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New Findings, Classification and Long-Term Follow-Up Study Based on MRI Characterization of Brainstem Encephalitis Induced by Enterovirus 71

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Abstract

Background

To report the diversity of MRI features of brainstem encephalitis (BE) induced by Enterovirus 71. This is supported by implementation and testing of our new classification scheme in order to improve the diagnostic level on this specific disease.

Methods

Neuroimaging of 91 pediatric patients who got EV71 related BE were hospitalized between March, 2010 to October, 2012, were analyzed retrospectively. All patients underwent pre- and post-contrast MRI scan. Thereafter, 31 patients were randomly called back for follow-up MRI study during December 2013 to August 2014. The MRI signal patterns of BE primary lesion were analyzed and classified according to MR signal alteration at various disease stages. Findings in fatal and non-fatal cases were compared, and according to the MRI scan time point during the course of this disease, the patients’ conditions were classified as 1) acute stage, 2) convalescence stage, 3) post mortem stage, and 4) long term follow-up study.

Results

103 patients were identified. 11 patients did not undergo MRI, as they died within 48 hours. One patient died on 14th day without MR imaging. 2 patients had postmortem MRI. Medical records and imaging were reviewed in the 91 patients, aged 4 months to 12 years, and two cadavers who have had MRI scan. At acute stage: the most frequent pattern (40 patients) was focci of prolonged T1 and T2 signal, with (15) or without (25) contrast enhancement. We...
observed a novel pattern in 4 patients having foci of low signal intensity on T2WI, with contrast enhancement. Another pattern in 10 patients having foci of contrast enhancement without abnormalities in T1WI or T2WI weighted images. Based on 2 cases, the entire medulla and pons had prolonged T1 and T2 signal, and 2 of our postmortem cases demonstrated the same pattern. At convalescence stage, the pattern observed in 4 patients was foci of prolonged T1 and T2 signal without contrast enhancement. Follow-up MR study of 31 cases showed normal in 26 cases, and demonstrated foci of prolonged T1 and T2 signal with hyper-intensity on FLAIR in 3 cases, or of prolonged T1 and T2 signal with hypo-intensity on FLAIR in 2 cases. Most importantly, MR findings of each case were thoroughly investigated and classified according to phases and MRI signal alteration.

Conclusions
This study has provided enhanced and useful information for the MRI features of BE induced by EV71, apart from common practice established by previous reports. In addition, a classification scheme that summarizes all types of features based on the MRI signal at the four different stages of the disease would be helpful to improve the diagnostic level.

1. Introduction
Enterovirus 71 (EV71) that induces brainstem encephalitis can cause severe cardio-respiratory symptoms and even results in death [1,2]. Neuroimaging characteristics of EV71 induced brainstem encephalitis in children have been described in previous studies based on information depicted by Table 1[3–6]. The typical MRI findings are foci with prolonged T1 and T2 signal intensity at junction between pons and medulla oblongata, which pertains to the T1 weighted images (T1WI) and T2 weighted images (T2WI) that we will analyze. Previous studies did not describe in detail the variations in MR patterns and did not include large series of patients and most not employed contrast enhancement routinely in MR imaging. In fact, most other MR signal patterns have not been reported. To the best of our knowledge, we only found one study with 7 cases of follow-up MRI examinations within 2 months [4], and deduced that the diagnosis of lesions to be permanent or transient have not yet been thoroughly studied. In previous studies, findings in fatal cases have not been compared to those in recovering patients. As such, it would be valuable to learn how this disease deteriorates under the revelation of MRI.

During an epidemics, EV71 related brainstem encephalitis is very rare and mostly occurs in the southern parts of China and in Taiwan. The interesting part is that morbidity is extremely high for patients under the age of 3 years, and can experience severe complications such as pulmonary edema, pulmonary hemorrhage, and cardiopulmonary failure. The clinical manifestations vary in terms of presentation and severity, for instance, myoclonus, ataxia, nystagmus, oculomotor palsies, and bulbar palsy presents themselves simultaneously with different degrees and combinations. The locations of EV71-induced brainstem encephalitis lesions may be detected using plain or enhanced MRI scans. Lesions based on the different stages of disease progression appears with different intensities in T1WI, T2WI, and enhanced MR images and their unique patterns can serve as a hallmark for identification of the disease stage.

