INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 24, No. 5, p. 238-252, 2024

RESEARCH PAPER OPEN ACCESS

Impact of COVID-19 on central nervous system biomarkers: A comparative analysis with multiple sclerosis and healthy controls in Erbil city, KRG, Iraq

Daldar Fares Jeejo*[1], Mayada Noori Iqbal[1], Nisreen Sherif Alyasiri[2]

[1]*Department of Medical Laboratory Techniques, College of Health and Medical Technology, Baghdad, Middle Technical University, Baghdad, Iraq*

[2]*Department of Medical Laboratory Techniques, Suwaira Technical Institute, Middle Technical University, Waist, Iraq*

Key words: COVID-19, Multiple sclerosis, Biomarkers, Neuron-specific enolase, Glial fibrillary acidic protein, Neurofilament light chain

http://dx.doi.org/10.12692/ijb/24.5.238-252 Article published on May 12, 2024

Abstract

The neurological implications of COVID-19 are becoming increasingly documented, Elevated serum biomarkers Neuron-Specific Enolase (NSE), Glial Fibrillary Acidic Protein (GFAP), and Neurofilament Light Chain (NfL) show a strong association with neurological injury in both COVID-19 and Multiple Sclerosis (MS) patients. However, the use of biomarkers in the diagnosis and progression of COVID-19 and Multiple Sclerosis (MS) in the Kurdistan Region of Iraq (KRG) remains understudied. This investigation aims to assess the levels of GFAP, NSE, and NfL in COVID-19 and MS patients and compare them with healthy controls to establish their potential utility as biomarkers. Levels of Serum NSE, GFAP, and NfL were calculated in 91 COVID-19 patients, 29 MS patients, and 50 healthy controls using ELISA tests. To evaluate the differences between groups, statistical analysis was used, which included the application of the Kruskal-Walli's test. NfL levels were noticeably greater in MS patients, while COVID-19 patients had significantly higher levels of GFAP and NSE than controls. There was a correlation between the severity of COVID-19 and elevated biomarker levels. According to the study's findings, NSE and GFAP are markedly raised in COVID-19 patients, suggesting that they could be used as biomarkers for neurological involvement. Likewise, NfL levels were markedly increased in MS patients, highlighting its function as a biomarker for injury to the neuroaxon. Further research is recommended to corroborate these findings in a larger population and to explore the predictive value of these biomarkers for clinical outcomes. It is also recommended to extend interdisciplinary research to understand the pathophysiological mechanisms driving these biomarker changes.

* **Corresponding Author:** Daldar Fares Jeejo ✉ dldarhozan@gmail.com

Introduction

In late 2019, SARS-CoV-2 emerged in Wuhan, China, leading to a worldwide pandemic. By February 2020, Najaf in Iraq reported its initial COVID-19 cases. Two weeks later, Iraq's Ministry of Health confirmed 101 positive cases and nine deaths across fourteen provinces, with nearly 40% in Baghdad (Al-Sarray and Shareef, 2022)

SARS-CoV-2 can cause a cytokine storm, leading to inflammation that compromises the blood-brain barrier. This may result in brain tissue damage and various neurological complications, although such severe outcomes are more common in serious cases of COVID-19 (Thepmankorn *et al.*, 2021). A cytokine storm is characterized by an excessive production of pro-inflammatory cytokines, including interleukin (IL)-6, IL-2, IL-7, and tumor necrosis factor (TNF)-α. Recent research has indicated that COVID-19 patients in intensive care units exhibit increased levels of IL-24 in their bloodstream (Nazzal and Sabbar, 2022).

In addition to post-recovery effects like “brain fog” severe COVID-19 case frequently result in cognitive disorders like confusion, delirium, and a low level of consciousness (Otani *et al.*, 2023). The virus also results in long-lasting neurological problems such as joint, chest pain, anosmia, and ageusia (Sarıoğlu *et al.*, 2023). COVID-19‘s respiratory side effects and damage might cause problems such brain hypoxia, which may result in long-term cognitive and physical deficits (Dondaine *et al.*, 2022). Furthermore, there is a notable influence on mental health, as seen by the high prevalence of anxiety, depression, and stress following recovery (Iqbal *et al.*, 2021). Additionally, there is a symptom overlap between myalgic encephalomyelitis /chronic fatigue syndrome (ME/CFS) and long-term COVID-19 effects (Sokocheva *et al.*, 2022). The virus disproportionately affects particular racial and ethnic populations and increases the risk for people with underlying diseases such diabetes, obesity, and cerebrovascular illness (Carethers, 2021).

According to recent studies, direct viral entry and inflammatory reactions are the ways that COVID-19 may affect the central nervous system (Spudich and Nath, 2022). According to studies, the virus may enter the central nervous system CNS through the blood-brain barrier or the olfactory bulb, which could explain a variety of neurological symptoms that patients experience. Acute cerebrovascular episodes, encephalitis, headaches, and anosmia are among these symptoms (Khatoon *et al.*, 2022). Additionally linked to CNS involvement and possibly causing neurological impairment in COVID-19 patients are inflammatory responses such cytokine storms (Saleki *et al.*, 2021). The increasing amount of data highlights the necessity for additional study to completely comprehend the neurological effects of COVID-19.

Important biomarkers for determining brain damage and neurological involvement include neurofilament light (NFL), tau, glial fibrillary acidic protein (GFAP), and neuron-specific enollase (NSE). Because NSE measures impairment to neuronal function directly, it is useful for assessing traumatic brain injury (TBI) (Mendoza *et al.*, 2020). Tau and GFAP are brain-specific protein biomarkers that have been thoroughly investigated in traumatic brain injury (TBI) and offer insights into neuronal and astrocyte damage (Dadas *et al.*, 2018; Nishimura *et al.*, 2022). Furthermore, blood tests for GFAP and NFL, a marker of axonal ingury, provide a straightforward way to diagnose and prognostic TBI, especially in mild instances (Zhang *et al.*, 2016). These biomarkers are critical for correct neurological condition assessment and management, as they play a major role in knowing the pathophysiological state of brain damage (Wang *et al.*, 2018; Kulbe and Geddes, 2015; Wilde *et al.*, 2023).

When evaluating brain damage and cognitive involvement in COVID-19 patients, neurofilament light (NFL) glial fibrillary acidic protein (GFAP), tau, and neuron-specific enolase (NSE) are essential markers. Study has demonstrated that these biomarkers, which indicate brain damage and endothelial injury with sex-specific changes, are increased in COVID-19 individuals exhibiting neurological symptoms (Sahin *et al.*, 2022; Needham *et al.*, 2022). These biomarkers can also clarify neuro-axonal and astrogliail injuries, offering information about the pathophysiological condition of brain

damage in COVID-19 affected individuals (Telser *et al.*, 2023). The importance of these biomarkers in determining brain injury in COVID-19 patients is highlighted by the correlation between elevated levels of these markers and poor outcomes and the severity of condition (Dekosky *et al.*, 2020; Silva *et al.*, 2023).

