Detection of SARS-CoV-2 nucleic acid in CSF by ultrahigh depth sequencing in a patient with COVID-19 and neurological dysfunction: a case report

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Case Report

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Abstract

Background: SARS-Coronavirus-2 (SARS-CoV-2), the pathogen of coronavirus disease 2019 (COVID-19), not only infects the respiratory tract, but also other organs. About a third of the inpatients of COVID-19 have neurological symptoms and in vitro experiments revealed that SARS-CoV-2 could infect human neural progenitor cells and brain organoids. However, the traditional test often reports negative owing to the low number of virus in the cerebrospinal fluid. To date, timely diagnosis of central nervous system infection of SARS-CoV-2 remains a challenge.

Case presentation: On day 14 of COVID-19, seizures**,** maxillofacial convulsions, intractable hiccups and significant increase in intracranial pressure developed in a 56-year-old man. The RT-PCR of SARS-CoV-2 was negative. SARS-CoV-2 nucleic acid were detected in cerebrospinal fluid (CSF) by ultrahigh depth sequencing. The patient was successfully treated after 14 days of mechanical ventilation and treatment of pneumonia and neurological dysfunction.

Conclusions: This case suggests SARS-CoV-2 can invade the central nervous system and relevant examinations with CSF including ultrahigh depth sequencing of SARS-CoV-2 are needed among COVID-19 patients with neurological dysfunction.

Background

Coronavirus disease 2019 (COVID-19) is a newly illness and has become a pandemic threat leading to more than 30 million confirmed cases and more than 950,000 deaths globally, as of September 18th, 2020. SARS-Coronavirus-2 (SARS-CoV-2), the pathogen of COVID-19, predominantly involves the lungs and causes respiratory illness [1]. However, this virus not only infects the respiratory tract, but also other organs, including intestinal tract, urinary system, blood and so on [2]. Furthermore, this virus has been also reported to be associated with meningitis/encephalitis [3]. In a study of hospitalized COVID-19 patients in Wuhan, China, 36.4% of patients were found to have neurological symptoms including headache, anosmia, ageusia, confusion, seizure, and encephalopathy [4]. Recently, a study reported that SARS-CoV-2 can productively infect human neural progenitor cells and brain organoids, highlighting the potential of direct viral involvement in neurological symptoms in COVID-19 patients [5]. However, proof of a direct involvement of SARS-CoV-2 is missing in the most cases of COVID-19 with central nervous system (CNS) symptoms have been reported until now, because the virus or nucleic acid was not detected in cerebrospinal fluid (CSF) [6-8]. Here, we report a case of involvement of the CNS by the SARS-CoV-2, which was confirmed by ultrahigh depth metagenomic next generation sequencing (mNGS).

Case Presentation

On 24 January 2020, a 56-year-old man was admitted to the hospital due to fatigue, dizziness for 7 days, and fever for 3 days (Fig. 1). The patient had hypertension and a history of travel to Wuhan 14 days prior

to hospitalization and one relative was diagnosed with COVID-19 two days ago. Computed tomography (CT) scan of the chest revealed a large area of ground-glass opacities (GGO) dominated by extraneous areas in both lungs (Fig. 2), and throat swab SARS-CoV-2 nucleic acid test by real-time reverse transcription-polymerase chain reaction (RT-PCR) was positive. Then COVID-19 with respiratory failure was confirmed in this patient. A nasal catheter was inserted and was oxygenated at 5 L/min. He was given antiviral therapy with lopinavir/ritonavir (500 mg twice daily) combined with interferon alfa-2b (5 million units twice daily, atomisation inhalation), moxifloxacin (0.4g once daily, intravenously) to prevent secondary infection (Fig. 1). After admission, the symptoms of dyspnea kept worsening. On day 10 of illness, chest CT showed an enlarged GGO area and partial opacities in both lungs. Short-term high-flow nasal oxygen was briefly administered with a gas flow rate of 50 L/min and oxygen concentration of 90%. The patient continued to exhibit respiratory distress accompanied with RR 50 times/min, SpO_2 85%. Therefore, endotracheal intubation was performed in the intensive care unit (ICU) and mechanical ventilation was conducted according to the respiratory ventilation protocol of severe acute respiratory distress syndrome.