Due to the very limited literature on enhanced MRI used for the diagnosis of brainstem encephalitis induced by EV71, we decided to embark on a medical study of children diagnosed
with BE. In our present study, we reviewed our 3 years of clinical and medical data in consecutive cases with EV71 at a 1000 beds regional medical center that served a population of 16

Table 1. Previous studies on MRI characteristics of EV71 induced brainstem encephalitis.

<table>
<thead>
<tr>
<th>Journal</th>
<th>Author</th>
<th>Year</th>
<th>PMID</th>
<th>Number of cases</th>
<th>Age</th>
<th>Major observation in the report</th>
<th>MRI Follow up study</th>
<th>Difference from our study</th>
</tr>
</thead>
<tbody>
<tr>
<td>The New England Journal of Medicine</td>
<td>CHAO-CHING HUANG</td>
<td>1999</td>
<td>10498488</td>
<td>24</td>
<td>&lt;5y</td>
<td>MRI showed 17 of the 24 patients had high signal intensity in the brain stem on T2WI. No abnormal enhancement on T1WI</td>
<td>No MRI follow-up study.</td>
<td>Described only one type MR signal pattern of major lesion at acute stage</td>
</tr>
<tr>
<td>American Journal of Neuroradiology</td>
<td>Wu-Chung Shen,</td>
<td>1999</td>
<td>10588115</td>
<td>20</td>
<td>2m-7y</td>
<td>15 patients had hyperintensity in the brain stem (medulla oblongata,pons, midbrain) and spinal cords on T2WI images.</td>
<td>2 W to 2 M: the lesions completely disappeared in five patients. The destruction in posterior medulla and pons in two patients. Most of midbrain was damaged in one patient.</td>
<td>No post contrast MRI study. Described only one type MR signal Pattern of major lesion at acute stage</td>
</tr>
<tr>
<td>Eur J Paediatr Neurol.</td>
<td>Chen F</td>
<td>2013</td>
<td>23561930</td>
<td>21</td>
<td>6-37m</td>
<td>Type I patch-like hyperintense T1and T2 signals restricted to the posterior brainstem in13 cases. Type II speckled hyperintense T1and T2 signals on vague pattern in 8 cases</td>
<td>No MRI follow-up study. Clinic follow-up study showed the prognosis of type II cases was better than that of type I cases.</td>
<td>No post contrast MRI study. Only two types MR patterns.</td>
</tr>
<tr>
<td>Int J Neurosci</td>
<td>Li J</td>
<td>2012</td>
<td>22248036</td>
<td>21</td>
<td>5-41m</td>
<td>21 cases of pontine encephalitis, long T1 and long T2 signal intensity was seen in the posterior portions of Brain stem.</td>
<td>No MRI follow up study.</td>
<td>Described only one type MR signal pattern of major lesion at acute stage</td>
</tr>
<tr>
<td>Neuroradiology.</td>
<td>Zeng H</td>
<td>2012</td>
<td>22200974</td>
<td>42</td>
<td>5m-11y</td>
<td>Plain T1WI showed iso-or hypointense signals, and T2WI showed iso-and hyperintense signals. 12 cases had slight to moderate enhancement</td>
<td>No MRI follow up study.</td>
<td>Only two types MR patterns. Focus on enhancement role.</td>
</tr>
<tr>
<td>Neuropediatrics.</td>
<td>Tsai JD</td>
<td>2014</td>
<td>24258524</td>
<td>46</td>
<td></td>
<td>Results from viral culture and MR imaging indicated that positive identification of EV71 infection was associated significantly with lesions on MR imaging</td>
<td>No MRI follow up study.</td>
<td>Described only one type MR signal pattern of major lesion at acute stage</td>
</tr>
<tr>
<td>Asian Pac J Trop Med.</td>
<td>Chen F</td>
<td>2013</td>
<td>23317889</td>
<td>9</td>
<td>4m-27m</td>
<td>The midbrain, pons, and medulla demonstrated abnormal signal in 3 cases.</td>
<td>No MRI follow up study.</td>
<td>Only reported 3 cases</td>
</tr>
<tr>
<td>Zhonghua Er Ke Za Zhi.</td>
<td>Peng BW</td>
<td>2012</td>
<td>22801224</td>
<td>16</td>
<td>7m-5y</td>
<td>Bilateral hyperintense lesions, symmetrical in the posterior portions of the medulla, pons. Enhancement occurred only in the early MRI examination.</td>
<td>6 cases did MRI follow study.</td>
<td>This study focus on spinal cord.</td>
</tr>
<tr>
<td>Int J Clin Exp Med.</td>
<td>Chen F</td>
<td>2014</td>
<td>25356127</td>
<td>12</td>
<td>6m-37m</td>
<td>MRI showed posterior brainstem abnormalities with hyperintense areas on T2WI and hypointense areas on T1WI.</td>
<td>No MRI follow up study.</td>
<td>Described only one type MR signal pattern of major lesion at acute stage</td>
</tr>
</tbody>
</table>

This table tabulates the key findings of previous studies, which were compared with our study.

