Serum Brain-Derived Neurotrophic Factor (BDNF) in COVID-19 Patients and its Association with the COVID-19 Manifestations

Ali Asgarzadeh[1] · Nasrin Fouladi[2,3] · Vahid Asghariazar[4,5] · Shahnaz Fooladi Sarabi[6] · Hamid Afzoun Khiavi[4] · Mahsa Mahmoudi[7] · Elham Safarzadeh[8]

Abstract

COVID-19 is a systematic disease that frequently implies neurological and non-neurological manifestations, predominantly by inducing hypoxia. Brain-derived neurotrophic factor (BDNF) is a key factor in regulating functions of nervous and respiratory systems and has been strongly related to hypoxia. Therefore, this study planned to investigate BDNF association with the COVID-19 manifestations especially neurological impairments and the infection-induced hypoxia. We enrolled sixty-four COVID-19 patients and twenty-four healthy individuals in this study. Patients were divided into two groups, with and without neurological manifestations, and their serum BDNF levels were measured by enzyme-linked immunosorbent assay (ELISA). COVID-19 patients had significantly lower BDNF levels than healthy individuals ($p = 0.023$). BDNF levels were significantly lower in patients with neurological manifestations compared to healthy individuals ($p = 0.010$). However, we did not observe a statistically significant difference in BDNF levels between patients with and without neurological manifestations ($p = 0.175$). BDNF's levels were significantly lower in patients with CNS manifestations ($p = 0.039$) and higher in patients with fever ($p = 0.03$) and dyspnea ($p = 0.006$). Secondly, BDNF levels have a significant negative association with oxygen therapy requirement ($p = 0.015$). These results strongly suggest the critical association between dysregulated BDNF and hypoxia in promoting COVID-19 manifestations, particularly neurological impairments.

Keywords COVID-19 · BDNF · Neurological manifestations · Hypoxia · Dyspnea

✉ Elham Safarzadeh
E.safarzadeh@arums.ac.ir

1. Students Research Committee, School of Medicine, Ardabil University of Medical Sciences, Ardabil, Iran

2. School of Medicine and Allied Medical Sciences, Ardabil University of Medical Sciences, Ardabil, Iran

3. Social Determinants of Health Research Center, Ardabil University of Medical Sciences, Ardabil, Iran

4. Immunology Research Center, Ardabil University of Medical Sciences, Ardabil, Iran

5. Deputy of Research and Technology, Ardabil University of Medical Sciences, Ardabil, Iran

6. Fellowship of Critical Care Medicine, Department of Anesthesiology, School of Medicine, Ardabil University of Medical Sciences, Ardabil, Iran

7. School of Medicine, Ardabil University of Medical Sciences, Ardabil, Iran

8. Department of Microbiology, Parasitology, and Immunology, School of Medicine, Ardabil University of Medical Sciences, Ardabil, Iran

Abbreviations

BDNF	Brain-derived neurotrophic factor
ELISA	Enzyme-linked immunosorbent assay
CNS	Central nervous system
COVID-19	Coronavirus disease 2019
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
ARDS	Acute respiratory distress syndrome
NGF	Nerve growth factor
NT-3	Neurotrophin-3
MS	Multiple sclerosis
RT-PCR	Real-time reverse transcriptase-polymerase chain reaction
ICU	Intensive care unit
GCS	Glasgow Coma Scale
HRP	Horseradish peroxidase
OD	Optic density
SD	Standard deviation
ORs	Odds ratios
CIs	Confidence intervals
IMV	Invasive mechanical ventilation

PNS   Peripheral nervous system
DM   Diabetes mellitus
IL   Interleukin

Introduction

Since December 2019, the unprecedented pandemic of coronavirus disease 2019 (COVID-19) that is responsible for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has infected more than 200 million people, with over 4 million death recorded till 21st August 2021. COVID-19 is normally featured by fever, cough, shortness of breath, and most importantly acute respiratory distress syndrome (ARDS), in severe cases (Zhu et al. 2020). Additionally, SARS-CoV-2 can induce an extended spectrum of neurological presentations in central and peripheral nervous systems, such as impaired consciousness, seizure, stroke, headaches, dizziness, and smell and taste alteration manifestations (Tancheva et al. 2020; Zirpe et al. 2020; Garg 2020).

For explaining neurological disorders induced by SARS-CoV-2 infection, several mechanisms, including direct neuro-invasive features of the virus (Paniz-Mondolfi et al. 2020; Eden et al. 2021), and its indirect impacts on the nervous system, such as hypoxia (Coen et al. 2020), inflammation (Espíndola et al. 2021; Kempuraj et al. 2020), and vascular accidents induced damages (Pillai et al. 2021), have been proposed and investigated (Tancheva et al. 2020; Zirpe et al. 2020). Although some studies have detected viral particles in the brain tissue, more recent reports consistently consider hypoxia and excessive inflammation of the nervous system as the predominant neurological inflicts for SARS-CoV-2 (Thakur et al. 2021). However, there is a paucity of literature on the role of BDNF in COVID-19 neuro-pathogenesis (Zhou et al. 2020).