After 96 hours of mechanical ventilation, frequent maxillofacial and oral spasms accompanied by persistent hiccoughs were observed during the reduction of midazolam and fentanyl citrate. Physical examination revealed positive neck-resistance, bilateral pupils of equal size (3 mm diameter) with slow response to light, increased muscle tension in the extremities, hyperreflexia in both knees, and positive bilateral Babinski sign and ankle clonus. The CSF pressure was greater than 330 mmH_2O with a clear colourless appearance. Three days later CSF test showed that the pressure was greater than 290 mmH_2O, the CSF cell count was 5/mL, the protein was 30mg/mL and glucose was 4.3mmol/L (Fig. 1), and no abnormalities were found in the brain CT (Fig. 3).

Total genomic DNA and RNA was extracted from the CSF for RT-PCR and mNGS to identify potential pathogens. The RT-PCR of SARS-CoV-2 was negative. Ultrahigh depth mNGS was done and, the full data set of 209,119,576 raw reads was obtained from the RNA library, which gave 10,116 sequences that showed 99.99% identity and covered 100% of the SARS-CoV-2 genome NC_045512.2|SARS-CoV-2|Wuhan-Hu-1 (GenBank accession no. NC_045512.2) with the average sequencing depth was 31.6 (Supplementary figure 1). Except for the SARS-CoV-2, no other pathogens were detected. Furthermore, assembled 29,003 bp and 15,790 bp SARS-CoV-2 genomes were obtained from sputum and blood samples respectively based on 61,224,674 and 8,800,232 raw reads, respectively. No SNP was found among the three assembled SARS-CoV-2 sequences from CSF, sputum and blood. Sequence comparison with the reference genome of Wuhan-Hu-1 revealed only three amino acid residue mutations dispersed in S2 subunit of spike protein, ORF3 and ORF8 (Supplementary figure 2). This CSF derived genome did not form special branch in the phylogenetic tree generated from the comparison of the epidemic virus genomes obtained from GenBank (Supplementary figure 3).

After 14 days of mechanical ventilation and treatment of pneumonia and neurological dysfunction, pulmonary lesions gradually improved, and neurological symptoms disappeared. The endotracheal intubation was removed on day 24 of illness, and the patient was discharged from ICU on day 32 of

illness. The head MR was examined on May 6 (day 82 of illness) and high signal shadows were found in the hippocampus and bilateral temporal lobe, which may be the affected lesion (Figure 1). The patient had no cognitive barriers and memory impairment after discharge.

Discussion And Conclusions

Human coronaviruses are recognized as respiratory viruses. However, among these recognized human respiratory pathogens, at least HCoV-OC43, HCoV-229E, and SARS-CoV could be associated with the triggering or the exacerbation of neurological diseases as viral RNA or infectious virus can be detected in human brains [9-11]. Preliminary reports showed some COVID-19 patients showed CNS manifestations, such as dizziness, headache, nausea, vomiting, impaired consciousness, acute cerebrovascular disease, ataxia, and seizure, which warned that SARS-CoV-2 could have neuroinvasive potential [4-13]. Similar to SARS-CoV, SARS-CoV-2 also bind to the angiotensin-converting enzyme 2 (ACE2) receptor to enter human cells [14]. Many types of cells in the brain, such as neurons and glial cells, expressing ACE2 and may act as targets and are thus vulnerable to SARS-CoV-2 infection, and in vitro experiments revealed that SARS-CoV-2 could infect human neural progenitor cells and brain organoids [5]. In our patients, the main neurological symptoms are maxillofacial convulsion, intractable burping, intracranial pressure significantly increased combined with neck resistance, positive bilateral Babinski sign and ankle clonus, which suggesting the presence of neurological dysfunction. Although our case does not confirm that these CNS symptoms are caused by the SARS-CoV-2, we can confirmed that SARS-CoV-2 had invaded the CNS, as whole genome of SARS-CoV-2 was obtained from CSF by mNGS.