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million in which several epidemics had occurred since 2008. Not only did we perform long-
term follow-up studies in our patients, we also took the rare opportunity to perform post mor-
tem imaging in some fatal cases as a confirmation of our final diagnosis. According to the MRI
study time point in the course of this disease, four phases were classified as 1) acute stage, 2)
convalescence stage, 3) post mortem stage, and 4) long term follow-up study. The MRI signal
patterns of brainstem encephalitis primary lesion were analyzed and classified according to
MR signal alteration at the four stages. If an accurate diagnostics and assessment of disease
severity was realized, it would help the children, especially those in the Asia–Pacific region of
the world, and contribute to the health quality of mankind.

2. Material and Methods

2.1 Ethical Standards and Patient Consent
We declare that all human and animal studies have been approved by the Shenzhen Children's
Hospital ethics committee and have therefore been performed in accordance with the ethical
standards laid down in the 1964 Declaration of Helsinki and its later amendments. We declare
that all patients gave informed consent prior to inclusion in this study.

2.2 Ethics Statement
This study was approved by the Ethics Committee of Shenzhen Children's Hospital and written
informed consent was obtained from parents/guardians for minors in this study. This was
cohort study and met its criteria. STROBE Statement—Checklist of cohort study was filled and
submitted.

Clinical and neuroimaging data of 91 critical pediatric hand foot mouth disease (HFMD)
and/or herpangina cases complicated with brainstem encephalitis were retrospectively ana-
yzed. All patients were confirmed with EV71 infection, and were then hospitalized in Pediatric
Intensive Care Unit (PICU) in Shenzhen Children's Hospital, China, from March 1st, 2010 to
October 31st, 2012. All patients were examined by a board-certified pediatric neurologist. For
this study, 31 of the 91 patients were randomly recalled to undergo follow up MRI study during
the period from December 2013 to August 2014.

Stage methods: the first two weeks after onset of the disease was clinically defined as acute
stage, and after that it was set as the convalescence stage. Postmortem stage, plain MRI scans
were performed immediately after death in two fatal cases. Parental consents were obtained
from their parents before MRI scan. Follow up stage, 31 patients did the flow-up study as previ-
ous described.

