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EVALUATION OF THE DEVELOPMENT OF NEUROLOGICAL DAMAGE IN SEVERE COVID-19 PNEUMONIA WITH SERUM CK-BB LEVELS

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Abstract

Aim: Despite numerous reports of various neurological symptoms observed in Coronavirus disease-2019 (COVID-19) disease, the role of severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) in the neuropathogenesis and tropism of the central nervous system (CNS) has yet to be fully elucidated. In this study, we evaluated the development of CNS damage related to the severity of COVID-19 pneumonia with creatine kinase BB (CK-BB) serum levels.

Material and Methods: The study included 55 patients hospitalized in the intensive care unit diagnosed with severe COVID-19 pneumonia, 79 in the inpatient service diagnosed with milder COVID-19 pneumonia, and 39 healthy volunteers. CK-BB levels were measured using a quantitative sandwich enzyme immunoassay technique with an Enzyme-Linked Immunosorbent Assay kit. Detection of SARS-CoV-2 was performed by real time-polymerase chain reaction from the respiratory tract (nasopharyngeal swab) according to current guidelines.

Results: While markers that were shown to predict disease severity were higher in patients with severe COVID-19 pneumonia compared with patients with milder pneumonia and the control group, serum levels of the neurological damage marker CK-BB were found to be similar between the groups [respectively 6.84 (5.05-16.2), 7.48 (4.7-22.5), 6.7 (3.8-16.2), p>0.005]. In addition, there was no difference in serum CK-BB levels between patients who developed neurological symptoms and those who did not [respectively 6.78 (5.07-17.21), 7.43 (4.7-22.5), p>0.005].

Conclusion: In COVID-19 pneumonia, serum CK-BB levels do not increase with disease severity and the development of neurological symptoms.

Keywords: COVID-19, central nervous system, creatine kinase BB

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INTRODUCTION

Since the onset of Coronavirus disease-2019 (COVID-19), many reports have been published on the neurological manifestations of severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2), the most common of which are encephalopathy, headache, anosmia, vertigo, ageusia, and seizure (1-4). However, insufficient information exists to explain the pathophysiological mechanisms of neurological symptoms. Neuropathological effects may be associated with hypoxia, proinflammatory cytokines, or a direct result of viral neuroinvasion (5-7). On the contrary, studies show that SARS-CoV-2, due to viral neuroinvasion or antibody levels measured intrathecally, are very low in cerebrospinal fluid (CSF) examinations performed in patients with neurological symptoms (8). Currently, the role of SARS-CoV-2 in central nervous system (CNS) neuropathogenesis and tropism remains unclear, and questions regarding the existence of CNS damage due to SARS-CoV-2 remain unanswered.

Creatine kinase BB (CK-BB), an isoform of CK, is found in the CNS. The transfer of phosphate groups from ATP to creatine phosphate is catalyzed by this enzyme, resulting in energy transfer in tissues with high energy needs, such as the brain. CK-BB, which is found in astrocytes in the CNS, is released into the environment in cases where brain tissue is damaged (9,10). Studies have shown that serum CK-BB levels increase in cases of acute head trauma (10-13) or the development of brain damage due to ischemia after cardiac arrest (14) and subarachnoid hemorrhage (15). In conclusion, many studies have reported that CK-BB is a reliable neurotoxicity biomarker (16-20).

This study was conducted to detect CNS damage that may develop in severe COVID-19 pneumonia. Therefore, we compared serum CK-BB levels between patients with severe COVID pneumonia in the intensive care unit, patients with milder COVID pneumonia in the inpatient service, and healthy volunteers.

MATERIAL AND METHODS

This study was conducted as an analytical case-control study between June 1, 2020 and June 1, 2022. The study included 55 intensive care unit patients and 79 inpatient service patients hospitalized in the Ankara City Hospital diagnosed with COVID-19 pneumonia using a positive nasal SARS-CoV-2 polymerase chain reaction (PCR) test. Forty healthy volunteers with a mean age and gender similar to those of patients with COVID-19 pneumonia were included in the control group. Patients with malignancy, those younger than 18 years of age, and pregnant women were excluded from the study. Individuals with diabetes mellitus, hypertension, coronary artery disease, or chronic lung disease were considered positive for the presence of comorbid diseases. Informed consent was obtained from the individuals included in the study and from the first-degree relatives of the patients who could not provide consent.