In summary, although a direct association between the symptoms of encephalopathy and SARS-CoV-2 requires further investigation, our case suggests SARS-CoV-2 can invade the CNS, which warns the physicians of patients who have CNS symptoms. Relevant examinations with CSF as well as nucleic acid test of SARS-CoV-2 are needed, which is beneficial for a more comprehensive understanding of SARS-CoV-2 infection, thereby further reducing the mortality of severely ill patients and the probable risk of transmission as a result of missed diagnosis.

List Of Abbreviations

SARS-CoV-2: SARS-Coronavirus-2; COVID-19: coronavirus disease 2019; CNS: central nervous system; CSF: cerebrospinal fluid; mNGS: metagenomic next generation sequencing; CT: computed tomography; GGO: ground-glass opacities; RT-PCR: reverse transcription-polymerase chain reaction; ICU: intensive care unit.

Declarations

Ethics approval and consent to participate

The study was approved by the Ethics Committee of The Beijing Ditan Hospital, Capital Medical University. The written consent to publish this information has been obtained from the study patient.

Consent for publication

Written informed consent was obtained from the patient for publication of this case report and any accompanying images.

Availability of data and materials

The data that support the findings of this study are available from the corresponding author (HZ) upon reasonable request.

Competing Interests

The authors declare that there are no conflicts of interest.

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Authors' contributions

JL, YW, BK, HZ design the study. PX, XX, LG, HW, HX, RL, LP, FJ, CL, MZ, JT, YS, YL, HG, JH, YW, JL supplied the clinical data. JL, PX, LG, HX, LP, CL, MZ, JT, YS, FZ, YL, HG, JH evaluated and treated the patients. XL, YX, TQ, HR, JY, JG, XC, HZ, FZ, X H, HZ did the next generation sequencing. HZ, PX, XX, XL, LG, ZL, BK analyzed the data. HZ, PX, XL, LG wrote the paper. BK, YW, JL reviewed the paper. All authors have read and approved the manuscript.

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References

1. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020; 395:497-506.
2. Wang W, Xu Y, Gao R, et al. Detection of SARS-CoV-2 in Different Types of Clinical Specimens. JAMA. 2020; 323:1843-4.
3. Moriguchi T, Harii N, Goto J, et al. A first case of meningitis/encephalitis associated with SARS-Coronavirus-2. Int J Infect Dis. 2020; 94:55-58.

4. Mao L, Jin H, Wang M, et al. Neurologic Manifestations of Hospitalized Patients With Coronavirus Disease 2019 in Wuhan, China. JAMA Neurol. 2020; 77:1-9.
5. Zhang B, Chu H, Han S, et al. SARS-CoV-2 infects human neural progenitor cells and brain organoids. Cell Res. 2020; Aug 4:1-4. Online ahead of print.
6. Ellul MA, Benjamin L, Singh B, et al. Neurological associations of COVID-19. Lancet Neurol. 2020; S1474-4422(20)30221-0.
7. Bernard-Valnet R, Pizzarotti B, Anichini A, et al. Two patients with acute meningoencephalitis concomitant with SARS-CoV-2 infection. Eur J Neurol. 2020; 10.1111/ene.14298.
8. Zoghi A, Ramezani M, Roozbeh M, et al. A case of possible atypical demyelinating event of the central nervous system following COVID-19. Mult Scler Relat Disord. 2020; 44:102324.
9. Arbour N, Day R, Newcombe J, et al. Neuroinvasion by human respiratory coronaviruses. J Virol. 2000; 74:8913-8921.
10. 10. Xu J, Zhong S, Liu J, et al. Detection of severe acute respiratory syndrome coronavirus in the brain: potential role of the chemokine mig in pathogenesis. Clin Infect Dis. 2005; 41:1089-1096.
11. Gu J, Gong E, Zhang B, et al. Multiple organ infection and the pathogenesis of SARS. J Exp Med. 2005; 202:415-424.
12. Carod-Artal FJ. Neurological complications of coronavirus and COVID-19. Rev Neurol. 2020; 70:311-22.
13. Li YC, Bai WZ, Hashikawa T. The neuroinvasive potential of SARS-CoV2 may play a role in the respiratory failure of COVID-19 patients. J Med Virol. 2020;92:552-555.
14. Lu R, Zhao X, Li J, et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. Lancet. 2020; 395:565-574.