These central nervous system(CNS) experiences demyelination and neurodegeneration in multiple sclerosis(MS) , a chronic inflammatory illness that causes a variety of neurological symptoms. Three factors contribute to the pathophysiology of MS: autoimmune, neurodegeneration, and CNS inflammation (Richter *et al.*, 2021). Encephalitis, acute cerebrovascular, and other central nervous system signs have been linked to COVID-19, a virus generated by the SARS-CoV-2 virus (Maury *et al.*, 2021). Both MS and COVID-19 can lead to neurological involvement, making MS a comparative model in studying COVID-19-related neurological issues (Schwartz *et al.*, 2022).

Similarities between the pathophysiology of MS and COVID-19-related neurological issues include the involvement of inflammatory processes in CNS damage. Both conditions can lead to neuroinflammation and neurodegeneration, contributing to neurological symptoms (Conway *et al.*, 2022). However, differences exist in the underlying mechanisms, with MS being an autoimmune disease affecting the CNS, while COVID-19 primarily involves viral infection and systemic inflammatory responses (Fernandes de Souza *et al.*, 2023). It was shown that individuals with MS may have worse COVID-19 outcomes compared to the general population, indicating a potential interaction between MS and COVID-19 (Barzegar *et al.*, 2023). The impact of COVID-19 on people with MS has been a subject of research, with some studies reporting an increased risk of hospitalization and mortality in MS patients (Sormani *et al.*, 2022). However, other studies have suggested that MS may not be associated with an increased risk for severe COVID-19 (Moreno-Torres *et al.*, 2021).

Biomarkers play a crucial role in the diagnosis, prognosis, and management of multiple sclerosis (MS). Neurofilament light (NFL), glial fibrillary acidic protein (GFAP), tau, and neuron-specific enolase (NSE) are some of the biomarkers that have been studied in MS. NFL, a marker of axonal injury, is elevated in MS patients, indicating neuroaxonal damage (Arneth and Kraus, 2022). Two brain-specific protein biomarkers that shed light on MS-related astroglial injury and neuronal impairment are tau and GFAP (Momtazmanesh *et al.*, 2020). NSE is useful in evaluating (MS) since it is a measure of impairment to neuronal function (Gyang and Rempe, 2021). The identification of MS, assessment of disease activity, and evaluation of therapy response can all be aided by these biomarkers (Serafeim and Anagnostouli, 2013). Furthermore, biomarkers can help distinguish between different MS subtypes, predict relapses, and suggest tailored treatment plans (Buck and Hemmer, 2014). In order to improve patient outcomes by early identification and efficient treatment of the disease, biomarkers must be used in MS management. (Arneth and Kraus, 2022).

This research fills a major knowledge gap about the effects of COVID-19 on the central nervous system (CNS) and draws comparisons with multiple sclerosis, with a focus on the particulars of Erbil City, KRG, Iraq. Comprehending these associations is essential for formulating focused treatments and handling tactics. The findings could offer important insights into the neurological manifestations of the virus and Multiple Sclerosis in other populations, perhaps directing global health responses, given the numerous ethnic and environmental conditions in Erbil City.

Investigating the effect of COVID-19 on central nervous system biomarkers and comparing it with the effects seen in MS patients and healthy controls is the main goal of this study. Our goal is to investigate the idea that, in contrast to what is seen in multiple sclerosis, COVID-19 induces notable changes in several biomarkers (NFL, GFAP, tau, and NSE) linked to brain injury. This research seeks to bridge the gap in understanding the neurological implications of COVID-19 in comparison to a well-characterized neurological disorder like MS, thereby contributing to the broader knowledge of virus-induced neurological damage.

Material and methods

Study participants

This research adopted a hospital-based, case-control, cross-sectional design, focusing on participants from Erbil city, Iraq, and conducted at the Laboratory Department of Erbil Central Emergency Hospital from March to September 2023. It involved 170 participants, including 120 patients (91 with COVID-19 and 29 with Multiple Sclerosis) and 50 healthy controls. In the patient group, there were 56 females and 64 males. The COVID-19 patients were categorized based on the WHO clinical progression scale, while MS patients were assessed using the EDSS. A healthy control group, matched for age and sex with the patient cohorts and with no history of COVID-19 or MS, was included to provide a baseline for comparisons.

The study included participants with confirmed SARS-CoV-2 infection for the COVID-19 group, those clinically diagnosed with MS for the MS group, and healthy individuals without a history of these conditions or significant neurological or inflammatory diseases for the control group. It excluded individuals with other neurological disorders, chronic inflammatory diseases, severe chronic illnesses potentially affecting biomarkers, and those vaccinated against COVID-19 in the COVID-19 group to eliminate confounding factors.

The study obtained ethical approvals. Participants gave verbal consent, following ethical guidelines to ensure respect for their autonomy. The research team prioritized confidentiality, handling data with utmost discretion to maintain privacy and comply with medical research ethical standards.

Structured questionnaires covering age, gender, medical history, and symptoms were used to collect demographic data. Nasopharyngeal and oropharyngeal swabs were used in the identification of SARS-CoV-2. Trained medical personnel collected the samples, which were then maintained in viral transport media for (PCR) testing. Blood samples were obtained in order to measure the levels of the proteins S100B and UCHL1. A blood sample of 5-10 ml was taken from each participant, and serum was separated and stored at the appropriate temperature in a lab for processing. Throughout the sample collection and handling process, strict aseptic technique and quality control procedures were utilized to ensure sample integrity and accurate results.

Laboratory investigation tests

Quantitative Polymerase Chain Reaction QPCR was used in this investigation to identify COVID-19 . RNA was extracted from swab samples using a kit based on the Magnetic Bead Method, and then (PCR) amplification was performed using an RT-QPCR kit. The kit is fluorescent probes made it possible to monitor data in real time and evaluate it using threshold assay ELISA tests (ELK Biotechnology , China) the levels of neurofilament light (NFL), tau , glial fibrillary acidic protein (GFAP) , and neuron-specific enolase (NSE) were determined for biomarker analysis . Through colorimetric changes, these procedures measured biomarkers and checked their accuracy against standard curves .Precise determination of biomarker levels was assured by this method, which is essential for comprehending their functions in COVID-19 and MS.

Statistical analysis

Descriptive statistics were first used in this study to highlight fundamental distributions and trends in the data. A p-value of less than 0.05 is regarded as statistically significant in this study, which used Turkey's Honestly Significant Difference HCD test for post hoc analysis. Pearson's Correlation test was used to assess relationships between continuous variable, while the t-test examined quantitative associations between two groups. For data not following a normal distribution, the Kruskal-Wallis test was applied. All statistical analyses were conducted using IBM SPSS Statistics 27 to ensure robust and accurate interpretation of the results.

Results

In this study, a total of 170 participants were involved, which comprised a patient group of 120 individuals and a healthy control group of 50. Among the patient group, COVID-19 patients were categorized based on disease severity: 23 (19.2%) with severe COVID-19, 31 (25.8%) with moderate COVID-19, and 37 (30.8%) with mild COVID-19. Additionally, 29 participants (24.2%) were diagnosed with Multiple Sclerosis, as shown in (Fig. 1). This distribution provides a comprehensive overview of varying disease severities, allowing for a detailed analysis of the impact of COVID-19 compared to Multiple Sclerosis.