Patients who developed symptoms such as confusion, impaired consciousness, sleepiness, agitation, hallucinations, syncope, acute muscle weakness, spasticity, hyperreflexia, Babinski symptoms, or acute central vertigo during the follow-up of patients hospitalized in the COVID-19 service or intensive care units were evaluated by neurology specialists through an in-hospital consultation system. These patients, considered to have developed symptoms localized to the CNS after neuroimaging, were included in the neurological symptom subgroup.

According to current guidelines (21) (Institut Pasteur; World Health Organization technical manual), SARS-CoV-2 detection was performed by real time (RT)-PCR of the respiratory tract (nasopharyngeal swab). The threshold detection limit of this assay, which targets two regions of the RNA polymerase gene in a viral RNA-dependent manner, was 500 copies/mL.

Ten milliliters of venous blood samples were collected in vacutainer tubes and centrifuged at 1300xg for 10 minute. The separated greenhouse was aliquots into Eppendorf tubes and stored at 80 °C until analysis. CK-BB levels were measured with an Enzyme-Linked Immunosorbent Assay kit (USCN, USCN, Wuhan, China; catalog number: SEC030Hu; lot number: L210602384) using a quantitative sandwich enzyme immunoassay technique. The concentration of CK-BB in the samples was calculated by comparing the samples' optical density (OD) to the standard curve. The detection range of the assay was 1.56-100 ng/mL. Intra- and interassay precision were <10% and <12%, respectively. CK levels were measured spectrophotometrically using an ADVIA Chemistry XPT autoanalyzer (Siemens Healthineers, Erlangen, Germany). Mass CK-MB levels were measured by chemiluminescent immunoassay on an ADVIA Centaur XPT autoanalyzer (Siemens Healthineers, Erlangen, Germany).

Statistical Analysis

Statistical analysis of the study was performed using SPSS 22.0. First, the suitability of the numerical variables, whose descriptive statistics will be given, to the normal distribution was examined using visual (histogram, probability graphs) and analytical methods (Kolmogorov-Smirnov/Shapiro-Wilk tests). Descriptive statistics were calculated using mean and standard deviation (mean ± SD) for normally distributed variables and median and (minimum-maximum) values for non-normally distributed variables. Categorical variables are expressed as numbers and percentages (%). After Bonferroni correction, comparisons between multiple groups were made using One-Way ANOVA post

hoc Tukey's test for normally distributed numerical variables and with Independent Samples Kruskal-Wallis test for non-normally distributed numerical variables. Independent Sample t-tests were used for normally distributed samples to compare continuous numerical variables between groups, and the Mann-Whitney U test was used for non-normally distributed models. The chi-square test was used to compare categorical data. The correlation relationship between continuous variables was evaluated using Spearman's correlation analysis. The level of relationship between the variables was interpreted as follows according to the r correlation coefficient results: low degree between 0.01 and 0.29, medium degree between 0.30 and 0.70, and high degree between 0.71 and 0.99. The lower limit considered significant was taken as $p<0.05$ in pairwise comparisons, and in multiple comparisons, it was decided according to the Bonferroni correction.

The Ankara City Hospital Ethics Committee approved the research protocol (approval number: E1-21-1813, date: 20.04.2022), and all patients provided written informed consent to participate in the study. Informed consent was obtained from the patients to participate in the study.

RESULTS

Demographic characteristics and laboratory values of the intensive care, inpatient service, and control groups are shown in Table 1. Regarding most values accepted as prognostic markers in COVID pneumonia, there was a significant difference in COVID-19 pneumonia groups compared with controls ($p<0.05$). However, no significant difference was found between the groups regarding CK-BB levels ($p>0.05$).

A comparison of CK-BB levels according to clinical condition and medical treatment type in patients with COVID-19 pneumonia is shown in Table 2. In patients with COVID-19 pneumonia, serum CK-BB levels were similar in the presence of different clinical conditions or treatment types ($p>0.05$).