2.3 Clinic data
Between March 1st, 2010 to October 31st, 2012, 217 children were hospitalized in Shenzhen
Children's Hospital, Shenzhen, China, to treat HFMD and/or herpangina infected by EV71. Of
these subjects, 103 patients were admitted into PICU because of neurological complication of
brainstem encephalitis. Among these patients, 13 of them deteriorated rapidly and quickly died
within 48 hours of hospitalization, which resulted in a lack of chance to perform MRI diagnost-
ics. Nevertheless, 2 of them underwent brain MRI scan immediately after death when parental
consent was obtained. Another 1 subject died on the 14th day of the course, we did not have
chance to perform MRI exam on this child. Therefore a total of 91 patients were consecutively
enrolled, including 54 males and 37 females with average age 2.6(±1.9) years, ranging from 4
months to 12 years. Table 2 illustrates the involved children demographics in this study.
Table 2. Results of demographics data, clinic data and MRI findings.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Classification</th>
<th>Number</th>
<th>Percentage in Stage</th>
<th>Age (M±Std)</th>
<th>Gender</th>
<th>Lesion Predominant MR features</th>
<th>clinical distinctive feature</th>
<th>Follow-up Number</th>
<th>Positive Follow up result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute stage</td>
<td>I</td>
<td>25</td>
<td>32.89%</td>
<td>2.61y (4m-7.1y)</td>
<td>M 14</td>
<td>Fleck Hypo- Hyper- Non</td>
<td>Myoclonus, tremor, and ataxia, dysfunction of cranial nerves</td>
<td>11</td>
<td>2 cases belongs to follow-up type I, 1 belongs to follow-up type II</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>15</td>
<td>19.74%</td>
<td>2.63y (9m-6.2y)</td>
<td>F 8</td>
<td>Fleck Iso/ Hypo- Hyper- Yes</td>
<td>Myoclonus, tremor, and ataxia, dysfunction of cranial nerves</td>
<td>6</td>
<td>1 case belongs to follow-up type I</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>10</td>
<td>13.16%</td>
<td>2.40y (11m-5Y)</td>
<td>M 6</td>
<td>Fleck Neg- Neg- Yes</td>
<td>Myoclonus, tremor, and ataxia, dysfunction of cranial nerves</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>IV</td>
<td>4</td>
<td>5.26%</td>
<td>1.09 (8m-2.1y)</td>
<td>M 3</td>
<td>Fleck Neg- Hypo- Yes</td>
<td>Myoclonus, tremor, and ataxia</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>V</td>
<td>2</td>
<td>2.63%</td>
<td>2.55y (2.3y-2.8y)</td>
<td>M 2</td>
<td>Whole Brainstem Hypo- Hyper- Non</td>
<td>Coma, neurogenic pulmonary edema</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>26.32%</td>
<td>3.15y (9m-12y)</td>
<td>M 11</td>
<td>Fleck Neg- Neg- Non</td>
<td>Myoclonus, tremor</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Conva. stage</td>
<td>A</td>
<td>4</td>
<td>30.77%</td>
<td>1.89y (8m-3.1y)</td>
<td>M 2</td>
<td>Fleck Hypo- Hyper- Non</td>
<td>Neurogenic pulmonary edema</td>
<td>1</td>
<td>1 case belongs follow-up type II</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>9</td>
<td>69.23%</td>
<td>2.11y (6m-3.6y)</td>
<td>M 6</td>
<td>Fleck Neg- Neg- Neg-</td>
<td>Myoclonus, tremor</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>FLAIR</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Follow up</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>3</td>
<td>9.68%</td>
<td>5.96y (3.9y-6.7y)</td>
<td>M 2</td>
<td>Fleck Hypo- Hyper- Hyper-</td>
<td>Neurogenic sequelia</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>2</td>
<td>6.45%</td>
<td>4.95y (4.6y-5.8y)</td>
<td>M 2</td>
<td>Fleck Hypo- Hyper- Hyper-</td>
<td>Irregular breath</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>26</td>
<td>83.67%</td>
<td>5.62y (3.7y-8.3y)</td>
<td>M 15</td>
<td>Fleck Neg- Neg- Neg-</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

This table summarizes the clinical data and classifies the MRI features.

doi:10.1371/journal.pone.0162877.t002
Throat swabs and stool specimens were collected from each patient for the detection of EV71 by using reverse transcriptase polymerase chain reaction (RT-PCR). Brainstem encephalitis was clinically diagnosed according to neurological symptoms associated with the brainstem function, including myoclonus with tremor and/or ataxia, myoclonus with cranial nerve involvement, and/or rapid cardiopulmonary failure. Although neuroimaging could support the diagnosis at that time, it was not a necessary criterion [7]. Clinical manifestations and symptoms were reviewed and analyzed. Acute stage was defined as disease infection within the first two weeks and convalescence stage was after this stage.

2.4 Magnetic resonance imaging study

2.4.1 Initial MRI study. All patients (89 cases) underwent pre- and post-contrast cranial MRI scans using the Signa Excite 1.5T imaging system (General Electric, Fairfield, USA). Pre-contrast scan included axial T1-weighted image (T1WI), axial T2-weighted image (T2WI), axial fluid-attenuated inversion recovery (FLAIR) and diffusion weighted imaging (DWI), and sagittal T1WI. Post-contrast scan included axial and sagittal T1WI. Thickness/spacing was 6.0/2.0 mm for all axial slices, and was 3.5/0.5 mm for all sagittal slices. Sequence parameters were as followings: T1WI employed T1-FLAIR sequence (TR = 2,307ms, TE = 10.6ms, TI = 620ms), T2WI used FRFSE-XL (TR = 4,000ms and TE = 104.4ms), FLAIR sequence parameters were TR = 8,420ms, TE = 127ms, and TI = 2,100ms, EPI DWI (TR/TE 6,000/82 ms, b-value = 800). Post-contrast T1WI employed a FSE sequence (TR = 500ms and TE = 20ms). The MRI contrast agent was gadopentetate dimeglumine (Bayer, Germany). Our injection dosage was 0.1 mmol/kg. Uncooperative patients were sedated with chloral hydrate enema (0.6 ml/kg) before the MRI scan.