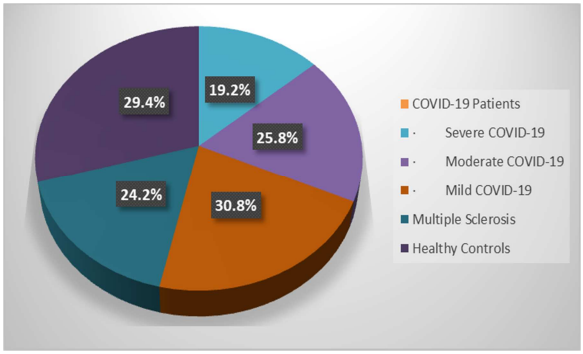

Fig. 1. Distribution of study participants by group and disease severity

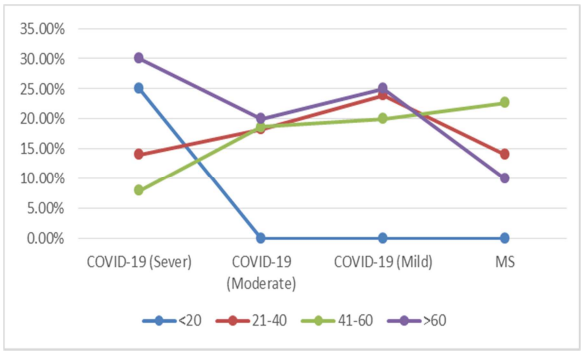

Fig. 2. Distribution of study participants by group and disease severity

In this study, of the total COVID-19 patients, 43 were female (47.3%) and 48 were male (52.7%). Specifically, severe COVID-19 cases included 10 females (23.3% of COVID-19 females) and 13 males (27.1% of COVID-19 males), moderate cases had 11 females (25.6% of COVID-19 females) and 20 males (41.7% of COVID-19 males), and mild cases comprised 22 females (51.2% of COVID-19 females) and 15 males (31.3% of COVID-19 males). In the MS group, there were 13 female (44.8% of MS patients) and 16 male (55.2% of MS patients) patients. The control group had an equal gender distribution, with 25 females (50%) and 25 males (50%). All these data are shown in (Table 1).

The chi-square test showed a significant association between gender and COVID-19 severity (chi-square value = 6.572, p-value = 0.037), indicating gender differences in severity levels (p, 0.05). However, the z-test comparing gender proportions in the MS and COVID-19 groups (z-score = 0.28, p-value = 0.78) showed no significant difference, suggesting similar gender distribution in both disease groups (>0.05). The data in Table 2 shows the distribution of COVID-19 severity, MS cases, and controls across different age groups. In the youngest age group (<20 years), there was only one case of severe COVID-19, accounting for 25% of individuals in that age bracket, with the remaining 75% being healthy controls. In the 21-40 age group, there were 10 severe cases (14.08%), 13 moderate cases (18.31%), and 17 mild cases (23.94%), with MS cases and controls at 14.08% and 29.58% respectively. Among 41-60-year-olds, severe cases were at 8%, moderate at 18.67%, mild at 20%, MS at 22.67%, and controls at 30.67%. In the >60 age group, severe cases constituted 30%, moderate 20%, mild 25%, MS 10%, and controls 15%.

Fig. 2 illustrates the dynamic trends in COVID-19 severity and MS incidence across various age groups. For individuals under 20, there is a notable spike in severe cases at 25%, with no moderate or mild cases reported, and a significant proportion of controls at 75%. The 21-40 age group shows a gradual increase across severities, peaking with mild cases at 23.94%, while controls slightly outpace MS cases. The pattern shifts in the 41-60 bracket, where moderate and mild cases are more prevalent at 18.67% and 20%, respectively, and MS cases peak at 22.67%. For those over 60, severe cases surge to 30%, with a decrease in other categories, highlighting a heightened vulnerability in this age group.

Table 1. Distribution of covid-19 severity and multiple sclerosis cases by gender

Condition	Female (No. and %)	Male (No. and %)	Total (No. and %)
COVID-19 (Total)	43 (47.3%)	48 (52.7%)	91 (100%)
Severe COVID-19	10 (23.3% of females)	13 (27.1% of males)	23 (25.3% of total)
Moderate COVID-19	11 (25.6% of females)	20 (41.7% of males)	31 (34.1% of total)
Mild COVID-19	22 (51.2% of females)	15 (31.3% of males)	37 (40.7% of total)
Multiple Sclerosis	13 (44.8% of MS)	16 (55.2% of MS)	29 (100% of MS)
Control Group	25 (50%)	25 (50%)	50 (100%)

Table 2. Gender and age group analysis of COVID-19 severity, multiple sclerosis incidence, and control distribution

Gender and age groups	COVID-19 No. (%)			MS No. (%)	Controls No. (%)
	Sever	Moderate	Mild		
<20	1 (25.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	3 (75.00%)
21-40	10 (14.08%)	13 (18.31%)	17 (23.94%)	10 (14.08%)	21 (29.58%)
41-60	6 (8.00%)	14 (18.67%)	15 (20.00%)	17 (22.67%)	23 (30.67%)
>60	6 (30.00%)	4 (20.00%)	5 (25.00%)	2 (10.00%)	3 (15.00%)

Table 3. Comparison of NSE, GFAP, and NFL levels among COVID-19, multiple sclerosis (MS), and control groups

Parameters	Group	No. of Cases	Mean Level (pg/mL)	Standard Deviation	P-value
NSE	COVID-19	91	33.70	30.50	p<0.001
	MS	29	35.19	14.31	
	Control	50	1.24	2.24	
GFAP	COVID-19	91	6.12	12.32	p<0.001
	MS	29	17.18	14.13	
	Control	50	1.85	2.4	
NFL	COVID-19	91	293.99	478.62	p<0.003
	MS	29	501.12	581.05	
	Control	50	17.97	5.48	

Table 4. COVID-19 severity-based distribution of NSE, GFAP, and NFL levels

Group	No. of cases	NSE (Mean ± SD)	GFAP (Mean ± SD)	NFL (Mean ± SD)
Covid-19-sever	23	50.87 ± 22.31	10.89 ± 20.99	736.06 ± 620.49
Covid-19-moderate	31	40.63 ± 40.91	5.19 ± 7.74	220.41 ± 397.64
Covid-19-mild	37	17.22 ± 10.91	3.92 ± 6.36	80.84 ± 171.58
P-value		<0.003*	p>0.05*	p<0.001*

Table 3 indicates that neuron-specific enolase (NSE) levels were significantly higher in COVID-19 patients (mean 33.70 pg/mL) compared to controls (mean 1.24 pg/mL), with a highly significant p-value ($p<0.001$). Similarly, glial fibrillary acidic protein (GFAP) levels were markedly elevated in the COVID-19 group (mean 6.12 pg/mL) against controls (mean 1.85 pg/mL), also showing statistical significance ($p<0.001$). Our current study found Neurofilament light chain (NFL) mean serum levels significantly higher in COVID-19 patients at 293.99 (SD 478.62) compared to healthy controls, who averaged 17.97 (SD 5.48), with a p-value of less than 0.003. Neurofilament light chain (NFL) levels were substantially raised in MS patients (mean 501.12 pg/mL) when compared to both COVID-19 patients and controls, with a notable p-value of less than 0.003, indicating a significant difference across the groups. The control group consistently showed the lowest mean levels across all parameters, underscoring the significant alteration of these biomarkers in disease states.

