

# Cerebrospinal Fluid Analysis in Patients with COVID-Induced Encephalopathy

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## Abstract

**Background:** The novel coronavirus has been found to have neurological manifestations, and cerebrospinal fluid (CSF) analysis could be helpful in the differential diagnosis of critically ill patients with neurological symptoms and act as a prognostic factor in these patients.

**Objectives:** In this study, we assessed the CSF of patients with COVID-19 who presented with encephalopathy to elucidate any common characteristics.

**Methods:** This cross-sectional study included 36 patients with positive COVID-19 PCR who presented with encephalopathy and were hospitalized. We evaluated RT-PCR, total cell counts, LDH, protein, and glucose levels of CSF samples. Clinical manifestations, neurological complaints, and outcomes (death or survival) were extracted from patients' files.

**Results:** A total of 36 patients underwent lumbar puncture and CSF analysis. RT-PCR for SARS-CoV-2 RNA was negative in all thirty-six cases. Fever was present in 29 (80.6%) patients, shortness of breath in 23 (63.9%), and cough in 14 (38.9%) as primary clinical manifestations. Seizures were reported in 12 (33.3%) patients as a neurological presentation. The mortality rate was 7 (19.4%) in our cases. Abnormalities in WBC count were found in 6 (16.6%) of CSF samples. The remaining patients had normal CSF results, indicating no specific pattern of CSF markers.

**Conclusion:** Our study did not reveal any usual patterns in CSF analysis. Moreover, we were unable to detect SARS-CoV-2 RNA in CSF samples definitively. These findings suggest that the indirect mechanisms of SARS-CoV-2 may play a significant role in the neurological manifestations associated with COVID-19.

**Keywords:** COVID-19, Encephalopathy, Cerebrospinal Fluid, Encephalitis

## 1. Background

COVID-19 is an infectious disease and a global clinical challenge that has displayed neurological manifestations in addition to respiratory, gastrointestinal, and cardiovascular involvement.<sup>1</sup> Neurological presentations may include any aspect of neurological function such as acute cerebrovascular events, impaired consciousness, and musculoskeletal injury. Meningoencephalitis, encephalopathy, encephalomyelitis, acute demyelination, Guillain-Barré Syndrome (GBS), acute stroke, loss of consciousness, dizziness, ataxia, seizures, anosmia and dysgeusia, neuralgia, and headache have been reported in COVID-19 patients.<sup>2</sup> Positive CSF Real-time RT-PCR has been reported only in a few published cases. However, like other viral CNS infections, a negative PCR test can't completely rule out brain viral contamination.<sup>3</sup> Most CSF analyses in COVID-19 patients with neurological manifestations showed elevated CSF protein with a lymphocyte dominant cell count. Moreover, the severity of neurological complications was related to the amount

of CSF protein elevation.<sup>4</sup> Although not specific, CSF results may aid in the diagnosis of CNS diseases and act as a useful prognostic marker in acutely ill patients.<sup>5</sup>

## 2. Objectives

In this study, we assessed the cerebrospinal fluid (CSF) of patients with COVID-19 who presented with symptoms of encephalopathy to identify any common characteristics that may exist.

## 3. Methods

This is a cross-sectional study that was conducted on patients who were hospitalized from March 30, 2020, to March 30, 2021, in two educational hospitals: Alzahra University Hospital in Isfahan and Shohadaye Tajrish University Hospital in Tehran, Iran. During the study period, 108 patients with signs and symptoms of encephalopathy and a positive COVID-19 PCR test of nasopharyngeal swab were admitted to the hospitals. Lumbar puncture was performed on 36 patients.

Confirmation of COVID-19 diagnosis by a positive PCR test and simultaneous association with encephalopathy are among the criteria for inclusion of patients in the study. Patients who did not undergo a lumbar puncture due to a lack of consent for sampling or the diagnosis of the treating physician that sampling is not required were excluded from the study. Also, patients who had systemic and pulmonary infections at the onset of the disease and hospitalization were excluded from the study.

Patients' data, including gender, age, past medical history, drug history, neurological complaints, hospitalization duration, and outcome (recovery or death), were documented. Common COVID-19 manifestations such as fever, shortness of breath, cough, weakness, myalgia, vomiting, and diarrhea were also documented. Headache, dizziness, seizure, slurred speech, agitation, bilateral blurred vision, visual impairment, facial paralysis, limb paresis, incontinence, and ataxia were all neurological complaints recorded. Laboratory tests such as RT-PCR for SARS-CoV-2 RNA, cell counts, and chemical analysis (LDH, protein, and glucose levels) on CSF samples were performed to investigate a common CSF analyzing pattern in positive COVID-19 patients with encephalopathy.

Statistical analysis of data was done using SPSS version 23. A *P*-value of less than 0.05 was considered statistically significant.

#### 4. Results

A total of 36 patients (23 males and 13 females) with a mean age of  $45.12 \pm 27.01$  years underwent lumbar puncture and CSF analysis. All patients tested positive for COVID-19 based on RT-PCR from nasopharyngeal swabs. Fever, shortness of breath, and cough were among the most common primary clinical manifestations of COVID-19 among the patients (29 (80.6%), 23 (63.9%), and 14 (38.9%), respectively). Seizure was the most common primary neurological presentation in our patients (12 cases, 33.3%). Other observed neurological presentations included agitation (5 cases, 13.9%), slurred speech (4 cases, 11.1%), persistent headache (3 cases, 8.3%), ataxia (2 cases, 5.6%), dizziness (2 cases, 5.6%), limb paresis (1 case, 2.8%), and visual impairment (bilaterally decreased vision) (1 case, 2.8%). Twenty-nine patients (80.5%) recovered completely and were discharged, while 7 patients (19.4%) died. RT-PCR of CSF in all patients was reported negative. Out of the six patients with abnormal CSF results based on a WBC cutoff below  $5 \text{ cells/mm}^3$ , five were males and one was a female. The CSF results of the female patient, aged 64, indicated meningoencephalitis (WBC = 5200, Protein = 185), which unfortunately resulted in her death. The remaining patients had normal CSF results, showing no consistent pattern of CSF markers. Despite having normal CSF results, five of these patients also succumbed to the disease.