BDNF is a member of the neurotrophin family, alongside the nerve growth factor (NGF), neurotrophin-3 (NT-3), and NT-4 (Dechant and Neumann 2002). BDNF is a pivotal regulator of neuroplasticity and fundamentally improves brain abilities such as memory, consciousness, and cognition (Kowiański et al. 2018). Different components of the human brain, mainly the hippocampus, brain cortex, olfactory bulb, and leukocytes and platelets, are the main central and peripheral sources of BDNF secretion (Anders et al. 2020; Le Blanc et al. 2020). The dysregulation of BDNF has been recognized as one of the primary mediators in the pathogenesis of abundant neurological diseases, such as multiple sclerosis (MS), Parkinson's disease, and Alzheimer's disease (Bathina and Das 2015).

Interestingly, BDNF is also a critical defensive factor against hypoxia and inflammation-induced neural damages (Lima Giacobbo et al. 2019; Chen et al. 2013). In addition, mounting numbers of studies have been implemented the role of dysregulated BDNF in the exhibition of various neurological disorders such as impaired consciousness (Bagnato et al. 2020), headache (Fischer et al. 2012), and altered smell and taste (McLean et al. 2001; Yee et al. 2003) that are also common neurological complications of COVID-19 patients. Additionally, multiple studies have addressed altered concentrations of BDNF in respiratory tract infections induced by both viral and bacterial pathogens (Chiaretti et al. 2013; Lommatzsch et al. 2007).

To date, few studies have been evaluated the roles of BDNF in the course of COVID-19. Azoulay D et al. conducted the first study to investigate this subject. They recorded a low serum concentration of BDNF in COVID-19 patients, which its restoration was associated with recovery from SARS-CoV-2 infection (Azoulay et al. 2020). Likewise, another cohort study done by Ong et al. demonstrated the higher levels of BDNF in recovered COVID-19 patients at 180 days post symptom onset (Ong et al. 2021). Moreover, a study done by Chan et al. displayed a correlation between the rising of BDNF levels and response to Remdesivier treatment in COVID-19 patients (Chan et al. 2021). Secondly, in a case report study, a decreased blood level of BDNF has been correlated with the neurological complications of a 4-year-old COVID-19 patient.

Although these studies uncovered the negative effect of the SARS-CoV-2 infection on BDNF levels and its association with the recovery of COVID-19 patients, they did not fully determine the extent of BDNF associations with the manifestations of COVID-19 patients, particularly their neurological impairments. Additionally, the effect of SARS-CoV-2 infection-induced hypoxia on BDNF levels is also unknown in these cases. Therefore, we planned this study to answer these questions by assessing serum levels of BDNF in hospitalized COVID-19 patients.

Methods and Materials

Study Design and Participants

Sixty-four patients diagnosed with COVID-19 and twenty-four healthy individuals were included in this study from October to December 2020. Healthy individuals were enrolled in the study to determine the normal ranges of the BDNF. Patients were officially tested positive with real-time reverse transcriptase-polymerase chain reaction (RT-PCR) test (One-step RT-PCR Kit, Pishtaz Teb Zaman Diagnostics, Tehran, Iran) through nasopharyngeal and oropharyngeal swabs, and then admitted at Imam Khomeini Hospital, a COVID-19 designated referral hospital in Ardabil Province, Iran. Healthy individuals were outpatients with negative RT-PCR test for SARS-CoV-2 that attended the same center over the same period. Healthy individuals with pre-existing

or history of neurological and respiratory disorders were excluded.

According to their manifestations, patients were divided into two distinct groups; with or without neurological manifestations. In this study, patients younger than 18 years, addicted patients, patients with active malignancy or a history of recent chemotherapy, patients using hemodialysis therapy, and pregnant women were excluded. Blood samples were collected from patients 24–48 h after admission or after presenting in-hospital neurological events.

Data Collection

The data was recorded from printed, electronic medical and nursing records using a standardized anonymized data collection form. Demographical and clinical data, including symptoms at admission and comorbidities, the history of a diagnosed disorders like cardiovascular, diabetes mellitus (DM), respiratory, and neurological diseases, were extracted. Subjective symptoms were also provided by the patients who were conscious and cognitively competent to respond to the interview. Any missing or uncertain data were collected and clarified through direct communication with health care clinicians and patients' families. Manifestations and physical examination were reviewed and confirmed by two trained physicians. Days from symptoms onset to hospital admission, requirement of intensive care unit (ICU) admission or invasive mechanical ventilation (IMV), and hospital outcomes were also documented. On admission, severity of the disease was calculated with the comorbidities, age, lymphocytes, and lactate dehydrogenase (CALL) scoring model (Ji et al. 2020).

Definitions

In this study, impaired consciousness, headache, and dizziness were defined as CNS, and smell and taste alteration and nerve pain were defined as peripheral nervous system (PNS) manifestations. Impaired consciousness was defined as the change of consciousness level, using a Glasgow Coma Scale (GCS) ≤ 13 points at presentation, and decline in consciousness content (confusion and delirium). Other neurological manifestations, including headache, dizziness, altered smell and taste, and nerve pain, were detected mainly by patients' subjective symptoms and the related examinations. New established neurologic presentations, such as impaired consciousness, seizures, and stroke, were defined as neurologic events during the hospital stay.

Blood Sample Preparation

The collection of samples was carried out based on the Ardabil University of Medical Sciences Ethics Committee's published guideline after informed consent. Five milliliters of blood samples was collected by a laboratory technician from the patients 24–48 h after admission in heparin-lithium and serum-separator tubes. The samples were processed within 1 h after collection, and isolated serums were immediately kept at -80 °C until the experimental analyses.