Table 4 showcases the severity-based distribution of biomarker levels in COVID-19 patients, with significant variances observed particularly in NSE and NFL levels. Severe cases exhibited the highest mean levels of NSE (50.87 pg/mL) and NFL (736.06

pg/mL), with both showing significant differences as indicated by the Kruskal-Wallis Test ($p<0.003$ and $p<0.001$, respectively). GFAP levels, although highest in severe cases (10.89 pg/mL), did not show a statistically significant variance across different severities ($p>0.05$). The marked elevation of NSE and NFL in severe cases compared to moderate and mild cases underscores the potential of these biomarkers in reflecting disease severity.

Table 5 displays Pearson correlation coefficients alongside p-values, examining the interrelations among NSE, GFAP, and NFL . While NSE and GFAP show no significant relationship (correlation coefficient = 0.085, p = 0.271), a significant and strong positive correlation is evident between NSE and NFL (correlation coefficient = 0.303, $p < 0.001$). This indicates that increases in NSE levels are associated with rises in NFL levels.

Table 5. Pearson correlation coefficients between (NSE, GFAP and NfL

Parameter		NSE	GFAP	NFL
NSE	Pearson Correlation	1	0.085	0.303**
	Sig. (2-tailed)		0.271	0.000
GFAP	Pearson Correlation	0.085	1	0.423**
	Sig. (2-tailed)	0.271		0.000
NfL	Pearson Correlation	0.303**	0.423**	1
	Sig. (2-tailed)	0.000	0.000	

**Correlation is significant at the 0.01 level (2-tailed), n=170

Discussion

In the present study, a cohort of 120 patients with COVID-19 and 29 individuals with Multiple Sclerosis (MS) were investigated to discern the relationship between disease severity and levels of key neurological biomarkers—Neurofilament light (NFL), Glial Fibrillary Acidic Protein (GFAP), and Neuron-Specific Enolase (NSE). The purpose was to understand how these biomarkers varied with disease progression and to explore their utility in clinical assessment. Our findings revealed significant associations that underscore the potential role of these biomarkers in reflecting the pathophysiological impact of both COVID-19 and MS.

The study found that among COVID-19 patients, severe cases included a higher percentage of males (27.1% of males) compared to females (23.3% of females). This aligns with several other studies showing worse COVID-19 outcomes in males compared to females. A meta-analysis of 11 studies with over 3000 COVID-19 patients found that males had 31% higher odds of developing severe COVID-19 infection compared to females (Jin *et al.*, 2020). Similarly, a study of over 30 million confirmed COVID-19 cases in the US found males had a mortality rate 10-20% higher than females (Siliezar, 2022). The higher COVID-19 severity and mortality risk for males has been observed across multiple studies and populations. According to current evidence, women seem to have a better immune response and less systemic inflammation, resulting in milder symptoms and a lower risk of blood clots when infected with COVID-19. On the other hand, men appear to have a weaker immune response, leading to more severe symptoms and a higher risk of blood clots (Pivonello *et al.*, 2021; La Vignera *et al.*, 2020; Asfahan *et al.*, 2020).

The distribution of COVID-19 severity across age groups in this study aligns with broader epidemiological findings indicating lower incidence and severity in younger populations. The study found that among the COVID-19 patients, the 41-60 age group had a balanced distribution of mild, moderate, and severe cases. This contrasts with the younger (21-40) and older (over 60) groups, which showed a skew towards moderate and severe cases, respectively. The higher prevalence of moderate cases in the 21-40 group aligns with global patterns of MS being most common in young adults (Farshbafnadi *et al.*, 2021). The skew towards more severe cases in the over 60 group reflects widely observed age-related increases in COVID-19 severity (Herrera-Esposito and de Los

Campos, 2022; Turke, 2020). The peak COVID-19 mortality risk in those over 75 versus 70-75 years old highlights the substantial increase in vulnerability in advanced age. Overall, the age-based distribution of COVID-19 severity categories mirrors population-level data indicating greater susceptibility to severe COVID-19 with older age. However, within age groups, the interplay between pre-existing conditions like MS and COVID-19 severity is complex and requires further research.

The current study found significantly higher serum NSE levels in COVID-19 patients compared to uninfected controls. Within the COVID-19 group, patients with respiratory distress and dyspnea showed even further elevated NSE levels. This aligns with other studies demonstrating increased NSE levels correlate with COVID-19 severity (Sahin, 2023). A study of 60 recovered COVID-19 patients also found higher NSE levels during the acute infection stage compared to follow-up (Heidari and Rezaei, 2023). Elevated NSE has been associated with lung injury in COVID-19 in multiple reports (Kokkoris *et al.*, 2022; Vretto *et al.*, 2022). The consistently observed relationship between high NSE levels and worse COVID-19 outcomes suggests neuronal damage or stress may be induced by SARS-CoV-2 infection, even in milder cases. This could indicate the virus' impact goes beyond the respiratory system (Morillo-Gonzalez *et al.*, 2020). Overall, the elevated serum NSE levels in COVID-19 patients, especially those with respiratory distress, mirrors findings from other studies linking increased NSE to greater COVID-19 severity. NSE may have utility as a biomarker for neurological involvement in COVID-19.

The significant elevation of glial fibrillary acidic protein (GFAP) in COVID-19 patients observed in this study is corroborated by similar findings in recent literature. GFAP, a brain injury biomarker, was elevated in COVID-19 patients with neurological symptoms during the acute phase of the disease, as reported in one study (Sahin *et al.*, 2022). This supports the notion that COVID-19 can affect the central nervous system, leading to potential brain injury. Another study noted that GFAP levels were found to change in patients with neurological manifestations of COVID-19, further suggesting its role as an indicator of axonal damage and neuronal degeneration in these patients (Bose, 2023). However, it is noted that while elevated GFAP was associated with disease severity, it may not directly correlate with neurological symptoms in COVID-19 patients, indicating that its elevation might represent a broader response to systemic inflammation rather than specific neuronal damage (Saraste, 2021). Lastly, increased levels of GFAP have been observed in the cerebrospinal fluid of patients with central neurological symptoms, which aligns with the increased levels noted in the serum of COVID-19 patients, emphasizing the potential utility of GFAP as a marker for central nervous system injury (Virhammar *et al.*, 2021)

The current study found significantly higher serum NFL levels in COVID-19 patients (mean 293.99 pg/mL) compared to healthy controls (mean 17.97 pg/mL). This aligns with several other studies showing increased serum NFL levels in COVID-19 patients versus controls. For example, two studies found 2-3-fold higher serum NFL levels in hospitalized COVID-19 patients compared to controls (Verde *et al.*, 2022). Higher NFL levels have also been associated with worse COVID-19 outcomes, including need for mechanical ventilation, prolonged ICU stay, and increased mortality (Giovannini, 2022). The consistently observed relationship between elevated serum NFL and greater COVID-19 severity suggests potential neuronal damage or stress induced by SARS-CoV-2 infection. However, the exact mechanisms are still unclear. Some possibilities include direct viral invasion of the CNS, hypoxia from respiratory distress, endothelial dysfunction, or inflammation (Hay *et al.*, 2021 ; Hidari and Rezaei 2023). Overall, the significantly increased serum NFL levels in COVID-19 patients, especially severe cases, mirrors findings from multiple other studies linking elevated NFL to COVID-19 severity. Serum NFL may have utility as a prognostic biomarker for neurological involvement in COVID-19.