The correlation between the biochemical markers predicting COVID-19 pneumonia severity and CC-BB is shown in Table 3. No significant correlation was found between CK-BB levels and other biomarkers ($p>0.05$).

DISCUSSION

In this study, we aimed to detect CNS damage that may develop in severe COVID-19 pneumonia. Therefore, we compared plasma CK-BB levels between patients with severe COVID-19 pneumonia in the intensive care unit, patients with milder COVID pneumonia in the inpatient service, and healthy volunteers. Our results showed that elevated CK-BB levels in blood-brain barrier damage remained within normal limits in patients with SARS-CoV-2 and severe pneumonia.

Although neurological symptom development has been widely reported since the onset of the COVID-19 pandemic, SARS-CoV-2 has rarely been reported to cause neuroinvasion. Neurological symptoms that develop in COVID-19 patients may generally result from ischemic injury due to hypoxia, stroke, toxic metabolic reactions, excessive cytokine release, or molecular similarity between COVID-19 antibodies and nervous system glycopeptides and cells (8). In addition, postmortem neuropathological examination results showed that it was not possible to distinguish whether symptoms were the result of hypoxia, viral neuroinvasion, multiple organ failure, decreased immune response, cytokine storm, or stroke. Therefore, it has been reported that large-scale cellular and molecular studies in CSF and brain tissue are needed, together with neurological evaluation and neuroimaging studies, to evaluate the neurological damage due to SARS-CoV-2 (22). One case report demonstrated the presence of SARS-CoV-2 in brain tissue through molecular testing and ultrastructural analysis despite having a negative SARS-CoV-2 PCR in CSF (23). On the other hand, a review study covering 43 postmortem brain tissue examinations reported that the severity of neuroimmune activation was not associated with SARS-CoV-2 in the brain tissue (24).

Although there have been reports of positive SARS-CoV-2 RNA detection in the CSF of COVID-19 patients with neurological symptoms, these reports generally come from a limited number of case reports (25-29). A review study was published by Lewis et al. (8) between December 1, 2019, and November 18, 2020, involving 430 COVID-19 patients with symptoms localized to the CNS and CSF examination. According to the results of this study, the SARS-CoV-2 PCR test was positive in 17 (6%) of 304 patients who underwent the CSF test. SARS-CoV-2 antibody positivity in CSF was observed in 7 (12%) of 58 patients, and evidence of intrathecal antibody synthesis was detected in 3 (2%) of 132 patients with oligoclonal bands. In the examination of CSF for autoimmune antibodies, positivity was found in 4 (5%) of 77 patients. In conclusion, this study reported that viral neuroinvasion and intrathecal antibody synthesis due to SARS-CoV-2 rarely occur in COVID-19 patients with neurological symptoms (8).

Although neuropathological examinations of brain tissue and CSF analysis studies partially explain the neurological symptoms developing in COVID-19 patients, data on the presence of neurological damage due to SARS-CoV-2 are still insufficient. CK-BB, which can be used as a reliable biomarker of neurotoxicity (16-20), has not been previously investigated in detecting

neurological damage that may develop due to COVID-19 pneumonia. In this study, we evaluated plasma CK-BB levels to detect neurological damage that may develop with the severity of COVID-19 pneumonia. In previous studies, parameters such as ferritin, CRP, pro-BNP, troponin-I, fibrinogen, d-dimer, neutrophil-lymphocyte ratio, procalcitonin, and IL-6 were found to be prognostic biomarkers in COVID-19 pneumonia (30-32). In our study, these biomarkers were found to be high in intensive care patients with severe pneumonia symptoms ($p<0.01$), while serum CK-BB levels did not change ($p>0.05$). In addition, our results showed no increase in serum CK-BB levels in COVID-19 patients who developed neurological symptoms compared with those who did not ($p>0.05$).

The small number of patients in the subgroup with neurological syndrome and the inability to evaluate CK-BB levels in CSF simultaneously with plasma CK-BB in this patient group are among the limitations of this study. Another limitation of our study is that serum CK-BB levels may be affected by other secondary infections accompanying COVID-19 pneumonia or immunosuppressive agents used in medical treatment.