2.4.2 Follow up MRI study. In 31 cases were performed on the 3.0T Skyra MRI (Siemens, Germany). Scan sequences included axial T1WI and T2WI, sagittal T1WI and FLAIR. T1WI employs T1_FLASH (Fast Low Angle Short) sequence, and T2WI employs T2_TSE sequence, FLAIR employs a t2_tirm_sag_dark-fluid. The parameters of above sequences were as follows: Axial and sagittal T1WI (T1_fl2d_tra,TR = 244ms, TE = 2.93ms, Flip angle = 70, Average = 2, Concatenation = 1, Distance factor = 20); Axial and sagittal T2WI (TSE, TR = 3,800ms, TE 112, Average 1, Concatenation 2, Flip angle 150, Turbo factor 19); axial FLAIR (t2_tirm_sag_dark-fluid TR = 900ms, TE = 81ms, TI = 2,500ms, Flip angle 150, Distance factor = 20, Average = 1, Concatenation = 2). For all axial images, scan thickness was 3mm, and distance factor was 20%. For all sagittal images, the implemented thickness was 3mm, and the distance factor was 15%.

2.4.3 MRI findings classification method. In this study, we focused on the MRI signal alteration of the primary lesion in the brainstem encephalitis. To the best of our knowledge, this has not been thoroughly investigated, and the medical community normally took it for granted that prolonged T1 or prolonged T2 signal were constant throughout the entire disease infection, which was mainly due to the limited cases for conclusion. However, from the records of our rare patient database over the two years, primary lesion MR signal changed along with the disease development. According to the course of disease, classically three stages were defined, acute stage (from onset to 14th day of disease), convalescence stage (after the 14th day of this episode) and long-term flow up (called back follow-up study). Some patients were very critical, and did not have a chance to do the MRI exam at acute stage. Therefore, in this study, MRI classification of the primary lesion of brainstem encephalitis mainly based on the signal change and disease stages. Additionally, post-mortem studies were performed on two fatal cases. The main pathology changes were inferred from the MR signal, inflammation or hemorrhage. Two experienced pediatric radiologists, who were blind to the clinic results, were
introduced to evaluate and classify the MRI findings independently. If there was a disagree-
ment, they were asked to discuss and to make a final consensus.

3. Results and Findings

From a total of 103 patients, 91 patients with MRI data were included in this study.

3.1 Clinical manifestations and symptoms

At the acute stage, fever, maculopapular lesions and blisters on the hand, foot, mouth, and butt-
tocks were the most common clinical manifestations. The three major neurological manifesta-
tions were myoclonus, tremor, and ataxia. 42 (46%) cases pertain to the involvement of cranial
nerves (VI, VII, IX, X, XI, and XII). The dysfunction of cranial nerves were facial palsy,
ophthalmoplegia, dysphagia, oculomotor palsy, et al. Mental status change varied from drowsi-
ness, lethargy, to coma. 19 (21%) patients experienced a different extent of fulminant pulmo-

nary edema and/or pulmonary hemorrhage. The fulminant pulmonary edema was also called
neurogenic pulmonary edema as the lesion damaged the respiratory and circulation control
center in medullar oblongata.

3.2 MRI findings and classification

The primary lesion of brainstem encephalitis that was induced by EV71 was a longitudinal
fleck, which located at the posterior junction region of the pons and medulla oblongata (Fig 1).
The alterations of MR signal of primary lesion varied greatly due to difference course and
severity of the disease. The MRI findings of primary lesion were summarized in Table 2, and
the typical MR images of each type were presented to help readers understand this concept
clearly.

3.3 MRI signal patterns in four stages

All patterns in the four stages of the disease were scientifically catalogued, which were summa-
rized in Table 2. In our paper, the use of enhancement pertains to the implementation of con-
trast agent to develop a post- enhanced image that can reveal the lesion with a higher
definition and clarity. In particular, we present more explanations as follows:

3.3.1 Acute stage. The primary lesion signal alterations consisted of 5 patterns, which
were listed in Table 2 and summarized as follows: 1) Prolonged T1 and T2 signal, without
enhancement, (n = 25, 32.9%); 2) Prolonged T1 and T2 signal, with enhancement (n = 15,
19.7%); 3) Negative on T1WI and T2WI, but having enhancement (n = 10, 13.2%); 4) Normal
on T1WI, Low intensity on T2WI, with enhancement (n = 4, 5.3%); and 5) Involvement of
whole brainstem, with prolonged T1 and T2 signal (n = 2, 2.6%). (Figs 2–6). Point 4) was novel
findings while compared with previous study in Table 1.

