords should be modified as follows: Syringomyelia; Umbilical cord-MSCs; Cell therapy; Allogenic stem cells; Chiari malformation -Discussion part should be rewritten more comprehend. -Figures 1 & 2 should be merged and the authors should be show the difference between before and after cell the

rapy.

Respond

- 1) We revised the abstract and keywords.
- 2) We merged the Figure 1 and 2.
- 3) We obtained the certificate of English.

Reviewer #3

We appreciate your comments. After your comment we have tried to obtain additional data. As a result, we were able to increase the scientific quality of our manuscript. Unfortunately, the data we were unable to obtain also allowed us to clearly see the limitations of our research. Thank you for using your precious time for us.

Below is the response to your comments.

Specific Comments to Authors:

In this case report, the authors tried to illustrate the treatment using uncultured umbilical cord-derived mesenchymal stem cells with a syringomyelia patient, which could be a new treatment alternative for syringomyelia. However, there are major specific points in this manuscript as shown in following comments: 1.Regarding treatment effect, it is recommended to add the Magnetic resonance imaging of the patient in May, 2016 before the stem cell treatment, which could draw a scientific conclusion. 2. Cerebrospinal fluid pressure measurement should be studied in this work. 3. In the aspect of treatment, How to exclude the failure for the surgery for the patient in 2010 if there were no evidences for the image and cerebrospinal fluid pressure measurement after surgery and before the cord-derived mesenchymal stem cells treatment? 4. What is the basis for the dose of the stem cell therapy?

Respond

- 1) Unfortunately, we were not able to secure a MRI taken just before stem cell treatment. Instead, since we acquired an additional MRI of 2012, we included it in the manuscript. And the opinion of the doctor in charge recorded

in the medical record was included in the manuscript.

- 2) Since our treatment goal was not syringomyelia, we did not measure the cerebrospinal fluid pressure. After checking the medical records of the hospital where the patient underwent surgery to treat the Chiari malformation, there were no records of pressure measurements.
- 3) We added additional MRI images of the patient after surgery and the opinion of her doctor on syringomyelia in the manuscript.
- 4) We based on the weight of the patient. We transplanted 1×10^6 MSCs per kg.