Figures

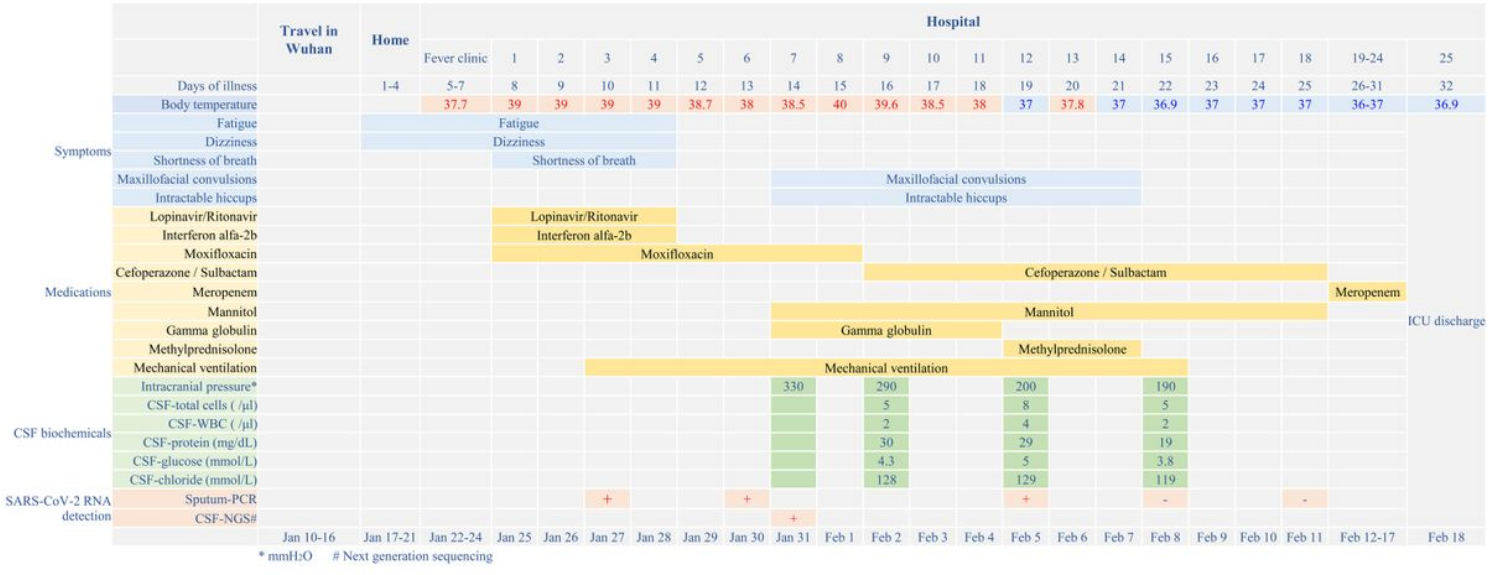

Figure 1

Timeline of disease course according to days from initial presentation of illness and days from hospital admission.