Table 1. Comparison of demographic characteristics and laboratory values between the intensive care, service, and control groups

Parameters	Intensive care	Inpatient service	Control	p-value[&]
Gender female/male, n	21\34, 55	32\47, 79	15\24, 39	>0.05
Age, mean ± SD (years)	56.05±13.23	51.48±15.17	52.2±14.1	>0.05
Body mass index, mean ± SD	26.23±4.02	24.34±3.36	27.4±4.69	>0.05
Presence of comorbid disease, n (%)	26 (47.2)	38 (46.9)	18 (43.5)	>0.05
WBC, median (min.-max.) [cells/mm^3]	10140 (1190-48610)*	7110 (22330-2630)	7130 (13670-3640)	**<0.001**
Hemoglobin, median (min.-max.) [g/dL]	10 (6.5-16.3)*	13 (7.2-16.3)	13.6 (8.2-17.2)	**<0.001**
PLT, median (min.-max.) [x10^9/L]	279 (26-788)	260 (137-623)	285 (155-478)	0.589
Creatinin, median (min.-max.) [U/L]	0.73 (0.21-15.1)	0.78 (0.44-1.73)	0.7 (0.1-1.02)	0.131
ALT, median (min.-max.) [U/L]	46.5 (20-3161)	38.5 (10-331)	18 (9-81)$^+$	**<0.001**
AST, median (min.-max.) [U/L]	37 (14-9549)	34 (12-170)	19 (11-42)$^+$	**<0.001**
LDH, median (min.-max.) [U/L]	483 (222-4134)	338 (118-775)	191 (32-242)$^+$	**<0.001**
CRP, median (min.-max.) [mg/L]	85 (0.5-277)*	23.5 (0.6-246)**	3 (0.5-7)	**<0.001**
ESR, mean ± SD [mm/h]	48.5±28.9*	35.05±21.05**	11 (6-54)	**<0.01**
Ferritin, median (min.-max.) [µ/L]	600 (55-68025)*	250 (3.48-3245)**	45 (2.4-135.7)	**<0.001**
NLR, median (min.-max.)	13.8 (2.5-215)*	5.11 (1.1-45.8)**	2.38 (1.27-5.97)	**<0.001**
Pro-BNP, median (min.-max.) [ng/L]	1065 (67-35000)*	219 (35-9614)**	12 (5-36)	**<0.010**
Troponin-I, median (min.-max.) [ng/mL]	22.3 (2.5-1483)*	3.03 (2-271.6)**	1.05 (0.4-2.5)	**<0.001**
CK-MB, median (min.-max.) [µg/L]	0.59 (0.1-4.21)	0.54 (0.1-29)	0,65 (0.12-4.12)	0.48
CK, median (min.-max.) [U/L]	104 (23-2181)*	70 (7-2152)	83 (38-890)	**0.014**
CK-BB, median (min.-max.) [ng/mL]	6.84 (5.05-16.2)	7.48 (4.7-22.5)	6.7 (3.8-16.2)	0.183
Fibrinogen, mean ± SD, [g/L]	5.29±1.83	4.93±1.5	2.1 (1.4-4.6)$^+$	**<0.01**
D-dimer, median (min.-max.) [mg/L]	1.8 (0.1-35.2)*	0.6 (0.01-13.1)**	0.03 (0.01-0.08)	**<0.001**
Procalcitonin, median (min.-max.) [µ/L]	0.2 (0.03-79)*	0.05 (0.03-26.9)**	0.01 (0.004-0.05)	**<0.001**
IL-6, median (min.-max.) [pg/mL]	22.1 (2.7-1268)*	6.08 (2.6-110.04)**	2.1 (1.1-7.3)	**<0.01**

Bold font indicates statistical significance. * Group different from inpatient service and control group,** group different from the control group, $^+$group different from the intensive care and inpatient service group, [&]the significantly lower limit accepted after Bonferroni correction was $p<0.0167$.
Note: After Bonferroni correction, comparisons between multiple groups were made using One-Way ANOVA post hoc Tukey's test for normally distributed numerical variables and with Independent Samples Kruskal-Wallis test for non-normally distributed numerical variables. WBC: White blood cells, CRP: C-reactive protein, ESR: Erythrocyte sedimentation rate, PLT: Platelet, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, LDH: Lactate dehydrogenase, NLR: Neutrophil/lymphocyte ratio, BNP: Brain natriuretic peptide, CK: Creatine kinase, IL-6: Interleukin-6, SD: Standard deviation, min.: Minimum, max.: Maximum