The current study found significantly elevated mean NSE levels in MS patients (35.19 pg/mL) compared to healthy controls (1.24 pg/mL). This appears to contradict some earlier small studies showing no difference or even lower NSE levels in MS patients versus controls (Rodat *et al.*, 1994; Forooghian *et al.*, 2007). However, more recent research has demonstrated elevated serum and CSF NSE levels correlate with MS relapses and disease activity, suggesting NSE may reflect ongoing neuronal injury (Yang *et al.*, 2022; Gelderblom *et al.*, 2013; Wang *et al.*, 2018). The higher NSE levels in relapsing MS patients align with the observation that NSE levels peak during acute inflammatory events causing axonal damage. However, the relationship between NSE and long-term disability progression in MS remains unclear (Koch *et al.*, 2015). While this study reinforces the potential for NSE as a biomarker of neuronal damage in MS, NSE levels alone may not reliably distinguish MS from controls due to significant overlap and variability. Overall, this study provides further evidence that NSE levels are elevated in MS patients compared to healthy individuals, reflecting ongoing neuroaxonal injury, but additional biomarkers are likely needed for accurate diagnosis and monitoring.

Similarly, the current study found substantially higher serum GFAP levels in MS patients (mean 17.18 pg/mL) compared to healthy controls (mean 1.85 pg/mL). This aligns with several other studies showing elevated GFAP levels in the blood and CSF of MS patients versus controls. A recent meta-analysis found mean serum GFAP was 97.7 pg/mL in MS patients versus 67.5 pg/mL in controls (Saraste *et al.*, 2021). Another study showed a mean CSF GFAP of 17.8 pg/mL in MS patients compared to 8.2 pg/mL in controls (Abdelhak *et al.*, 2018). Higher GFAP levels have also been associated with greater disease severity and progression in MS, especially in progressive forms (Sharquie *et al.*, 2018; Högel *et al.*, 2020). The consistently observed relationship between elevated GFAP and MS suggests glial activation and astrocyte injury may play a role in disease pathology. However, GFAP alone may have limited utility as a diagnostic biomarker for MS due to variability and overlap with controls (Sun *et al.*, 2021). Overall, this study provides further evidence of increased GFAP levels in the blood of MS patients compared to healthy individuals, reflecting ongoing astrocyte activation or damage.

The current study found markedly elevated serum NfL levels in MS patients (mean 501.12 pg/mL) compared to healthy controls (mean 17.97 pg/mL). This aligns with numerous other studies showing significantly increased serum and CSF NfL levels in MS patients versus healthy controls. A recent meta-analysis found blood NfL levels were on average 76% higher in MS patients compared to non-matched controls (Ning and Wang, 2022). Another study found mean serum NfL of 30.5 pg/mL in MS patients versus 16.9 pg/mL in controls (Haines *et al.*, 2011). The consistently observed elevation of NfL in MS suggests ongoing neuroaxonal damage and loss. Higher NfL levels have also been associated with greater MS disease severity and progression, including conversion to secondary progressive MS (Kuhle *et al.*, 2019; Bittner *et al.*, 2021). This indicates the marked NfL increase in MS patients reflects more advanced neurodegeneration. Overall, the dramatic elevation of serum NfL in MS cases compared to controls in this study mirrors results from many other reports, further confirming NfL utility as a biomarker for neuroaxonal involvement and damage in MS.

The current study found no significant correlation between serum NSE and GFAP levels (correlation coefficient = 0.085, p = 0.271). This aligns with several other studies that also did not find a significant association between these two biomarkers across different neurological conditions. For example, studies in traumatic brain injury and cardiac arrest patients found no correlation between serum NSE and GFAP levels (Czeiter *et al.*, 2020; Gill *et al.*, 2018). The lack of correlation suggests NSE and GFAP may reflect different pathological processes, with NSE indicating acute neuronal damage and GFAP indicating astrocyte activation or injury.

In contrast, the study found a significant and strong positive correlation between serum NSE and NFL levels (correlation coefficient = 0.303, $p < 0.001$). Higher NSE levels were associated with increased NFL levels. This aligns with multiple other studies demonstrating significant positive correlations between serum or CSF levels of NSE and NFL across various neurological diseases, including Alzheimer's disease, multiple sclerosis, and traumatic brain injury (Leister *et al.*, 2021). The consistent correlation suggests both NSE and NFL can indicate the extent of acute axonal injury. However, NSE elevation tends to be more transient while NFL remains elevated longer, making NFL a potential indicator of ongoing neurodegeneration (Youssef *et al.*, 2023). Overall, the correlation results between NSE, GFAP, and NFL align with findings from other studies showing association between markers of neuronal injury like NSE and NFL but not astrocyte activation like GFAP.

Our study's significant findings on elevated biomarkers among COVID-19 and MS patients have critical implications for clinical practice. The marked increase in NFLE, NSE, and GFAP levels in MS patients compared to healthy controls suggests these biomarkers could potentially serve in the early detection and monitoring of central nervous system involvement. For COVID-19, the notable elevation of these biomarkers indicates a broader impact of the virus, possibly extending to neurological damage. These insights could pave the way for more targeted therapeutic strategies and a better understanding of the pathophysiological mechanisms underpinning both diseases. This is , as far as we are aware , the first investigation into the potential applications of neuron-specific enolase (NSE) , glial fibrillary acidic protein (GFAP) , and neurofilament light chain (NFL) as biomarkers in the context of multiple sclerosis and COVID-19 in the Kurdistan Region of Iraq (KRG)

Conclusion

Our study ‘s findings highlight the importance of raised serum biomarkers in identifying the neurological effects of COVID-19 and Multiple Sclerosis(MS) including Neurofilament light chain(NFLE), neuron-specific enzyme (NSE) and glial fibrillary acidic protein (GFAP). In particular, a possible function for these biomarkers in clinical diagnosis and tracking disease progression is suggested by the significant increase in NFLE levels in MS patients and the significant rise in NSE and GFAP levels in COVID-19 patients as compared to healthy controls. These results add to the increasing amount of information suggesting that COVID-19 may cause neurological issues in addition to respiratory symptoms. Our findings could guide future research that could enhance patient care by identifying patients with neurologic symptoms of these illnesses early and developing specialized treatment plans for them.