3.3.2 Convalescence stage. The primary lesion of 4 cases displayed as prolonged T1 and
T2 signal, and without enhancement (n = 4). The other 11 cases had no abnormal MRI finding.
(Fig 7)

3.3.3 Post-mortem stage. MRI findings revealed the posterior portion of whole brainstem
that was involved with prolonged T1 and T2 signal, with restricted diffusion in 2 cases. (Fig 8)
This was also a novel finding.

3.3.4 Long-term follow up MRI study. 26 cases (84%) were negative. Smaller lesions at
their original place with prolonged T1 and T2 signal in 3 cases, hyper-intensity on FLAIR. 2 of
the 3 cases’ MRI signal belonged to Type I, and one belonged to Type II in acute stage. MRI
findings of these three cases suggested that it was gliosis change. Also, malacia cavities at
previous lesion region were found in 2 patients, which belonged to Type I in acute stage and Type A at convalescence stage separately. These five patients had special neurological sequelae, including irregular breath and involuntary extreme tremor during sleeping at night. (Fig 9)

4. Discussion

In the study, the clinical manifestations of 91 critical cases with brainstem encephalitis induced by EV71 from PICU were summarized. Also, MR findings of these cases were thoroughly investigated; classifications were also made according to phases and MRI signal alteration. To the best of our knowledge, after comparing our findings with previous reports (see Table 1),
ours is the most comprehensive study with the world’s largest number of cases. Furthermore and most importantly, we have focused on detailed information of MR signal alteration, made relevant and useful classification, as well as validated and confirmed the clinical applicability of our scheme using a long-term follow up study.

4.1 Clinic manifestations

EV71 has been proven to be a causative agent of severe and fatal cases in several large outbreaks of HFMD or herpangina in Asia [2,7]. A small portion of the patients especially those in the critical condition tend to have neurological complications, including aseptic meningitis, acute flaccid paralysis, and brainstem encephalitis. According a recent review report, brainstem encephalitis was the most common neurological complication, accounting for 58%, followed by aseptic meningitis [7]. Brainstem encephalitis, which can cause neurogenic pulmonary
edema/hemorrhage and cardiorespiratory failure, or even fatality, was considered as the most critical neurological complication, drawing great attention from the medical community[1]. Results of multivariate analysis indicated RR.26/min, Age < 4yo, GLU > 8.3 mmol/L, LYM > 40% were risk factors [8]. In our present study, the average age was 2.6 (±1.9) years, 54 were males, accounting for 59%. The demographics features were consistent with the other studies in Asian countries and regions [8–10]. The most common neurological manifestations of brainstem encephalitis induced by EV 71 were myoclonus and tremor in this research, which were similar to the other studies [1,11,12]. 19 (21%) patients complicated a fulminant pulmonary edema; the incident rate was lower than previous study in our hospital located within Shenzhen, and also in Taiwan. This was mainly because of the valuable experience learned from an EV71 outbreak in 2008 by applying positive end-expiratory pressure ventilation in the early phase of disease infection.

![Fig 4. Acute stage type III, Female, 2.5 years old, on the 7th day of the disease.](image)

There was no abnormal signal at the brainstem seen from sagittal T1WI (A) and T2WI (B); Post-contrast sagittal T1WI demonstrated a patchy, moderate enhancement region (C, white arrow).

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![Fig 5. Acute stage type IV, Male, 6 months old, on the 6th day of the disease.](image)

There was no abnormal signal at the brainstem seen from sagittal T1WI (A). T2WI showed a hypo-intensive lesion at the posterior junction region of the pons and medulla (B, black arrow), which had moderate enhancement (C, white arrow).