Table 2. Comparison of CK-BB levels according to the clinical condition and medical treatment type in patients with COVID-19 pneumonia

Clinical condition		n	CK-BB, median (min.-max.)	p-value
Intubated	Yes	24	7.56 (5.07-16.25)	0.153
	No	110	7.87 (6.42-21.53)	
Non-invasive mechanical ventilator or high O^2 requirement	Yes	39	7.16 (5.7-11.5)	0.571
	No	95	7.33 (4.5-20.4)	
Low O^2 requirement	Yes	36	8.09 (5.23-22.51)	0.483
	No	98	8.41 (6.42-21.53)	
No O^2 support	Yes	35	7.24 (4.7-16.7)	0.694
	No	99	7.32 (4.9-22.45)	
Vasopressor requirement	Yes	15	7 (5.07-16.2)	0.603
	No	119	7.21 (4.7-22.51)	
Neurological symptom	Yes	29	6.78 (5.07-17.21)	0.074
	No	105	7.43 (4.7-22.5)	
Death	Yes	30	6.78 (5.07-16.25)	0.408
	No	104	7.21 (4.7-22.51)	
Medical treatment				
Medium-dose steroid	Yes	9	7.16 (5.45-16.2)	0.569
	No	125	7.38 (5.79-19.1)	
High-dose steroid	Yes	54	7.29 (5.07-22.51)	0.614
	No	80	7.41 (6.92-20.57)	
Pulse steroid	Yes	14	7.29 (5.39-13.1)	0.746
	No	120	7.36 (6.45-19.9)	
IL-1 blocker	Yes	8	7.02 (5.07-14.97)	0.215
	No	126	7.49 (5.32-17.4)	
O^2 therapy only	Yes	49	7.11 (4.91-16.57)	0.102
	No	85	7.49 (5.12-18.59)	

Note: Pairwise comparisons were performed using the Mann-Whitney U test. Bold font indicates statistical significance. CK-BB: Creatine kinase BB, COVID-19: Coronavirus disease-2019, IL: Interleukin, O_2: Oxygen, min.: Minimum, max.: Maximum

Table 3. Correlation between biochemical markers predicting COVID-19 pneumonia severity and CC-BB levels

Parameter (p)	CK	CRP	IL-6	D-dimer	Pro-BNP	Troponin-I	Ferritin	NLR	Creatinin
CK-BB	0.057 (0.454)	-0.073 (0.347)	-0.013 (0.881)	0.054 (0.524)	-0.066 (0.447)	0.037 (0.670)	0.143 (0.077)	-0.003 (0.974)	-0.027 (0.7329)
CK		0.108 (0.164)	0.202 **(0.019)**	0.142 (0.091)	0.083 (0.341)	0.164 (0.056)	0.080 (0.325)	0.097 (0.214)	0.171 **(0.032)**
CRP			0.388 **(<0.0001)**	0.463 **(<0.0001)**	0.248 **(0.004)**	0.484 **(<0.0001)**	0.527 **(<0.0001)**	0.559 **(<0.0001)**	0.074 (0.357)
IL-6				0.350 **(<0.0001)**	0.250 **(0.004)**	0.285 **(0.001)**	0.125 **(0.149)**	0.257 **(0.003)**	0.016 (0.860)
D-dimer					0.421 **(<0.0001)**	0.624 **(<0.0001)**	0.360 **(<0.0001)**	0.555 **(<0.0001)**	-0.001 (0.986)
Pro-BNP						0.446 **(<0.0001)**	0.235 **(<0.006)**	0.433 **(<0.0001)**	0.150 (0.096)
Troponin-I							0.415 **(<0.0001)**	0.494 **(<0.0001)**	0.128 (0.150)
Ferritin								0.566 **(<0.0001)**	0.164 **(0.049)**
NLR									0.133 (0.097)