Recommendation(s)

Our study’s findings highlight the importance of raised serum biomarkers in identifying the neurological effects of COVID-19 and Multiple Sclerosis(MS) including Neurofilament light chain(NFLE), neuron-specific enzyme (NSE) and glial fibrillary acidic protein (GFAP). Inparticular, a possible function for these biomarkers in clinical diagnosis and tracking disease progression is suggested by the significant increase in NFLE levels in MS patients and the significant rise in NSE and GFAP levels in COVID-19 patients as compared to healthy controls. These results add to the increasing amount of information suggesting that COVID-19 may cause neurological issues in addition to respiratory symptoms. Our findings could guide future research that could enhance patient care by identifying patients with neurologic symptoms of these illnesses early and developing specialized treatment plans for them.

Acknowledgments

Gratitude is extended to the Laboratory Department staff at Erbil Central Emergency Hospital for their indispensable assistance and cooperation in conducting this study. The authors also thank the participants for their vital contribution to the research.

References

Abdelhak A, Huss A, Kassubek J, Tumani H, Otto M. 2019. Serum GFAP as a biomarker for disease severity in multiple sclerosis. Sci Rep. **8**(1), 14798.
DOI: 10.1038/s41598-018-33158-8.

Al-Sarray AM, Shareef RH. 2022. Awareness of Healthcare Providers About COVID-19 Vaccinations in Karbala City. Journal of Techniques **4**(Special Issue), 91-96.
https://doi.org/10.51173/jt.v4i33.654,

Arneth B, Kraus J. 2022. Laboratory biomarkers of Multiple Sclerosis (MS). Clin Biochem 99, 1-8. https://doi.org/10.1016/j.clinbiochem.2021.10.004.

Asfahan S, Deokar K, Dutt N, Niwas R, Jain P, Agarwal M. 2020. Extrapolation of mortality in COVID-19: exploring the role of age, sex, co-morbidities and health-care related occupation. Monaldi Arch Chest Dis. **90**(2).

Barzegar M, Sindarreh S, Manteghinejad A, Javanmard SH, Shaygannejad V, Nasirian M. 2023. Multiple sclerosis is associated with worse COVID-19 outcomes compared to the general population: A population-based study. Mult Scler Relat Disord. **79**, 104947.
https://doi.org/10.1016/j.msard.2023.104947.

Bittner S, Oh J, Havrdová EK. 2021. The potential of serum neurofilament as biomarker for multiple sclerosis. Brain. **144**(10), 2954-2963.
DOI: 10.1093/brain/awab241.

Bose P. 2023. Are plasma levels of axonal damage and neuronal degeneration makers altered in COVID-19 patients without major neurological manifestations? News-Medical. Net. 2023 Feb 3. Available from: https://www.news-medical.net/news/20230203/

Buck D, Hemmer B. 2014. Biomarkers of treatment response in multiple sclerosis. Expert Rev Neurother.**14**(2), 165-72.
DOI: 10.1586/14737175.2014.874289.

Carethers JM. 2021. Insights into disparities observed with COVID-19. J Intern Med. **289**(4), 463-473. https://doi.org/10.1111/joim.13199.

Conway SE, Healy BC, Zurawski J, Severson C, Kaplan T, Stazzone L. 2022. COVID-19 severity is associated with worsened neurological outcomes in multiple sclerosis and related disorders. Mult Scler Relat Disord. **63**, 103946.
DOI: 10.1016/j.msard.2022.103946.

Czeiter E, Amrein K, Gravesteijn BY. 2020. Blood biomarkers on admission in acute traumatic brain injury: Relations to severity, CT findings and care path in the CENTER-TBI study. EBioMedicine. 56, 102785.
DOI: 10.1016/j.ebiom.2020.102785.

Dadas A, Washington J, Diaz-Arrastia R, Janigro D. 2018. Biomarkers in traumatic brain injury (TBI): a review. Neuropsychiatr Dis Treat., 14:2989-3000.
DOI: 10.2147/NDT.S125620.

DeKosky ST, Kochanek PM, Valadka AB, Clark RSB, Chou SH-Y, Au AK, 2021. Blood Biomarkers for Detection of Brain Injury in COVID-19 Patients. J Neurotrauma. **38**(1), 1.
DOI: 10.1089/neu.2020.7332.

Dondaine T, Ruthmann F, Vuotto F, Carton L, Gelé P, Faure K. 2022. Long-term cognitive impairments following COVID-19: a possible impact of hypoxia. J Neurol. **269**(8), 3982-3989.
DOI: 10.1007/s00415-022-11077-z.

Farshbafnadi M, Kamali Zonouzi S, Sabahi M, Dolatshahi M, Aarabi MH. 2021. Aging & COVID-19 susceptibility, disease severity, and clinical outcomes: The role of entangled risk factors. Exp Gerontol. **154**, 111507.
DOI: 10.1016/j.exger.2021.111507.

Fernandes de Souza WD, Fonseca DMD, Sartori A. 2023. COVID-19 and Multiple Sclerosis: A Complex Relationship Possibly Aggravated by Low Vitamin D Levels. Cells. **12**(5), 684.
DOI: 10.3390/cells12050684.

Forooghian F, Cheung RK, Smith WC, O'Connor P, Dosch HM. 2007. Enolase and arrestin are novel nonmyelin autoantigens in multiple sclerosis. J Clin Immunol. **27**(4), 388-96.
DOI: 10.1007/s10875-007-9091-1.

Gelderblom M, Daehn T, Schattling B, Ludewig P, Bernreuther C, Arunachalam P. 2013. Plasma levels of neuron specific enolase quantify the extent of neuronal injury in murine models of ischemic stroke and multiple sclerosis. Neurobiol Dis. **59**, 177-82.
DOI: 10.1016/j.nbd.2013.07.017.

Gill J, Latour L, Diaz-Arrastia R. 2018. Glial fibrillary acidic protein elevations relate to neuroimaging abnormalities after mild TBI. Neurology. **91**(15), e1385-e1389.
DOI: 10.1212/WNL.0000000000006321.

Giovannini G, Bedin R, Ferraro D, Vaudano AE, Mandrioli J, Meletti S. 2022. Serum neurofilament light as biomarker of seizure-related neuronal injury in status epilepticus. Epilepsia. **63**(1), e23-e29.
DOI: 10.1111/epi.17132.

Gyang T, Rempe T. 2021. The State of Biomarkers in Multiple Sclerosis. NeurologyLive. 4(6). Available from: https://www.neurologylive.com/view/the-state-of-biomarkers-in-multiple-sclerosis.

Haines JD, Inglese M, Casaccia P. 2011. Axonal damage in multiple sclerosis. Mt Sinai J Med. 78(2), 231-43.
DOI: 10.1002/msj.20246.

Hay M, Ryan L, Huentelman M, Konhilas J, Hoyer-Kimura C, Beach TG. 2021. Serum Neurofilament Light is elevated in COVID-19 Positive Adults in the ICU and is associated with Co-Morbid Cardiovascular Disease, Neurological Complications, and Acuity of Illness. Cardiol Cardiovasc Med. **5**(5), 551-565.
DOI: 10.26502/fccm.92920221.

Heidari A, Rezaei N. 2023. The role of neurofilament light chain in COVID-19: A potential prognostic biomarker. Acta Neurobiol Exp (Wars). **83**(2), 111-126.
DOI: 10.55782/ane-2023-011.

