Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Short Communication

Cerebrospinal fluid analysis of pregnant women at early stages of COVID-19

Lizandra Paravidine Sasaki, Geraldo Magela Fernandes, Angelo Pereira da Silva, Felipe Motta, Clara Siracusa, Isadora Pastrana Rabelo, Agenor dos Santos Junior, Paulo Sergio França, Patricia Kurisky, Rosana Maria Tristão, Cleandro de Albuquerque, Ciro Gomes, Licia Maria da Mota, Alberto Zaconeta

Department of Obstetrics and Gynecology, University of Brasília, Brasília, Brazil
Postgraduate Program in Medical Sciences, University of Brasília, Brasília, Brazil
Department of Pediatrics, University of Brasília, Brasília, Brazil
Regional Hospital of Asa Norte, Health Department, Brasília, Brazil
Central Laboratory of Public Health of the Federal District, Brasília, Brazil

Objective: To determine the presence or absence of SARS-CoV-2 in the cerebrospinal fluid of pregnant women at early stages of COVID-19.

Materials and methods: We conducted a prospective observational study with pregnant women undergoing cesarean section and real-time polymerase chain reaction to SARS-CoV-2 was performed in the cerebrospinal fluid in the early stages of COVID-19.

Results: Fourteen pregnant women, whose COVID-19 symptoms started between four to 18 days prior to delivery, were included. Eleven of the women reported anosmia, dysgeusia, and headaches and there were two fatal cases. SARS-Cov-2 was not present in the cerebrospinal fluid of these COVID-19 patients with early neurological symptoms, even in severe cases.

Conclusion: Our study suggests that peripheric cell damage and parainfectious phenomena may predominate over direct central nervous system injury in the pathophysiology of COVID-19 related early neurological symptoms on pregnant women.

© 2022 Taiwan Association of Obstetrics & Gynecology. Publishing services by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Introduction

Several neurological signs and symptoms have been described in patients with coronavirus disease (COVID-19), but it is unknown whether they are due to direct nervous system invasion by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) or are secondary to inflammatory and immunological processes. Typically, headaches, anosmia, and dysgeusia occur in the initial disease stages, while more severe neurological conditions such as encephalitis, stroke, and seizures may appear later [1].

As most studies have not detected SARS-CoV-2 in the cerebrospinal fluid (CSF) of patients with severe neurological disorders [2,3], Panciani et al. proposed the interesting hypothesis that early symptoms are caused by direct neurological injury when the viruses are present in the CSF, whereas later symptoms are due to immune-mediated damage upon SARS-CoV-2 clearance from the CSF [4]. However, as CSF analysis is usually reserved for patients with more severe neurological symptoms, it is currently unknown whether SARS-CoV-2 is actually present or not in the CSF in the early stages of COVID-19.

In pregnant women, hospitalizations due to COVID-19 occur mainly in the third trimester [5]; therefore, spinal anesthesia for those undergoing cesarean section is a great opportunity to assess the presence of SARS-CoV-2 in the CSF. We performed a study to determine the presence or absence of SARS-CoV-2 in the CSF of pregnant women at early stages of COVID-19.

* Corresponding author. Department of Obstetrics and Gynecology, Faculty of Medicine, University of Brasília, Campus Darcy Ribeiro, Asa Norte, Brasília, DF, CEP 70297-400, Brazil.
E-mail address: lizandra.sasaki@ebserh.gov.br (L.P. Sasaki).

https://doi.org/10.1016/j.tjog.2022.03.043
© 2022 Taiwan Association of Obstetrics & Gynecology. Publishing services by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
Materials and methods

This study is part of a larger project - PROUDEST: Effects of COVID-19 on pregnancy, childbirth, puerperium, neonatal period and child development: prospective, multicenter cohort study registered on the Brazilian Register of Triangular Trials (RBBE), RBR65QXS2, [https://ensaiosclínicos.gov.br/rg/RBR-65qxs2](https://ensaiosclínicos.gov.br/rg/RBR-65qxs2) and approved by the Research Ethics Committee of the University of Brasilia School of Medicine ([http://www.fm.unb.br/cep-fm - CAAE 32359620.0.0000.5558](http://www.fm.unb.br/cep-fm - CAAE 32359620.0.0000.5558)) [6]. All participants provided written, informed consent.

In this segment, we conducted a prospective observational study in women undergoing cesarean section at a COVID-19 referral hospital in Brasilia, Brazil, from June 20 to August 18, 2020. COVID-19 was diagnosed based on the following criteria: (a) positive SARS-CoV-2 real-time polymerase chain reaction (RT-PCR) on a nasopharyngeal swab specimen, (b) identification of serum antibodies to SARS-CoV-2 concomitantly with a chest computed tomography (CT) compatible with COVID-19, or (c) clinical symptoms suggestive of COVID-19 and a chest CT compatible with COVID-19.