Note: The correlation relationship between continuous variables was evaluated using Spearman's correlation analysis. Bold font indicates statistical significance.
CK-BB: Creatine kinase BB, CRP: C-reactive protein, IL-6: Interleukin-6, BNP: Brain natriuretic peptide, NLR: Neutrophil/lymphocyte ratio, COVID-19: Coronavirus disease-2019

CONCLUSION

This study showed no increase in serum CK-BB levels, considered an indicator of CNS damage, depending on the severity of COVID-19 pneumonia. Currently, no concrete evidence definitively demonstrates the existence of neurological damage due to SARS-CoV-2, and our results suggest no increase in neurological damage shown by serum CK-BB levels in patients with COVID-19 pneumonia.

However, further studies using imaging methods, CSF studies, and histopathological techniques are needed to clarify the nature of SARS-CoV-2-induced CNS damage and evaluate its relationship with disease.

Ethics

Ethics Committee Approval: The Ankara City Hospital Ethics Committee approved the research protocol (approval number: E1-21-1813, date: 20.04.2022).

Informed Consent: Informed consent was obtained from the individuals included in the study and from the first-degree relatives of the patients who could not provide consent.

Authorship Contributions

Surgical and Medical Practices: D.E., R.G., Concept: Y.M., A.K., E.A., D.E., Ş.E., Design: Y.M., A.K., İ.D., R.G., Ö.E., Data Collection or Processing: Y.M., H.E.K., K.O., Analysis or Interpretation: Y.M., E.A., E.F.O., K.O., Ö.E., Ş.E., Literature Search: H.E.K., İ.D., E.F.O., Writing: A.K., Y.M.

Conflict of Interest: The authors have no conflicts of interest to declare.

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REFERENCES

1. Mao L, Jin H, Wang M, et al. Neurologic manifestations of hospitalized patients with coronavirus disease 2019 in Wuhan, China. JAMA Neurol. 2020;77:683-90.
2. Helms J, Kremer S, Merdji H, et al. Neurologic features in severe SARS-CoV-2 infection. N Engl J Med. 2020;382:2268-70.
3. Lu L, Xiong W, Liu D, et al. New-onset acute symptomatic seizure and risk factors in coronavirus disease 2019: a retrospective multicenter study. Epilepsia. 2020;61:49-53.
4. Frontera JA, Sabadia S, Lalchan R, et al. A prospective study of neurologic disorders in hospitalized patients with COVID-19 in New York City. Neurology. 2021;96:e575-86.

5. Koralnik IJ, Tyler KL. COVID-19: a global threat to the nervous system. Ann Neurol. 2020;88:1-11.

6. Desforges M, Le Coupanec A, Brison E, et al. Neuroinvasive and neurotropic human respiratory coronaviruses: potential neurovirulent agents in humans. Adv Exp Med Biol. 2014;807:75-96.

7. Desforges M, Le Coupanec A, Dubeau P, et al. Human coronaviruses and other respiratory viruses: underestimated opportunistic pathogens of the central nervous system? Viruses. 2019;12:14.

8. Lewis A, Frontera J, Placantonakis DG, et al. Cerebrospinal fluid in COVID-19: A systematic review of the literature. J Neurol Sci. 2021;421:117316.

9. Lonare M, Kumar M, Raut S, et al. Evaluation of imidacloprid-induced neurotoxicity in male rats: a protective effect of curcumin. Neurochem. Int 2014;78:122-9.

10. Sharma R, Rosenberg A, Bennett ER, et al. A blood-based biomarker panel to risk-stratify mild traumatic brain injury. PLoS One. 2017;12:e0173798.

11. Phillips JP, Jones HM, Hitchcock R, et al. Radioimmunoassay of serum creatine kinase BB as index of brain damage after head injury. Br Med. J 1980;281:777-9.

12. Cooper PR, Chalif DJ, Ramsey JF, et al. Radioimmunoassay of the brain type isoenzyme of creatine phosphokinase (CK-BB): a new diagnostic tool in the evaluation of patients with head injury. Neurosurgery. 1983;12:536-41.