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4.2 Pathology and MRI findings

Prior to discussion of the MRI findings, it is important to review the histopathology change revealed by previous studies for a better understanding of basis behind the MRI. Autopsy demonstrated that inflammation in the central nervous system (CNS) was most severe in the gray matter of spinal cord and brain stem. In our present study, the case presented in Fig 6 and the postmortem case in Fig 8, where MRI showed that the brainstem was seriously affected. The affected neurons were mostly destroyed and infiltrated by inflammatory cells [13]. Autopsy

**Fig 6. Acute stage type V, Male, 28 months old, on the 11th day of the disease.** Whole brain stem was involved in this case, from midbrain to medullar oblongata, demonstrating as prolonged T1 (A) and prolong T2 signal (B, black arrow), without enhancement (C). This subject was hospitalized in PICU for 20 days, and final discharged. Follow-up study showed that there were neurologic sequelae, presenting as irregular breath and extremities tremor during sleeping.

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**Fig 7. Convalescence Stage, Male, 4 years old, on the 28th day of the disease.** MR images showed there is a longitudinal malacia cavity at the posterior junction region of the pons and medulla, with prolonged T1 (A, white arrow) and prolonged T1 (B, black arrow) signal, without enhancement (C, white arrow). This subject had had impaired function of breath and recurrent pneumonia after discharge.

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studies revealed that histopathology changes included neuronal degeneration and necrosis, neuronophagia, perivascular cuffing and diffuse or nodular hyperplasia of macrophages/microglia, which were caused by virus neurotropism rather than by ischemic injury [13,14]. Previous study showed that EV71 virus can be isolated from different CNS tissues, including the pons, medulla, cerebellum, and spinal cord [13]. EV71 antigen was positive in the cerebella and brainstem, evidence from immunohistochemical aspect. Researchers also found that viral loads were highest in brainstem among all tissues [14]. There are accumulating evidence support that EV71 can invade the brainstem directly and cause brainstem encephalitis, especially those with respiratory distress, cyanosis, poor peripheral perfusion, shock, coma[1,13]. Another study pointed out that cytokines, lymphocytes and monocytes contribute significantly to pathogenesis [15].

![Fig 8. Postmortem stage, Male, 37 months old, on the 2nd day of the disease. Sagittal FALIR (A, black arrow) and axial T2WI (B, black arrow) showed the whole posterior portion of the brainstem was involved, demonstrating hyper-intensive signal, with restricted diffusion (C, black arrow). doi:10.1371/journal.pone.0162877.g008](image1)

Fig 8. Postmortem stage, Male, 37 months old, on the 2nd day of the disease. Sagittal FALIR (A, black arrow) and axial T2WI (B, black arrow) showed the whole posterior portion of the brainstem was involved, demonstrating hyper-intensive signal, with restricted diffusion (C, black arrow).

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![Fig 9. Follow-up study of previous case (Female, 6 years 8 months old, from Fig 2) in 3 years 8 months. Sagittal MR images revealed the lesion was smaller while compared with Fig 2, demonstrating prolonged T1 (A, white arrow) and prolonged T2 (B, black arrow) signal, and hyper-intensive signal on FLAIR (C, white arrow). This subject had special neurological sequelae, which were irregular breath and involuntary extremity tremor during sleeping at night. doi:10.1371/journal.pone.0162877.g009](image2)

Fig 9. Follow-up study of previous case (Female, 6 years 8 months old, from Fig 2) in 3 years 8 months. Sagittal MR images revealed the lesion was smaller while compared with Fig 2, demonstrating prolonged T1 (A, white arrow) and prolonged T2 (B, black arrow) signal, and hyper-intensive signal on FLAIR (C, white arrow). This subject had special neurological sequelae, which were irregular breath and involuntary extremity tremor during sleeping at night.