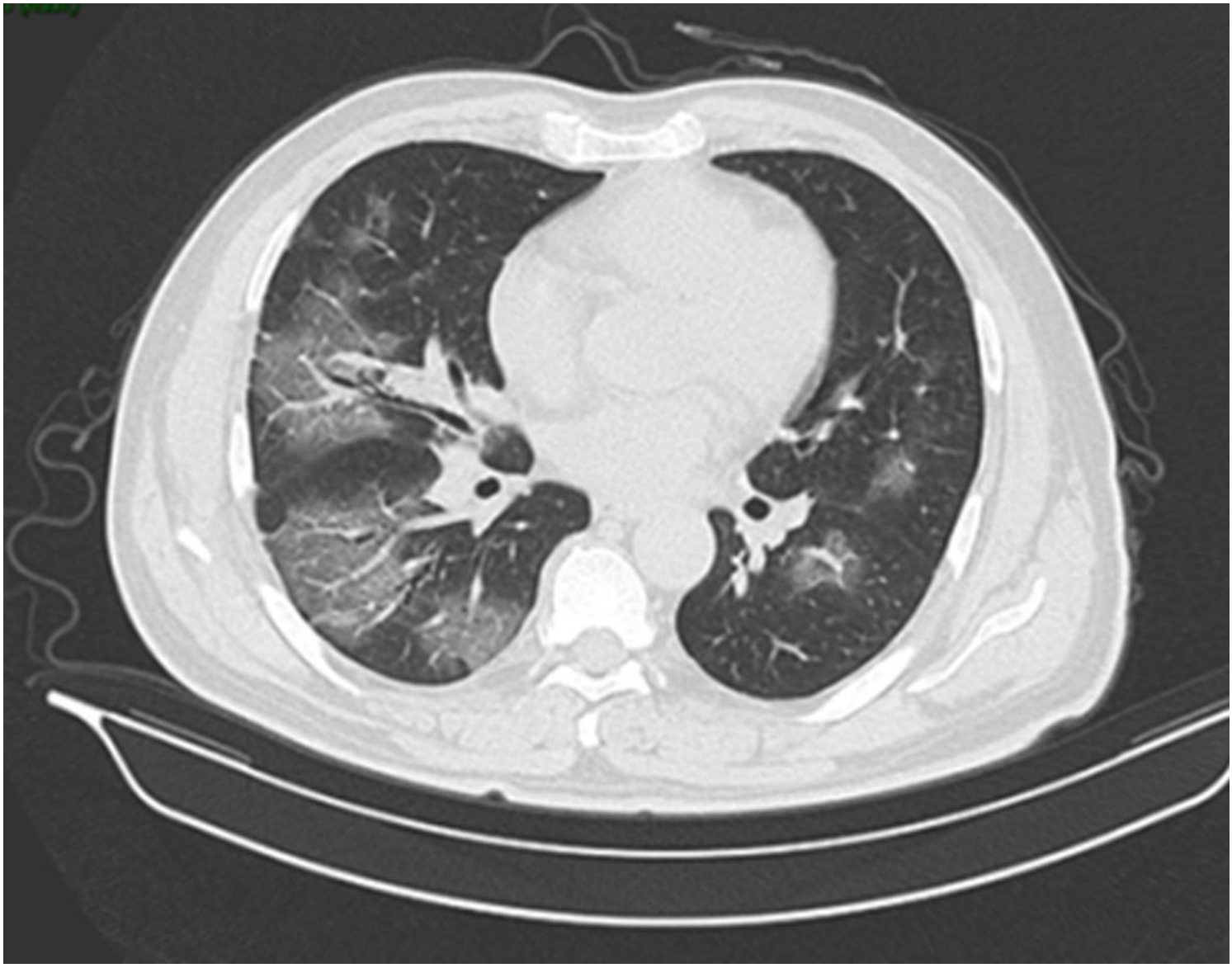

Figure 2

CT scan of the chest. Both lungs show scattered and patchy flakes of ground-glass opacities (GGO) on 24 January (day 7 of illness).