13. Kaste M, Hernesniemi J, Somer H, et al. Creatine kinase isoenzymes in acute brain injury. J Neurosurg. 1981;55:511-5.

14. Tirschwell DL, Longstreth WT, Rauch-Matthews ME, et al. Cerebrospinal fluid creatine kinase BB isoenzyme activity and neurologic prognosis after cardiac arrest. Neurology. 1997;48:352-7.

15. Coplin WM, Longstreth WT, Lam AM, et al. Cerebrospinal fluid creatine kinase-BB isoenzyme activity and outcome after subarachnoid hemorrhage. Arch Neurol. 1999;56:1348-52.

16. QureshiG A, Bibi S, Baloch S, et al. The role of excitatory amino acids and its neurotoxic impact in severe head injury patients. World Appl Sci. J 2011;15:909-14.

17. Kruse A, Cesarini KG, Bach FW, et al. Increases of neuron-specific enolase, S-100 protein, creatine kinase and creatine kinase BB isoenzyme in CSF following intraventricular catheter implantation. Acta Neurochir (Wien). 1991;110:106-9.

18. Secer HI, Izci Y. The CSF creatine kinase-BB isoenzyme activity in experimental lumbar spinal stenosis model. J Spinal Disord Tech. 2008;21:148-52.

19. Zandbergen EG, de Haan RJ, Hijdra A. Systematic review of prediction of poor outcome in anoxic-ischaemic coma with biochemical markers of brain damage. Intensive Care Med. 2001;27:1661-7.

20. Carr ME Jr, Masullo LN, Brown JK, et al. Creatine kinase BB isoenzyme blood levels in trauma patients with suspected mild traumatic brain injury. Mil Med. 2009;174:622-5.

21. Hirotsu Y, Mochizuki H, Omata M. Double-quencher probes improve detection sensitivity toward Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) in a reverse-transcription polymerase chain reaction (RT-PCR) assay. J Virol Methods. 2020;284:113926.

22. Al-Sarraj S, Troakes C, Hanley B, et al. Invited review: The spectrum of neuropathology in COVID-19. Neuropathol Appl Neurobiol. 2021;47: 3-16.

23. Paniz-Mondolfi A, Bryce C, Grimes Z, et al. Central nervous system involvement by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). J Med Virol. 2020;92:699-702.

24. Matschke J, Lütgehetmann M, Hagel C, et al. Neuropathology of patients with COVID-19 in Germany: a post-mortem case series. Lancet Neurol. 2020;19:919-29.

25. Moriguchi T, Harii N, Goto J, et al. A first case of meningitis/encephalitis associated with SARS-Coronavirus-2. Int J Infect Dis. 2020;94:55-8.

26. Domingues RB, Mendes-Correa MC, de Moura Leite FBV, et al. First case of SARS-COV-2 sequencing in cerebrospinal fluid of a patient with suspected demyelinating disease. J Neurol. 2020;267:3154-6.

27. Mardani M, Nadji SA, Sarhangipor KA, et al. COVID-19 infection recurrence presenting with meningoencephalitis. New Microbes New Infect. 2020;37:100732.

28. Huang YH, Jiang D, Huang JT. SARS-CoV-2 detected in cerebrospinal fluid by PCR in a case of COVID-19 encephalitis. Brain Behav Immun. 2020;87:149.

29. Khodamoradi Z, Hosseini SA, Gholampoor Saadi MH, et al. COVID-19 meningitis without pulmonary involvement with positive cerebrospinal fluid PCR. Eur J Neurol. 2020;27:2668-9.

30. Aljohani FD, Khattab A, Elbadawy HM, et al. Prognostic factors for predicting severity and mortality in hospitalized COVID-19 patients. J Clin Lab Anal. 2022;36:e24216.

31. Orsucci D, Trezzi M, Anichini R, et al. Increased creatine kinase may predict a worse COVID-19 outcome. J Clin Med. 2021;10:1734.

32. Rasyid H, Sangkereng A, Harjianti T, et al. Impact of age to ferritin and neutrophil-lymphocyte ratio as biomarkers for intensive care requirement and mortality risk in COVID-19 patients in Makassar, Indonesia. Physiol Rep. 2021;9:e14876.