Nanotechnology Perceptions
ISSN 1660-6795
www.nano-*ntp.com*

Comparative Analysis Of IL-6 And The Cytokine TNF-A Across COVID-19 Severities, And Multiple Sclerosis In Erbil City, KRG, Iraq: Implications For Disease Prognosis And Inflammatory Responses

Daldar Fares Jeejo[1], Mayada Noori Iqbal[2], Nisreen Sherif Alyasiri[3]

[1, 2] Middle Technical University/ College of Health and Medical Technology.
[3] Middle Technical University/ Suwaira Technical Institute.
**Corresponding author E-mail: dldarhozan@gmail.com*

Objectives: This study sought to compare IL-6 the biomarker TNF-α values across various severities of COVID-19, in multiple sclerosis cases, and healthy individuals, exploring the link between cytokines.

Methods: A type of cross-sectional analysis was done with 91 COVID-19 patients (categorized by severity), 29 MS patients, and 50 healthy controls. Serum IL-6 and the cytokine TNF-α values are tested, and Pearson correlation coefficients were used to assess their relationship.

Results: Results: COVID-19 patients, especially those with severe symptoms, showed higher IL-6 and the cytokine levels of TNF-α in comparison with controls, a trend also seen in MS patients. A moderate positive correlation ($r = 0.479$, $p < 0.001$) was detected between these cytokines in COVID-19 patients. Cytokine level variations in COVID-19 severities weren't statistically significant, suggesting variability within each group.

Conclusions: The elevated IL-6 and the cytokines TNF-α in COVID-19 and MS patients underscore their role in the disease mechanisms, especially in COVID-19 inflammation. Future research should observe these biomarkers' levels, their important on persons treatments, and how cytokine treatment affects disease progression and results.

Keywords: COVID-19 Severity, IL-6 -TNF-α Levels, Multiple Sclerosis, Cytokine Correlation, Inflammatory Biomarkers, Immune Response, Disease Pathogenesis

1. Introduction

1.1 Review on the SARS-CoV-2

In end of the 2019 year , the world faced a challenge with the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), firstly appeared in China(Lai, Shih, Ko, Tang, & Hsueh, 2020; Organization, 2023). This virus quickly went into a worldwide health emergency

outbreak, leading to many infections and a high number of deaths around the world. In February 2020, Iraq found its first case(Al-Malkey, Al-Sammak, & disease, 2020; Al-Sarray & Shareef, 2022), putting additional strain on its medical system, which was dealing with limited resources and conflict. The virus, known for disseminate fast between people in close contact, caused several lines of infection, each with more strong versions of the virus(Beig Parikhani et al., 2021). These new variants, which had changed in their spike proteins, raised concerns about how well the vaccines developed could work against them(Singh, Dahiya, Yadav, Sehrawat, & Microbiology, 2022).

The SARS-CoV-2 virus, perhaps its cunning tactics, attaches itself to ACE2 receptors in the body. This relation not only reduces the effectiveness of ACE2 but also makes a lessen in anti-inflammatory of cytokines and an elevated value in Angiopoietin-2. This triggers waves of pro-inflammatory cytokines(Tanzadehpanah et al., 2023). This strong increase can cause to a cytokine storm, a severe immune reaction appeared as elevated levels of cytokines such as IL-6 values and the biomarker TNF-α. In risky COVID-19 cases, this result can turn into dangerous hyperinflammation(Qudus et al., 2023). This cytokine storm is a strong immune reaction that can lead to failure of many organs and may be death. It's detected by an high releasing of cytokines before inflammation, that including the interleukin such as the IL-6, the cytokines IL-2, IL-7, and the biomarker tumor necrosis factor (TNF)-α(Montazersaheb et al., 2022). Recent studies have found that COVID-19 patients in intensive care have increased the serum values of cytokines such as IL-24(Bordon, 2023; Nazzal & Sabbar, 2022).

The signs of COVID-19 differ largely, occur from no signs at all to severe viral pneumonia and respiratory failure. The disease also can resemble sepsis of blood or septic shock, which also result in dysfunction of many organs and may be death(Huang et al., 2020). The markers of the severity of COVID-19 are immunological markers, such as IL-6 and the cytokine TNF-α, because they causing inflammation. The higher levels of these cytokines can make clear aggressive immune response, which leading to severe signs, that complications of all the body, and a possibly deadly cytokine storm(Qin et al., 2020).