Measurement of Serum BDNF

BDNF level was measured using a human BDNF, ELISA, and ZellBio GmbH (Gmbh Ulm, Germany, Cat No.: ZB-11302C-H9648) according to the manufacturer's instructions. In brief, 96-well plates were coated with 50 μl standard (bound to biotin-conjugated antibodies) and 50 μl streptaverdin-horseradish peroxidase (HRP) as standard solution wells, and then 40 μl sample plus 10 μl anti-BDNF biotin-labeled antibody with 50 μl streptaverdin-HRP as sample wells. The samples and BDNF standards were incubated for 60 min at 37 °C after the washing step. It was then incubated with 100 μl chromogen solution for 10 min at 37 °C. The addition of 50 μl stop solution stopped the reaction. The optic density (OD) was measured at 450 nm wavelength with an automated microplate reader within 10 min after addition of stop solution. BDNF concentration was calculated using a standard curve according to the defined instruction.

Ethical Statement

The study was performed according to the Declaration of Helsinki principles and approved by the Ethics Committee of Ardabil University of Medical Sciences, Ardabil, Iran (IR.ARUMS.REC.1399.378). Signed informed consent was obtained from all considered patients and healthy individuals or next-of-kin before inclusion in the study.

Statistical Analysis

Data were analyzed by IBM SPSS Statistics software version 26 (IBM Corp., Armonk, NY, USA) using descriptive and analytical statistics. Descriptive statistics were applied to summarize the demographic and clinical characteristics related to COVID-19 for each group of patients. Continuous variables were described as mean with standard deviation (SD) or median with interquartile range (IQR) as appropriate and categorical variables were expressed as absolute and relative frequencies. Analyses of differences for independent groups between categorical variables were performed with the chi-square test and for continuous variables with the one-way analysis of variance (ANOVA), independent t-test, and Mann-U Whitney test as appropriate. Odds ratios (ORs) and 95% confidence intervals (95% CIs) were also

calculated. Differences were considered statistically significant at a p-value < 0.05.

Results

Demographic and Clinical Characteristics

A total of sixty-four (32 females and 32 males, age: 64.4 ± 12.5) hospitalized patients with confirmed SARS-CoV-2 infection and 24 healthy individuals (10 females and 14 males, age: 45.7 ± 19.2) were included in the study. Thirty-three cases (51.6%) experienced neurological manifestations (16 females, age: 65.6 ± 12.3), while 31 of the patients (48.4%) had no neurological manifestations (16 females, age: 63.2 ± 12.7) at the sampling period. The median number of days from symptoms onset to admission was 4 days for both groups.

Dyspnea, fever, cough, and chest pain as the most frequent non-neurological symptoms were detected over the admission process in 57 (89.1%), 33 (51.6%), 25 (39.1%), and 18 (28.1%) of the patients, respectively.

There were no significant differences in presenting the non-neurological symptoms between the groups. The most common neurological manifestation at sampling was impaired consciousness in 22 (66.7%), followed by dizziness and altered smell and taste in 9 patients (27.3%), and headache in 5 (15.1%) patients.

Twelve (36.4%) patients developed impaired consciousness as an in-hospital neurological event, and in 10 (30.3%) patients, impaired consciousness was observed at the admission time as their primary manifestations. Mean GCS levels for these patients were 12.36 ± 2.30 at the time of sampling. Sampling from patients with impaired consciousness was carried out in ICU.

Forty-seven (73.4%) of the considered patients had at least one comorbidity. The most common comorbidity was cardiovascular diseases in 53 cases (82.8%), followed by diabetes in 17 (26.6%). Six infected cases (18.2%) represented some pre-existing neurological disorders, such as three cases with a history of stroke, two patients with Alzheimer's disease, and a case with a history of seizure.

There were no significant differences in distribution of the underlying diseases between the groups, except for pre-existing neurologic disorders, which were significantly more frequent in patients with neurological manifestations (100% vs. 0%; $p = 0.025$).

The major treatment of the patients was oxygen therapy with the continuation of their previously prescribed medications for their underlying diseases. Forty-four of the patients (68.7%) were using supplemental oxygen therapy at the time of sampling. Twenty-six of the infected cases (40.6%) were using oxygen masks, and 18 of them (28.1%) were under invasive mechanical ventilation (IMV). The recorded data indicated that patients with neurological manifestations required more supplemental oxygen relative to those without neurological impairments (23 (69.7%) vs. 21 (67.7%); $p = 1.000$). Furthermore, lower SpO_2 levels were monitored for patients with neurological manifestations at admission than those without neurological presentations ($81.4 \pm 12.5\%$ vs. $85.4 \pm 10.9\%$; $p = 0.126$).

The obtained data revealed that 46 infected patients (71.9%) required ICU treatment, with more frequency in those who experienced neurological manifestations (26 (78.8%) vs. 20 (64.5%); $p = 0.269$). Forty-five of the patients (70.3%) were discharged during the study period, which was significantly higher in patients without neurological manifestations (26 (83.9%) vs. 19 (57.6%); $p = 0.029$). Additionally, 10 cases (15.6%) were expired during the study period, which was higher in patients with neurological manifestations (6 (18.2%) vs. 4 (12.9%)). Based on CALL scoring model, 28 (43.7%) patients had low and 17 (26.6%) patients had high risks of developing severe type of the disease on admission. The former was significantly higher for the patients without neurological manifestations (18 (58.1%) vs. 10 (30.3%); $p = 0.043$) and the latter for the patients with neurological manifestations (13 (39.4%) vs. 4 (12.9%); $p = 0.023$). Additionally, there was statistically significant difference between platelet counts of the patients with and without neurological manifestations ($151.12 \pm 77.30 \times 10^3/$ μl vs. $204.77 \pm 77.57 \times 10^3/\mu l$; $p = 0.008$). However, this difference was not signifiant for their lymphocytes counts ($1.45 \pm 0.39 \times 10^3/\mu l$ vs. $1.06 \pm 0.36 \times 10^3/\mu l$; $p = 0.743$) (Supplementary data 1). More detailed demographic and clinical characteristics are summarized in Table 1.