Herrera-Esposito D, de Los Campos G. 2022. Age-specific rate of severe and critical SARS-CoV-2 infections estimated with multi-country seroprevalence studies. BMC Infect Dis. **22**(1), 311.
DOI: 10.1186/s12879-022-07262-0.

Högel H, Rissanen E, Barro C. 2020. Serum glial fibrillary acidic protein correlates with multiple sclerosis disease severity. Mult Scler J. **26**(2), 210-219.
DOI: 10.1177/1352458518819380.

Iqbal A, Iqbal K, Ali SA, Azim D, Farid E, Baig MD. 2021. The COVID-19 sequelae: a cross-sectional evaluation of post-recovery symptoms and the need for rehabilitation of COVID-19 survivors. Cureus. **13**(2).

Jin JM, Bai P, He W, Wu F, Liu XF, Han DM. 2020. Gender Differences in Patients With COVID-19: Focus on Severity and Mortality. Front Public Health. **8**, 152.
DOI: 10.3389/fpubh.2020.00152.

Khatoon F, Prasad K, Kumar V. 2022. COVID-19 associated nervous system manifestations. Sleep Med. **91**, 231-236.
DOI: 10.1016/j.sleep.2021.07.005.

Koch MW, George S, Wall W, Yong VW, Metz LM. 2015. Serum NSE level and disability progression in multiple sclerosis. J Neurol Sci. **350**(1-2), 46-50.
DOI: 10.1016/j.jns.2015.02.009.

Kokkoris S, Stamataki E, Emmanouil G, Psachoulia C, Ntaidou T, Maragouti A. 2022. Serum inflammatory and brain injury biomarkers in COVID-19 patients admitted to intensive care unit: A pilot study. eNeurologicalSci. 29:100434. https://doi.org/10.1016/j.ensci.2022.100434

Kuhle J, Kropshofer H, Haering DA. 2019. Blood neurofilament light chain as a biomarker of MS disease activity and treatment response. Neurology. **92**(10), e1007-e1015.
DOI: 10.1212/WNL.0000000000007032.

Kulbe JR, Geddes JW. 2016. Current status of fluid biomarkers in mild traumatic brain injury. Exp Neurol. **275**(Pt 3):334-52.
DOI: 10.1016/j.expneurol.2015.05.004.

La Vignera S, Cannarella R, Condorelli RA, Torre F, Aversa A, Calogero AE. 2020. Sex-Specific SARS-CoV-2 Mortality: Among Hormone-Modulated ACE2 Expression, Risk of Venous Thromboembolism and Hypovitaminosis D. Int J Mol Sci. **21**(8), E2948.
https://doi.org/10.3390/ijms21082948.

Leister I, Altendorfer B, Maier D, Mach O, Wutte C, Grillhösl A. 2021. Serum Levels of Glial Fibrillary Acidic Protein and Neurofilament Light Protein Are Related to the Neurological Impairment and Spinal Edema after Traumatic Spinal Cord Injury. J Neurotrauma. **38**(24), 3431-3439.
DOI: 10.1089/neu.2021.0264.

Maury A, Lyoubi A, Peiffer-Smadja N, de Broucker T, Meppiel E. 2021. Neurological manifestations associated with SARS-CoV-2 and other coronaviruses: A narrative review for clinicians. Rev Neurol (Paris). **177**(1-2), 51-64.
https://doi.org/10.1016/j.neurol.2020.10.001.

Mendoza DA, López KD, Echeverri RA, Pastor L, Rueda S, Fernández LL. 2020. Utility of biomarkers in traumatic brain injury: a narrative review. Colombian Journal of Anesthesiology **48**(3), 155-161.
DOI: 10.1097/CJ9.0000000000000165.

Momtazmanesh S, Shobeiri P, Saghazadeh A, Teunissen CE, Burman J, Szalardy L. 2021. Neuronal and glial CSF biomarkers in multiple sclerosis: a systematic review and meta-analysis. Rev Neurosci. **32**(6), 573-595.
https://doi.org/10.1515/revneuro-2020-0145.

Moreno-Torres I, Meca Lallana V, Costa-Frossard L, Oreja-Guevara C, Aguirre C, Alba Suárez EM. 2021. Risk and outcomes of COVID-19 in patients with multiple sclerosis. Eur J Neurol. **28**(11), 3712-3721. DOI: 10.1111/ene.14990.

Morillo-González J, Hernández-Huerta D, Guillama-Henríquez A, Correa-Palacio A, Pereira-Nogueira P. 2020. Beyond the Respiratory System: A Case Report Highlighting the Impact of COVID-19 in Mental Illness and Its Physical Consequences. J Clin Psychiatry **81**(4), 317.

Nazzal AG, Sabbar AG. 2022. Estimation of Some Biomarkers in Recovered COVID19 Patients. Journal of Techniques **4**(special issue), 21-27.
https://doi.org/10.51173/jt.v4i33.587.

Needham EJ, Ren AL, Digby RJ, Norton EJ, Ebrahimi S, Outtrim JG. 2022. Brain injury in COVID-19 is associated with dysregulated innate and adaptive immune responses. Brain. **145**(11), 4097-4107.
DOI: 10.1093/brain/awac321.

Ning L, Wang B. 2022. Neurofilament light chain in blood as a diagnostic and predictive biomarker for multiple sclerosis: A systematic review and meta-analysis. PLoS One. **17**(9), e0274565.
DOI: 10.1371/journal.pone.0274565.

Nishimura K, Cordeiro JG, Ahmed AI. 2022. Advances in Traumatic Brain Injury Biomarkers. Cureus. **14**(4), e23804. DOI:10.7759/cureus.23804.

Otani K, Fukushima H, Matsuishi K. 2023. COVID-19 delirium and encephalopathy: Pathophysiology assumed in the first 3 years of the ongoing pandemic. Brain Disord. **10**, 100074.
DOI: 10.1016/j.dscb.2023.100074.

Pivonello R, Auriemma RS, Pivonello C, Isidori AM, Corona G, Colao A, Millar RP. 2021. Sex Disparities in COVID-19 Severity and Outcome: Are Men Weaker or Women Stronger? Neuroendocrinology **111**(11), 1066-1085.
DOI: 10.1159/000513346.

Richter D, Faissner S, Bartig D. 2021. Multiple sclerosis is not associated with an increased risk for severe COVID-19: a nationwide retrospective cross-sectional study from Germany. Neurol Res Pract. **3**, 42. https://doi.org/10.1186/s42466-021-00143-y.

Rodat T, Cunningham JI, Morrow CF, Johnston KD, Buchanan KD. 1994. Serum neurone-specific enolase concentrations in patients with neurological disorders. Clin Chim Acta. **230**(2), 117-24.
https://doi.org/10.1016/0009-8981(94)90264-x.

Sahin BE, Celikbilek A, Kocak Y, Ilanbey B, Saltoglu GT, Konar NM, Hizmali L. 2023. Neurological symptoms and neuronal damage markers in acute COVID-19: Is there a correlation? A pilot study. J Med Virol. **95**(1).
DOI: 10.1002/jmv.28240.

Sahin BE, Celikbilek A, Kocak Y, Saltoglu GT, Konar NM, Hizmali L. 2022. Plasma biomarkers of brain injury in COVID-19 patients with neurological symptoms. J Neurological Sci., 17. Available from: https://doi.org/10.1016/j.jns.2022.120324.