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The MRI findings and classification based on three stages were discussed. Some of MRI findings of brainstem encephalitis induced by EV71 have been reported by many studies [1,4–6,11,16]. The prominent MRI feature of brainstem encephalitis was the location of primary lesion, which was set on the posterior of the pons and medulla oblongata. The most common alteration of MRI signal was prolonged T1 and T2 signal of the lesion. The figures in Table 2 of our study also confirmed this finding. Studies also demonstrated that in some cases, the lesion can be enhanced on post-contrast MRI scan [5,6]. Our previous study recommended that routine pre- and post-contrast MRI was an optimal choice because this method can reveal the lesions that might be unnoticed in plain MRI scan [5]. In this study, a new type (Type IV) signal alteration in acute stage was reported, presenting as normal on T1WI and hypo-intensive on T2WI and having enhancement. The average age of the type was 1.09 (8m-2.1y), which was much lower than other group. Combining the pathology change from the above paragraph and MR signal, the possible explanation for hypo-intensity on T2WI might be that intracellular deoxyhemoglobin in red blood cell occurs in acute stage (1–3 days). There are about 25% patients (20 of 76) with negative MRI results in acute stage. The MRI signal patterns in convalescence stage in this investigation are similar to our previous report[5]. Here, 5 types of the MR signal patterns in acute stage could help the pediatric radiologists to achieve a clear map of the signal variation. It was also necessary to be reminded that brainstem encephalitis was diagnosed clinically and a MRI negative result was not an exclusive criterion. As the pediatrician make the diagnosis of the EV71 related brainstem encephalitis, especially for those who were hospitalized in PICU, it is extremely important and necessary for physician to have clear image of the brainstem, to what extent the brainstem was involved. Furthermore, both our previous study [5] revealed that there was positive correlation between MRI scores and clinic scores. Plusing the results from this study, it was very safe to say that MRI plays a very important role in the evaluation of the EV71 related brainstem encephalitis, not just for the diagnosis but also for the treatment and prognosis prediction.

To the best of our knowledge, this was the first report on postmortem stage MRI findings. From 2 fatal cases, this postmortem stage MRI showed that the whole brainstems was swelling, with mixed MR signal uneven prolonged T1 and T2 signal. This information has helped us to understand the pathology for development of brainstem encephalitis, which results in the deterioration of the situation and eventual death. It was really a pity that these two cases did not undergo autopsy. Regrettably, if we had compared MRI findings from these two cases with autopsy from the existing study, we would have found consistency in both examinations[17].

The long-term MRI follow-up study demonstrated that 5 of the 31 cases had positive MRI results. From the morphological view, sagittal MR images (see Fig 9) demonstrated that the lesion narrowed with hypo-intensive signal on T1WI, and hyper-intensive signal on both T2WI and FLAIR in 3 of the cases. This MRI finding suggested that it was gliosis repairmen rather than encephalomalacia. The other 2 cases were encephalomalacia changes. These 5 patients had neurological sequelae, such as irregular breath and involuntary extremity tremor during sleeping at night. This would indicate that the permanent tissue destroy of brainstem and cause functional defect. Other neurological sequelae, such as respirator-dependent and V, VI, VII, IX nerves dysfunctions were also reported by previous study[4].

To sum up, there are three main reasons for MRI study of brainstem encephalitis induced. Firstly, a clear image of the brainstem is very important to physicians, to severity of brainstem encephalitis. Secondary, MRI provides valuable information for prognosis of this disease. For example, if you see the patient’s MRI meet type A of convalescence stage, the patient is likely to have brainstem dysfunction. Finally, the follow-up MRI study is also necessary for those who have Neurological sequelae.
4.3 Limitations

There are several limitations in this study that need to be declared. Firstly, all patients enrolled in this study were from PICU, and therefore, some patients with minimal brainstem encephalitis were excluded methodologically. Secondly, due to the objective condition restriction, only 31 subjects were randomly called to do the follow up MRI exams. Thirdly, the two cases with postmortem MRI did not underwent autopsy, and so the comparison study between MRI and pathology cannot be achieved. Finally, as this study only focuses on the primary lesion with follow up MRI scans, other lesions in the brain are not examined and discussed. While the use of visual analysis has been practical and successful in evaluation of diseases related to EV71, there may exist the issue of inconsistency when tuning the range of intensity values for the MR image. As such, an intensity histogram analysis for the T1WI and T2WI and derivation of their statistical properties may be useful.

5. Conclusion

In summary, from this study, we have provided enhanced and useful information for the MRI features of brainstem encephalitis induced by EV71 that correspond with clinical features apart from common practice established by previous reports. Furthermore, we established a classification scheme that summarizes all of our discovered types of features based on the MRI scans at the four different stages of the disease. Novel MRI findings of this study include Type IV in acute stage presenting as normal on T1WI, low intensity on T2WI, with enhancement, and whole brainstem involved in post-mortem stage. Furthermore, post-contrast can reveal the lesion missed by plain MRI scan. Clinically, radiologists should be aware of all types of MRI characters of brainstem encephalitis induced by EV71. MRI is a useful and effective modality both for diagnosis and follow-up evaluation.

Supporting Information

S1 File. This file (PDF format) contains STROBE_checklist_cohort for this paper. (PDF)

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