BDNF Levels in COVID-19 Patients and Healthy Individuals

In this study, the level of BDNF in serum was evaluated and compared between COVID-19-infected and healthy groups. The results revealed a considerably low level of BDNF in serum samples of the COVID-19 patients compared to healthy individuals (5407.66 ± 1500.6 pg/ml vs. 6402.08 ± 1827.6 pg/ml; $p = 0.023$) (Fig. 1).

BDNF Association with Neurological Manifestations of COVID-19 Patients

Serum levels of BDNF were compared between the patients with and without neurological manifestations to evaluate the possible associations between its levels and the existence of neurological manifestations in COVID-19 patients.

The data indicated no significant difference in BDNF levels in the cases with or without neurological manifestations, though a low level of BDNF was recorded in the former

Table 1 Characteristics of patients hospitalized with COVID-19

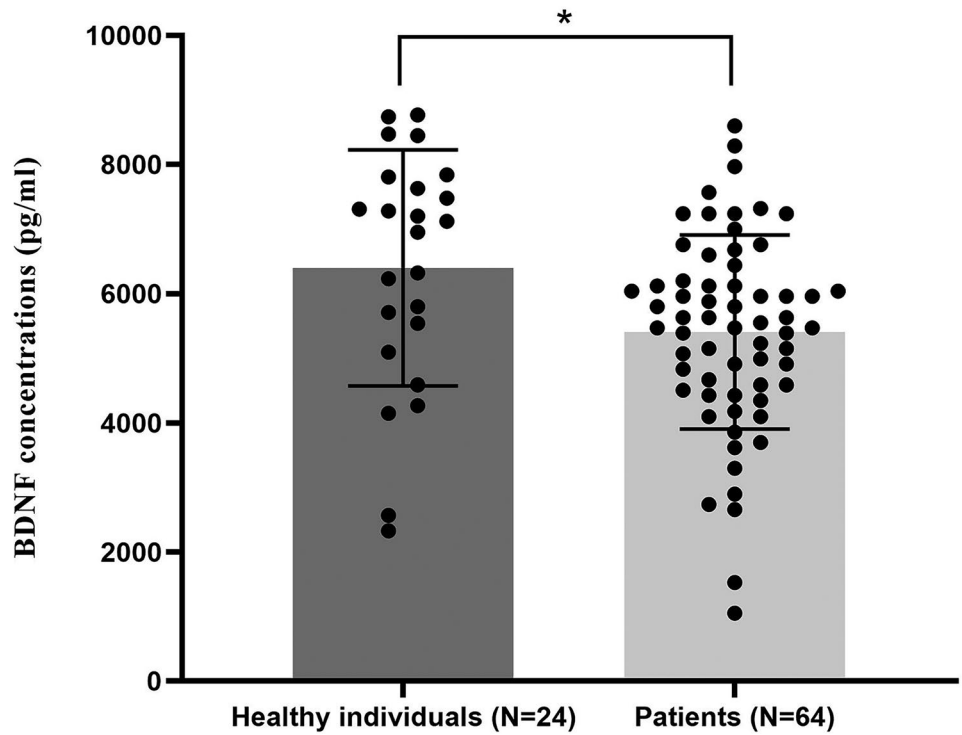|		All patients (N = 64)	With neurological manifestations (N = 33)	Without neurological manifestations (N = 31)	p-value
Age (years): mean ± SD (range)		64.4 ± 12.5 (48)	65.6 ± 12.3 (48)	63.2 ± 12.7 (48)	0.449
Sex, N (%)	Male	32 (50%)	17 (51.5%)	15 (48.4%)	1.000
	Female	32 (50%)	16 (48.5%)	16 (51.6%)	
Smoking (yes/no)		2/62	2/31	0/31	0.239
Time from symptoms onset to admission, median (IQR), days		4 (3–5)	4 (3–5)	4 (3–6)	0.068
Non-neurological manifestations at admission (yes/no)	Dyspnea	57/7	27/6	30/1	0.105
	Fever	33/31	17/16	16/15	1.000
	Cough	25/39	14/19	11/20	0.616
	Chest pain	18/46	8/25	10/21	0.581
	Fatigue/muscle pain	32/321	18/15	14/17	0.617
	Other	2/62	1/32	1/30	1.000
Neurological manifestations at sampling (yes/no)	Impaired consciousness	22/42	22/11	_	NA
	Dizziness	9/55	9/24	_	NA
	Headache	5/59	5/28	_	NA
	Sleep disorder	2/62	2/31	_	NA
	Altered smell	9/55	9/24	_	NA
	Altered taste	9/55	9/24	_	NA
	Nerve pain	2/62	2/31	_	NA
Comorbidities (yes/no)	CVD	53/11	27/6	26/5	0.467
	DM	17/47	9/24	8/23	1.000
	ND	6/58	6/27	0	**0.025**
	RD	5/59	2/31	3/28	0.667
	Renal dysfunction	5/59	3/30	2/29	1.000
	BPH	4/60	3/30	1/30	0.614
	Other	5/59	2/31	3/28	NA
SpO2 at admission (mean ± SD)		83.4 ± 11.8%	81.4 ± 12.5%	85.4 ± 10.9%	0.126
Supplemental oxygen requirement, N (%)	Oxygen mask	26 (40.6%)	11 (33.3%)	15 (48.3%)	0.309
	IMV	18 (28.1%)	12 (36.4%)	6 (19.3%)	0.169
ICU admission (yes/no)		46/18	26/7	20/11	0.269
Hospital outcomes, N (%)	Discharged	45 (70.3%)	19 (57.6%)	26 (83.9%)	**0.029**
	Death	10 (15.6%)	6 (18.2%)	4 (12.9%)	0.734
CALL score	Low risk, N (%)	28 (43.7%)	10 (30.3%)	18 (58.1%)	**0.043**
	Intermediate risk, N (%)	19 (29.7%)	10 (30.3%)	9 (29%)	1.000
	High risk, N (%)	17 (26.6%)	13 (39.4%)	4 (12.9%)	**0.023**