#### 5. Discussion

A spectrum of neurological manifestations has been reported in patients with COVID-19, involving either the central nervous system (CNS) or the peripheral nervous system (PNS). Hypothetically, SARS-CoV-2 may enter the cerebrospinal fluid (CSF) and/or the CNS parenchyma through three possible routes: the hematogenous pathway, the lymphatic pathway via the brain glymphatic system, and neuronal retrograde and trans-synaptic dissemination. This dissemination could start from a viral infection of the PNS and spread to the CNS, for example, through the olfactory nerve endings in the nasal neuroepithelium.<sup>6</sup> Given the ability of SARS-CoV-2 to induce hypercoagulability, impaired hemostasis, endothelial cell injury, and secondary intracranial hemorrhages, the hematogenous entry of SARS-CoV-2 into the CSF may seem more likely than other suspected pathways. Experimental data have shown modest replication of SARS-CoV-2 in neuronal cells *in vitro*.<sup>7,8</sup> However, observational studies and laboratory analyses of patients' CSF samples do not necessarily reflect an association between the virus presence in the CSF and neurological manifestations of COVID-19. An early report from Japan described the first case of COVID-19-associated meningitis; although SARS-CoV-2 RNA was not detected in the nasopharyngeal swab, it was detected in the CSF.<sup>3</sup> In another study on 58 consecutive patients with COVID-19-associated acute respiratory distress syndrome (ARDS), seven patients underwent lumbar puncture. All seven CSF samples were negative for SARS-CoV-2, using RT-PCR assays. One sample showed elevated levels of protein and IgG.<sup>9</sup> A retrospective screening of CSF samples from 23 COVID-19 patients –not necessarily presenting with neurological manifestations– was conducted at the virology laboratory of Lyon University Hospital. Samples from 21 patients were negative for SARS-CoV-2; the other two were only slightly positive and were considered probable blood contaminated CSF samples, given that brain biopsies from those two patients were negative for the virus and an available blood sample from one of them showed SARS-CoV-2 viremia.<sup>10</sup>

Later studies examining the CSF samples of a total of 69 COVID-19 patients with neurological manifestations have also yielded no evidence of SARS-CoV-2 presence in the CSF. In this study, we provided CSF analysis of 36 patients with confirmed COVID-19 infection who presented with signs and symptoms of encephalopathy. However, we did not find a similar pattern of cell count in the patients, and only one had CSF results consistent with meningoencephalitis. The majority of patients were male, and most had normal CSF results.<sup>11-13</sup> In a published case report, a 31-year-old male with altered mental status and abnormal behavior is presented. Based on imaging and clinical features, the patient was

diagnosed with acute encephalitis. The patient developed symptoms of COVID-19 two days before admission. COVID-19 encephalitis was confirmed with a positive CSF fluid PCR. However, the WBC and protein values of the CSF analysis were normal and similar to the majority of our patients.<sup>14</sup> In another study, out of 23 patients with encephalopathy who underwent CSF analysis, only 2 (9%) had high levels of WBC (>4/mm<sup>3</sup>).

However, nine of these patients (39%) had prominently elevated CSF protein levels.<sup>15</sup> Moghimi et al.<sup>16</sup> assessed the CSF results of nine patients with meningoencephalitis, and all except one had normal WBC counts, with only one patient having elevated protein levels (>45 mg/dl). This study further implies that CSF analysis cannot be solely used to identify COVID-19 unless PCR is undertaken. In a review comprising 56 isolated cases, encephalitis was more common among elderly individuals. Data reported mixed results and inconsistency between CSF findings. Patients either presented with completely normal CSF results or had raised protein levels, increased opening pressures, and lymphocytic pleocytosis.<sup>17</sup>

These studies and other available research have not successfully identified a link between CSF results and COVID-induced encephalitis. Lymphocytic pleocytosis and elevated protein levels are not seen in most patients, and the diagnosis of COVID-induced encephalitis has to be made based on clinical and imaging findings. Overall, despite earlier assumptions, our results, along with findings from other recent studies, may imply that the etiopathology of COVID-19-associated neurological presentations may more likely involve indirect SARS-CoV-2 pathogenesis rather than a direct presence of the virus in the CNS tissue and neuroinvasion.<sup>18</sup>

## 6. Conclusion

Our study did not reveal any usual patterns in CSF analysis. Moreover, we were unable to detect SARS-CoV-2 RNA in CSF samples definitively. Therefore, this study highlights the potential indirect role of SARS-CoV-2 in the development of neurological manifestations in COVID-19 patients.

### Research Highlights

#### What Is Already Known?

CSF analysis could be helpful in the differential diagnosis of critically ill patients with neurological symptoms and act as a prognostic factor in these patients.

#### What Does This Study Add?

These studies and other available research have not successfully identified a link between CSF results and COVID-induced encephalitis.

### Author Contributions

Authors contributed equally to this work.

### Conflict of Interest Disclosures

All authors declared that they have no conflict of interest.

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