We reserved a CSF sample from each woman when a lumbar puncture for spinal anesthesia was performed during the cesarean section. RT-PCR was performed with Molecular Research Institute of Paraná (Paraná, Brazil) or VIASURE SARS-CoV-2 (CerTest Biotech, Zaragoza, Spain) kits, whose detection limit is 4 copies per reaction.

Results

Fourteen pregnant women, whose COVID-19 symptoms started 4–18 days prior to delivery, were included. Among them, ten, three, and one had mild, severe, and critical diseases, respectively. Eleven reported anosmia, dysgeusia or headaches (Table 1).

There were two fatal cases, a critical disease patient who died 18 days postpartum due to respiratory failure and a severe disease patient whose respiratory condition was improving when she suddenly developed recurrent generalized tonic-clonic seizures and died 13 days postpartum. Angiotomography findings indicated posterior reversible encephalopathy syndrome, with no signs of thrombosis or hemorrhage. The CSF was SARS-CoV-2 RNA negative in all patients.

**Table 1**

Clinical features of pregnant women with COVID-19 and results of SARS-CoV-2 RNA testing of the cerebrospinal fluid.

| No. | Diagnostic criteria | Severity | Neurological symptoms (at any time) | Days from first symptoms to delivery and CSF analysis | Interval between the nasopharyngeal and CSF PCR results | CSF PCR results |
|-----|---------------------|----------|-------------------------------------|------------------------------------------------------|------------------------------------------------------|---------------|
| 1   | PCR | Severe | None | 4 | Same day | Negative |
| 2   | PCR | Mild | Anosmia, dysgeusia | 5 | Same day | Negative |
| 3   | PCR | Mild | Headaches | 6 | 3 days | Negative |
| 4   | PCR | Mild | Headaches, anosmia, dysgeusia | 6 | 3 days | Negative |
| 5   | PCR | Mild | Headaches | 7 | 1 day | Negative |
| 6   | PCR | Severe | Headaches, anosmia, dysgeusia | 7 | Same day | Negative |
| 7   | Ab and CT | Critical | None | 7 | NA | Negative |
| 8   | CT | Mild | Headaches, anosmia, dysgeusia | 8 | NA | Negative |
| 9   | CT | Mild | None | 9 | NA | Negative |
| 10  | PCR | Severe | Anosmia. Generalized tonic-clonic seizures. Brain CT: Posterior reversible encephalopathy syndrome | 12 | Same day | Negative |
| 11  | CT | Mild | Anosmia, dysgeusia | 13 | NA | Negative |
| 12  | PCR | Mild | Headaches | 13 | 11 days | Negative |
| 13  | Ab and CT | Mild | Headaches, anosmia, dysgeusia | 14 | NA | Negative |
| 14  | PCR | Mild | Anosmia, dysgeusia | 18 | 18 days | Negative |

Discussion

Brain invasion by SARS-CoV-2 has been reported on humans and animal models but remains unclear whether and how the virus crosses the blood–brain barrier (BBB) [7–9]. Since severe neurological manifestations of COVID-19 typically occur after the acute phase, microbiological examination of the CSF was rarely performed in the early stages of the disease [10]. However, during the first wave of the COVID-19 we had the opportunity to analyze the CSF of pregnant women whose symptoms had started between four and eighteen days.

Our results indicate that SARS-CoV-2 is not present in the CSF of COVID-19 patients with early neurological symptoms and therefore these symptoms may be not attributable to direct viral injury to the central nervous system.

As headache is a ubiquitous, non-specific symptom frequently seen in other infectious diseases, it can be a systemic effect of the SARS-CoV-2 infection. On the other hand, anosmia and dysgeusia are recognized as cardinal symptoms of early stages of the disease and have been used as diagnostic markers. They are most often transient, with a regaining of smell and taste after several days to weeks. This sudden onset, followed by a rapid recovery, is a strong argument against neuronal damage as a cause, given the time course of neuronal regeneration. Additionally, there is currently no evidence that the SARS-CoV-2 virus itself can reach the brain in the acute phase of anosmia [11]. Therefore, since SARS-CoV-2 enters human cells via the angiotensin-converting enzyme 2 (ACE 2) receptor that is expressed in the sustentacular cells of the olfactory epithelium but not in the olfactory sensory neurons, it has been proposed that anosmia and dysgeusia are secondary to olfactory epithelium damage [11].