The bold emphasis on Table 1 entries related to p-values that are statistically significant

BPH benign prostatic hypertrophy, *CALL* comorbidities, age, lymphocytes, and lactate dehydrogenase, *CVD* cardiovascular diseases, *DM* diabetes mellitus, *ICU* intensive care unit, *IMV* invasive mechanical ventilation, *NA* non-applicable, *ND* neurological diseases, *RD* respiratory diseases, *SD* standard deviation

patients (5160.9 ± 1560.9 pg/ml vs. 5670.32 ± 1411.27 pg/ml; $p = 0.175$). However, the collected data represented that the level of BDNF was remarkably lower in the patients with neurological manifestations than healthy individuals (5160.9 ± 1560.9 pg/ml vs. 6402.08 ± 1827.57 pg/ml; $p = 0.010$). BDNF levels between healthy individuals and patients without neurological manifestations were not different (5670.32 ± 1411.27 pg/ml vs. 6402.08 ± 1827.57 pg/ml; $p = 0.112$) (Fig. 2A).

Furthermore, the associations of BDNF levels with the exhibition of each of the different neurological manifestations of the patients were separately evaluated. BDNF levels were lower in the patients with impaired consciousness, dizziness, sleep disorder, altered taste, and nerve pain, while the levels of BDNF were higher in the cases with headache and altered smell (Table 2). There was no significant association between different levels of BDNF in the patients and the presentation of the considered manifestations.

Fig. 1 BDNF levels in COVID-19 patients and healthy individuals. A significant difference was observed in serum BDNF levels between patients and healthy individuals. *$p < 0.05$
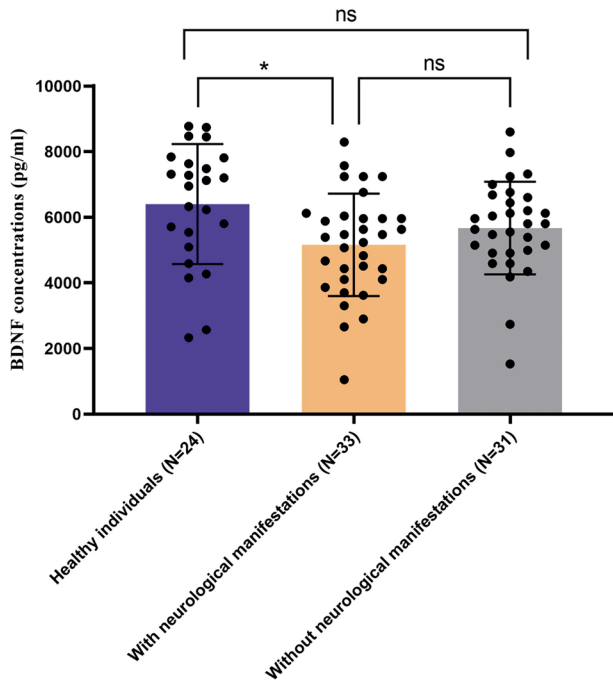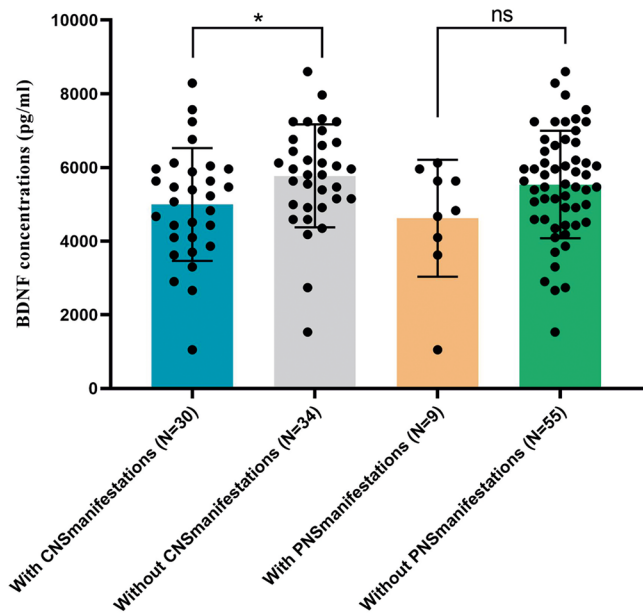