Saleki K, Banazadeh M, Miri NS, Azadmehr A. 2022. Triangle of cytokine storm, central nervous system involvement, and viral infection in COVID-19: the role of sFasL and neuropilin-1. Rev Neurosci., **33**(2), 147-160.
https://doi.org/10.1515/revneuro-2021-0047.

Saraste M, Bezukladova S, Matilainen M. 2021. Increased serum glial fibrillary acidic protein associates with microstructural white matter damage in multiple sclerosis: GFAP and DTI. Mult Scler Relat Disord. **50**, 102810.
DOI: 10.1016/j.msard.2021.102810.

Sarıoğlu E, Sarıaltın SY, Çoban T. 2023. Neurological complications and effects of COVID-19: Symptoms and conceivable mechanisms. Brain Hemorrhages. **4**(3), 154-173.
https://doi.org/10.1016/j.hest.2023.02.001.

Schwartz CE, Rapkin BD, Bonavita S, Bossa M, Buscarinu MC, Grasso MG. 2022. The impact of COVID-19 on people with multiple sclerosis: A comparison of Italian and United States cohorts. Mult Scler Relat Disord. **63**, 103888.
https://doi.org/10.1016/j.msard.2022.103888.

Serafeim K, Anagnostouli M. 2013. Biomarkers in Multiple Sclerosis: An Up-to-Date Overview. Mult Scler Int. **2013**, 340508. DOI: 10.1155/2013/340508.

Sharquie IK, Gawwam GA, Abdullah SF. 2020. Serum Glial Fibrillary Acidic Protein: A Surrogate Marker of the Activity of Multiple Sclerosis. Medeni Med J. **35**(3), 212-218.
DOI: 10.5222/MMJ.2020.48265.

Siliezar J. 2022. GenderSci Lab finds social factors, not biological difference between sexes, play biggest role by far. Harvard Gazette. 2022 Jan 20. Available from:
https://news.harvard.edu/gazette/story/2022/01/.

Silva RC, da Rosa MM, Leão HI, Silva EDL, Ferreira NT, Albuquerque APB. 2023. Brain damage serum biomarkers induced by COVID-19 in patients from northeast Brazil. J Neurovirol. **29**(2), 180-186.
DOI: 10.1007/s13365-023-01119-1.

Sormani MP, Schiavetti I, Carmisciano L, Cordioli C, Filippi M, Radaelli M. 2022. COVID-19 Severity in Multiple Sclerosis: Putting Data Into Context. Neurol Neuroimmunol Neuroinflamm. **9**(1), e1105.
https://doi.org/10.1212/NXI.0000000000001105.

Spudich S, Nath A. 2022. Nervous system consequences of COVID-19. Science. **375**(6578), 267-269. DOI: 10.1126/science.abm2052.

Sukocheva OA, Maksoud R, Beeraka NM, Madhunapantula SV, Sinelnikov M, Nikolenko VN. 2022. Analysis of post COVID-19 condition and its overlap with myalgic encephalomyelitis/chronic fatigue syndrome. J Adv Res. **40**, 179-196.
DOI: 10.1016/j.jare.2021.11.013.

Sun M, Liu N, Xie Q. 2021. A candidate biomarker of glial fibrillary acidic protein in CSF and blood in differentiating multiple sclerosis and its subtypes: A systematic review and meta-analysis. Mult Scler Relat Disord. **51**, 102870.
DOI: 10.1016/j.msard.2021.102870.

Telser J, Grossmann K, Weideli OC, Hillmann D, Aeschbacher S, Wohlwend N. 2023. The role of serum brain injury biomarkers in individuals with a mild-to-moderate COVID infection and Long-COVID - results from the prospective population-based COVI-GAPP study. medRxiv. 15:2023.02.15.23285972.
https://doi.org/10.1101/2023.02.15.23285972.

Thepmankorn P, Bach J, Lasfar A, Zhao X, Souayah S, Chong ZZ, Souayah N. 2021. Cytokine storm induced by SARS-CoV-2 infection: The spectrum of its neurological manifestations. Cytokine. **138**, 155404.

Turke PW. 2020. Five reasons COVID-19 is less severe in younger age-groups. Evol Med Public Health. **9**(1), 113-117. DOI: 10.1093/emph/eoaa050.

Verde F, Milone I, Bulgarelli I, Peverelli S, Colombrita C, Maranzano A. 2022. Serum neurofilament light chain levels in Covid-19 patients without major neurological manifestations. J Neurol. **269**(11), 5691-5701. DOI: 10.1007/s00415-022-11233-5.

Virhammar J, Nääs A, Fällmar D, Cunningham JL, Klang A, Ashton NJ. 2021. Biomarkers for central nervous system injury in cerebrospinal fluid are elevated in COVID-19 and associated with neurological symptoms and disease severity. Eur J Neurol. **28**(10), 3324-3331. DOI: 10.1111/ene.14703.

Vrettou CS, Vassiliou AG, Pratikaki M, Keskinidou C, Tsipilis S, Gallos P. 2022. Comparative evaluation and prognostic utility of neuronal injury biomarkers in covid-19 patients: a prospective study. Shock **58**(6), 507-13.

Wang KK, Yang Z, Zhu T, Shi Y, Rubenstein R, Tyndall JA, Manley GT. 2018. An update on diagnostic and prognostic biomarkers for traumatic brain injury. Expert Rev Mol Diagn. **18**(2), 165-180.
DOI: 10.1080/14737159.2018.1428089.

Wang S, Zha X, Zhu X. 2018. Metabolic syndrome and its components with neuron-specific enolase: a cross-sectional study in large health check-up population in China. BMJ Open. **8**(4), e020899. DOI: 10.1136/bmjopen-2017-020899.

Wilde EA, Wanner IB, Kenney K, Gill J, Stone JR, Disner S. 2022. A Framework to Advance Biomarker Development in the Diagnosis, Outcome Prediction, and Treatment of Traumatic Brain Injury. J Neurotrauma **39(7-8)**.
https://doi.org/10.1089/neu.2021.0099.

Yang J, Hamade M, Wu Q, Wang Q, Axtell R, Giri S, Mao-Draayer Y. 2022. Current and Future Biomarkers in Multiple Sclerosis. Int J Mol Sci. **23**(11), 5877. DOI: 10.3390/ijms23115877.

Youssef P, Hughes L, Kim WS, Halliday GM, Lewis SJG, Cooper A. 2023. Evaluation of plasma levels of NFL, GFAP, UCHL1 and tau as Parkinson's disease biomarkers using multiplexed single molecule counting. Sci Rep. **13**(1), 5217.
DOI: 10.1038/s41598-023-32480-0.

Zhang J, Puvenna V, Janigro D. 2016. Biomarkers of Traumatic Brain Injury and Their Relationship to Pathology. In: Laskowitz D, Grant G, editors. Translational Research in Traumatic Brain Injury. Boca Raton (FL): CRC Press/Taylor and Francis Group; 2016. Chapter 12. Available from: https://www.ncbi.nlm.nih.gov/books/NBK326724/.