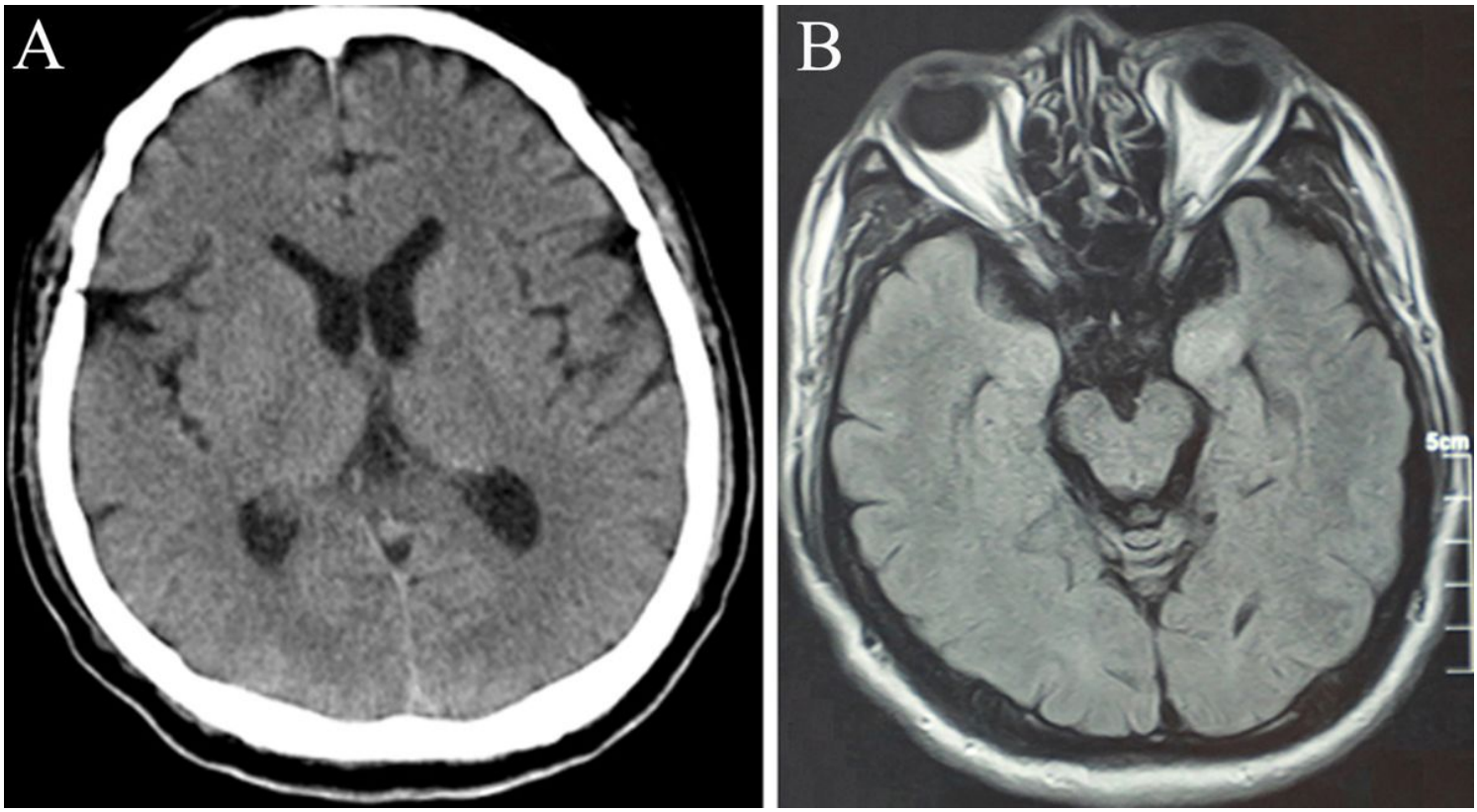

Figure 3

(A) CT scan of the brain showed no abnormally high or low opacity on 31 January (day 14 of illness). (B) MR scan of the brain after discharge showed abnormalities in the bilateral temporal lobe and hippocampus on 5 May (day 81 of illness).

Supplementary Files

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- AppendixFigure1.jpg
- AppendixFigure2.jpg
- AppendixFigure3.jpg