Fig. 2 BDNF levels in patients with neurological manifestations. **A** A significant difference was observed in serum BDNF levels between patients with neurological manifestations and healthy individuals. **B** BDNF serum levels in patients with and without CNS or PNS mani-festations. A significant difference was observed in serum BDNF levels between patients with and without CNS manifestations. *ns* not significant, *$p < 0.05$

Table 2 BDNF serum levels in different neurological manifestations of COVID-19 patients

Neurological manifestations	Status	N (%)	BDNF level (pg/ml) (mean ± SD)	p-value
Impaired consciousness	Positive	22 (34.4%)	5008.64 ± 1691.81	0.154
	Negative	42 (65.6%)	5616.67 ± 1365.25	
Headache	Positive	5 (7.8%)	5538 ± 1372.27	0.846
	Negative	59 (92.2%)	5396.61 ± 1521.34	
Dizziness	Positive	9 (14.1%)	5167.78 ± 1222.98	0.499
	Negative	55 (85.9%)	5446.9 ± 1547.37	
Sleep disorder	Positive	2 (3.1%)	4185 ± 459.62	0.143
	Negative	62 (96.9%)	5447.1 ± 1507.18	
Altered smell	Positive	9 (14.1%)	5660 ± 1179.34	0.602
	Negative	55 (85.9%)	5366.36 ± 1552	
Altered taste	Positive	9 (14.1%)	4623.33 ± 1586.96	0.139
	Negative	55 (85.9%)	5536 ± 1461.1	
Nerve pain	Positive	2 (3.1%)	4590 ± 113.14	0.287
	Negative	62 (96.9%)	5434.03 ± 1517.5	

As previously referred, the neurological presentations were divided into CNS and PNS manifestations, and then the level of BDNF was monitored in the considered cases (Fig. 2B). The lower levels of BDNF were recorded in both groups, though a significant negative association between BDNF level and exhibition of CNS manifestations was observed (4995.7 ± 1529.73 pg/ml vs. 5771.18 ± 1397.14 pg/ml; $p = 0.039$).

The Associations of BDNF Level with Non-neurological Manifestations of COVID-19 Patients

In this section, the possible relationships of BDNF serum levels and presentation of non-neurological manifestations such as dyspnea, cough, and fatigue were evaluated. The evaluation of the recorded data showed that those with dyspnea, fever, and diarrhea had higher serum levels of BDNF, while a low level of BDNF was monitored in the patients who experienced cough, chest pain, and fatigue/muscle pain (Table 3). The data represented that there was a statistically significant relationship between serum level of BDNF and dyspnea (5605.61 ± 1365.91 pg/ml vs. 3795.71 ± 1681.38 pg/ml; $p = 0.006$) and fever (5802.12 ± 1270.84 pg/ml vs. 4987.74 ± 1628.72 pg/ml; $p = 0.03$) (Table 3). However, there were no statistically significant associations between serum BDNF level and presentation of other manifestations.

BDNF Associations with the Requirement of Supplemental Oxygen in COVID-19 Patients

The relationship of serum levels of BDNF with the requirement of supplemental oxygen as a marker of hypoxia was

measured in this section. The collected data illustrated that those cases with lower levels of BDNF needed more supplemental oxygen than the other patients (5134.54 ± 1576.12 pg/ml vs. 6008.5 ± 1136.55 pg/ml; $p = 0.015$) (Fig. 3A).

Additionally, we wondered if there is an association between serum levels of BDNF in patients that presented CNS manifestations, dyspnea, and fever, three manifestations that had a significant negative association with BDNF levels, and their need for supplemental oxygen. Thereby, we selected these cases and ran the analysis for them. Interestingly, serum BDNF levels were significantly lower in the subgroup of each of the groups of the patients with CNS manifestations and dyspnea who more likely required supplemental oxygen compared to the other subgroup of these groups (4583.5 ± 1472.17 pg/ml vs. 5820 ± 1353.65 pg/ml; $p = 0.033$) (5357.18 ± 1435.92 pg/

Table 3 BDNF serum levels in different non-neurological manifestations of COVID-19 patients