Our data are in line with the growing evidence suggesting that parainfectious immune-mediated mechanism predominate over direct neurological injury in the pathophysiology of COVID-19 related neurological symptoms [1,12,13]. However, more studies are needed to determine the real entry routes of SARS-CoV-2 into the central nervous system, which pathogenic responses are triggered in infected cells and whether these mechanisms are influenced by pregnancy.
**Funding**

This research did not receive any funding.

**Declaration of competing interest**

The authors have no conflicts of interest relevant to this article.

**References**

[1] Pezzini A, Padovani A. Lifting the mask on neurological manifestations of COVID-19. Nat Rev Neurol 2020;16:636–44. https://doi.org/10.1038/s41582-020-0398-3.

[2] Espíndola OM, Siqueira M, Soares CN, Lima M, Leite A, Araujo A, et al. Patients with COVID-19 and neurological manifestations show undetectable SARS-CoV-2 RNA levels in the cerebrospinal fluid. Int J Infect Dis 2020;96:567–9. https://doi.org/10.1016/j.ijid.2020.05.123.

[3] Helms J, Kremers S, Merdji H, Clere-Jehl R, Schenck M, Kummerlen C, et al. Neurologic features in severe SARS-CoV-2 infection. N Engl J Med 2020;382:2268–70. https://doi.org/10.1056/NEJMoa2008597.

[4] Panciani PP, Saraceno G, Zanin L, Renisi G, Signorini L, Battaglia L, et al. SARS-CoV-2: “Three-steps” infection model and CSF diagnostic implication. Brain Behav Immun 2020;87:1288–9. https://doi.org/10.1016/j.bbi.2020.05.002.

[5] Knight M, Bunch K, Vousden N, Morris E, Simpson N, Gale C, et al. Characteristics and outcomes of pregnant women admitted to hospital with confirmed SARS-CoV-2 infection in UK: national population based cohort study. BMJ 2020;369:m2107. https://doi.org/10.1136/bmj.m2107.

[6] Fernandes GM, Motta F, Sasaki LMP, Silva APD, Miranda AM, Carvalho AO, et al. Pregnancy outcomes and child development effects of SARS-CoV-2 infection (PROUDEST trial): protocol for a multicenter, prospective cohort study. JMIR Res Protoc 2021;20(4):e26477. 10, https://doi: 10.2196/26477.

[7] Zhang L, Zhou L, Bao L, Liu J, Zhu H,Lv Q, et al. SARS-CoV-2 crosses the blood–brain barrier accompanied with basement membrane disruption without tight junctions alteration. Signal Transduct Targeted Ther 2021;6:337. https://doi.org/10.1038/s41392-021-00719-9.

[8] Moriguchi T, Harii N, Goto J, Harada D, Sugawara H, Takamine J, et al. A first case of meningitis/encephalitis associated with SARS-Coronavirus-2. Int J Infect Dis 2020;94:55–8. https://doi.org/10.1016/j.ijid.2020.03.062.

[9] Puelles VG, Litgehtehmann M, Lindenmeyer MT, Sperhake JP, Wong MN, Allweiss L, et al. Multiorgan and renal tropism of SARS-CoV-2. N Engl J Med 2020;383(6):590–2. https://doi:10.1056/NEJMoa2014600.

[10] Neumann B, Schmidbauer ML, Dimitriadis K, Otto S, Knier B, Niesen WD, et al. PANDEMIC and the IGNITE study groups. Cerebrospinal fluid findings in COVID-19 patients with neurological symptoms. J Neurol Sci 2020;418:117090. https://doi.org/10.1016/j.jns.2020.117090.

[11] Butowt R, van Bartheld C. Anosmia in COVID-19: underlying mechanisms and assessment of an olfactory route to brain infection. Neuroscientist 2021 Dec;27(6):582–603. https://doi:10.1177/1073858420956905.

[12] Baig AM, Khaleeq A, Ali U, Syeda H. Evidence of the COVID-19 virus targeting the CNS: tissue distribution, host–virus interaction, and proposed neurotropic mechanisms. ACS Chem Neurosci 2020;11(7):995–8. https://doi:10.1021/acschemneuro.0c00122.

[13] Karnik M, Beeraka NM, Uthaiah CA, Nataraj SM, Bettadapura ADS, Aliev G, et al. A review on SARS-CoV-2-induced neuroinflammation, neurodevelopmental complications, and recent updates on the vaccine development. Mol Neurobiol 2021;58(3):4535–63. https://doi:10.1007/s12035-021-02399-6.