| Non-neurological manifestations	Status	N (%)	BDNF level (pg/ml) (mean ± SD)	p-value
Dyspnea	Positive	57 (89.1%)	5605.61 ± 1365.91	**0.006**
	Negative	7 (10.9%)	3795.71 ± 1681.38	
Fever	Positive	33 (51.6%)	5802.12 ± 1270.84	**0.03**
	Negative	31 (48.4%)	4987.74 ± 1628.72	
Cough	Positive	25 (39.1%)	5286.8 ± 1048.66	0.571
	Negative	39 (60.9%)	5485.13 ± 1738.66	
Chest pain	Positive	18 (28.1%)	4942.22 ± 1532.01	0.135
	Negative	46 (71.9%)	5589.78 ± 1464.69	
Fatigue/muscle pain	Positive	32 (50%)	5163.75 ± 1618.5	0.196
	Negative	32 (50%)	5651.56 ± 1354.2	
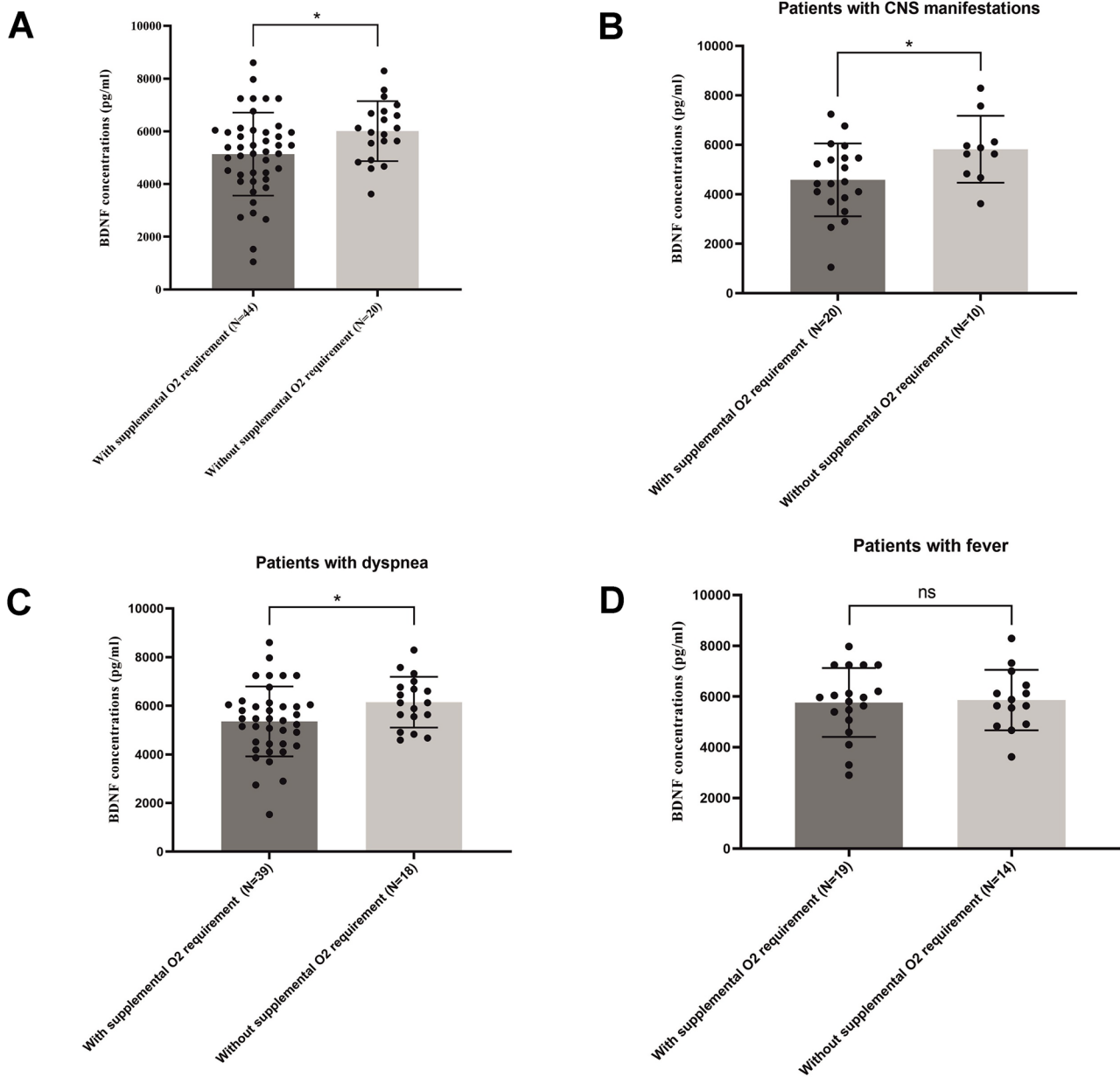
Fig. 3 BDNF serum levels in patients with different requirement of supplemental oxygen. A significant difference was observed in serum BDNF levels between patients, who required supplemental oxygen compare to other patients (**A**). BDNF serum levels in patients with CNS manifestations (**B**), dyspnea (**C**), and fever (**D**) with different requirement of supplemental oxygen. Significant differences were observed in serum BDNF levels between patients with CNS manifestations and dyspnea, who required supplemental oxygen compared to who do not required supplemental oxygen. *ns* not significant, *$p < 0.05$

ml vs. 6143.9 ± 1043.35 pg/ml; $p = 0.024$) (Fig. 3B, C). However, this association was not significant for fever (5761.05 ± 1358.07 pg/ml vs. 5857.86 ± 1189.93 pg/ml; $p = 0.829$) (Fig. 3D). We could not establish the mentioned relationship for other manifestations.

The Relationship of BDNF Levels with Different Comorbidities of COVID-19 Patients

In this study, the possible association of the patient's comorbidities and the serum level of BDNF was

investigated, regardless of their neurological or non-neurological manifestations.

To this purpose, the patients were divided into four different groups based on the four major frequent comorbidities in our study (diabetes mellitus (DM), cardiovascular, neurological, and respiratory diseases). The results showed higher serum levels of BDNF in the patients with neurological and respiratory diseases, though lower levels of BDNF were recorded in the patients with cardiovascular diseases and DM (Table 4). According to the results, no significant association was monitored between the serum level of BDNF and patients' comorbidities.

Discussion

This study found significantly lower serum levels of BDNF in hospitalized COVID-19 patients than healthy individuals, which was consistent with previous studies (Azoulay et al. 2020). Additionally, BDNF levels for the patients and healthy individuals were consisted with previously reported ranges of the BDNF for COVID-19 patients and healthy individuals (Azoulay et al. 2020; Amadio et al. 2017). However, our result was in contrast to what Ong et al. and Chan et al. found, which demonstrated higher concentrations of BDNF in COVID-19 patients compared to healthy controls (Ong et al. 2021; Chan et al. 2021). Different study design and ethnicity of involved individuals in our study than those of Ong et al. and Chan et al. done study might explain the discrepancy. Conducting covariance test demonstrated independency of the BDNF levels from age in the enrolled groups of our study ($p = 0.390$).

Dysregulated BDNF is a prominent finding in neurological diseases, particularly CNS disorders (Mariga et al. 2017). Likewise, we observed a significant negative association between BDNF levels and the presentation of CNS

Table 4 BDNF serum levels in different comorbidities of COVID-19 patients

Patients comorbidities	Status	N (%)	BDNF level (pg/ml) (mean ± SD)	p-value
CVD	Positive	53 (82.8%)	5368.11 ± 1544.2	0.617
	Negative	11 (17.2%)	5598.18 ± 1318.52	
DM	Positive	17 (26.6%)	5150.59 ± 1577.84	0.432
	Negative	47 (73.4%)	5500.64 ± 1478.11	
ND	Positive	6 (9.4%)	5876.67 ± 2174.43	0.536
	Negative	58 (90.6%)	5359.14 ± 1431.27	
RD	Positive	5 (7.8%)	5666 ± 1862.06	0.576
	Negative	59 (92.2%)	5385.76 ± 1483.43	

CVD cardiovascular diseases, *DM* diabetes mellitus, *RD* respiratory diseases, *ND* neurological diseases

manifestations in COVID-19 patients. Furthermore, by demonstrating a significant association between BDNF levels and supplemental oxygen requirements in the patients with CNS manifestations, we determined the negative effect of the hypoxia on BDNF levels, which culminated in the exhibition of CNS manifestations. These results add another proof to the mounting evidence of BDNF protective role against hypoxia-induced injuries in CNS (Chen et al. 2013; Xiong et al. 2021), addressing hypoxia-induced dysregulated BDNF as a potential pathological pathway for the neurological impairments of the COVID-19 (Coen et al. 2020).

Although altered levels of BDNF were recorded in the COVID-19 patients with various neurological manifestations, separate analysis between BDNF levels and presentation of each of the different neurological manifestations of the patients did not yield any significant associations. These results might be attributable to the high proportion of patients with symptom-based neurological manifestations, like altered smell and taste, and short-lasting neurological manifestations, like impaired consciousness in our study. In addition, the possible interference of patients' medication with the BDNF release and its measurements cannot be completely excluded.

Notably, patients with a high requirement of supplemental oxygen treatment commonly experience severe SARS-CoV-2 infection (Zhu et al. 2020). Since our study showed that patients with an increased need for supplemental oxygen had lower BDNF levels, its decreased serum concentrations may act as criteria for starting supplemental oxygen treatment. Accordingly, a recently done cohort study revealed a negative correlation between BDNF levels and disease severity, serving as a tool for predicting adverse clinical outcomes of the COVID-19 patients (Minuzzi et al. 2021).

Furthermore, significant positive associations were found between BDNF levels and presentation of dyspnea and fever, major non-neurological manifestations of COVID-19 patients in our study. These associations extend the essential protective features of the BDNF in non-neurological manifestations, especially in viral respiratory diseases, as demonstrated before in H1N1 infection (Chiaretti et al. 2013).

The significantly high concentrations of BDNF observed in the COVID-19 patients with dyspnea were in contrast to the study by Lommatzsch et al., which reported lower serum levels of BDNF in patients with respiratory tract infection that presented dyspnea (Lommatzsch et al. 2007). This disagreement might be attributable to the different etiology of the investigated respiratory diseases, as they enrolled patients with bacterial-induced respiratory tract infections. Secondly, a significant negative association demonstrated between BDNF levels and supplemental oxygen requirements in the COVID-19 patients with dyspnea suggests hypoxia-induced impaired levels of BDNF as a critical mediator for the presentation of dyspnea. Thus, our findings

indicate BDNF protective role in dyspnea, which is in keeping with recent discoveries of Paris A et al. that determined BDNF signaling as a significant pathway in the regulation of epithelial regeneration (Paris et al. 2020).

Moreover, the positive association of BDNF levels with the presentation of fever in the COVID-19 patients, which were not associated with their need for supplemental oxygen, might be suggestive of BDNF interplay with other mediators like pro-inflammatory cytokines such as interleukin (IL)-6 and IL-1β, which are crucial mediators of fever (Chen et al. 2019). However, cytokine levels were not monitored in this study, and further studies are required to evaluate their possible link with BDNF in promoting COVID-19 manifestations.

Conclusion

Taking together, we proposed dysregulated levels of the BDNF in association with the impaired oxygen delivery as a crucial contributor to the COVID-19 manifestations, suggesting its distinct role in the pathogenesis of COVID-19, particularly in its neurological impairments. Further studies with larger participants are required to verify BDNF possible associations with different manifestations of the COVID-19 and its long-term consequences and to assess BDNF potential use as a prognostic biomarker for COVID-19. Additionally, further experimental researches are required to investigate BDNF molecular interplay with SARS-CoV-2-induced hypoxia.

Data Availability The data that support the findings of this study are available on request from the corresponding author.

Declarations

Ethics Approval and Consent to Participate This study was approved by the approved by the Ethics Committee of Ardabil University of Medical Sciences, Ardabil, Iran (IR.ARUMS.REC.1399.378). All patients signed informed consent before any study-related procedures were performed.

Consent for Publication The patients were informed of the purpose of this study and an informed written consent has been obtained from the patients to publish this paper.

Competing Interests The authors declare no competing interests.

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