Studies suggest that SARS-CoV-2 might harm the CNS by attachment to ACE-2 receptors or through bloodstream transmission(Ellul et al., 2020; Haidar et al., 2022; Nath & Smith, 2021). CNS damage often results from adverse immune responses, leading to immediate or progressive demyelination(Li, Bai, & Hashikawa, 2020; Lima et al., 2020; Pattanaik, Bhandarkar B, Lodha, & Marate, 2023).

Emerging research shows that SARS-CoV-2, along with various proinflammatory cytokines (such as TNF-α , IL-10, IL-8, IL-2, IL-4, IFN-γ and the cytokine IL-6,), can passing the barrier between blood and brain. Once inside, they infect critical CNS cells of immune system such as, microglia, macrophages and astrocytes, triggering increased inflammation(Han et al., 2020).

1.2 Multiple Sclerosis

The CNS disorder of the Multiple Sclerosis (MS) emerges as a formidable autoimmune adversary, specifically assaulting the myelin of the central nervous system. This condition

manifests in a spectrum of symptoms of neurological, hallmarked by a trio of challenges: inflammation, neurodegeneration, and demyelinating lesions across both white and gray brain matter(Shah et al., 2023). The complex of Multiple Sclerosis (MS) is increased by the important effect of genetic role in its increasing(Abd El Ghaffar, Mohamed, Zidan, Alhadi, & Emad, 2023). Because it is the leading cause of non-traumatic disability among young persons, MS make a big challenge to public health due to its progression that cannot detected and many different responses in peoples, that making its management and therapy highly complicated(Bijoux Leist, Leist, & Medicine, 2022). Many risk factors, that including deficiency of the vitamin D, and smoking, and obesity, all dangerous to the vascular health that associated with the appearance of MS. Vascular issues such as destroy to the blood-brain barrier, microbleeds, and poor blood circulations in the brain are common in MS and may lead to its gradual and often unnoticed onset(Cashion, Young, & Sutherland, 2023).

1.3 Cytokines in Disease Progression

The cytokines have the crucial role in the body's inflammatory responses in COVID-19 and Multiple Sclerosis (MS) disease(Simões, de Araujo, & Bagatini, 2021). These small but strong proteins, are important for cell-to-cell reaction in the immune system, including the important ones such as the IL-6 and cytokines TNF-α. Scientists are concentrated on these cytokines because they’re the key role in the causing of inflammation and regulating the immune responses. In the COVID-19, high levels of IL-6 and the cytokine of TNF-α are related to the severity of the disease, making them important indicators for predicting how patients will fare(Halim, Mirza, & Sari, 2022). These cytokines similarly, in MS, significantly effect on the disease’s appearance and the results of treatments(Šiško Markoš et al., 2023).

While there's growing research on IL-6 and TNF-α in COVID-19 patients of different severities in Erbil City, KRG, Iraq, the study of the cytokines in the MS is still in the early stages(Al-Naseria, Salmanb, & Ad’hiahc, 2017).

A comparative study of these markers across both ailments holds the promise of shedding light on the underlying inflammatory mechanisms. This is particularly relevant in Erbil City, where, amidst an expanding repository of COVID-19 research, there remains a significant void in comprehensive epidemiological data regarding cytokine profiles in MS and their potential prognostic value.

1.4 Rationale for the Study

Investigating the IL-6 and the cytokine TNF-α in individuals with COVID-19 and MS assumes paramount importance, given the pivotal influence these cytokines exert in fueling inflammation and disease progression(MacDougall, El-Hajj Sleiman, Beauchemin, & Rangachari, 2022). In the realm of COVID-19, anomalous levels of IL-6 and biomarker of TNF-α have a marked correlation with the severity and eventual outcomes of the disease, positing them as key biomarkers for patient classification and the development of tailored treatments(Del Valle et al., 2020). In the case of MS, these cytokines are integral to the disease’s pathology, notably affecting the inflammatory response and neurodegeneration. Crucial to MS’s pathophysiology are microglial cells, agents of tissue destruction through

various means such as the appearance of the cytokines such as the cytokines of TNF-α, and IL-6, IL-1, and the cytokine of IFN-γ)(Correale, Marrodan, & Ysrraelit, 2019).

Erbil City, KRG, Iraq, presents a unique context for this study. The region has experienced a significant burden of COVID-19 cases(Barzinji, Jaff, & Ismael, 2022), yet, despite recent research progress, comprehensive data on cytokine profiles in MS remains sparse. By comparing IL-6 and the cytokine TNF-α levels across different severities of COVID-19 and in MS, our study aims to bridge this knowledge gap. Such comparison could reveal shared or distinct inflammatory pathways, informing personalized treatment approaches and improving prognostic accuracy for both conditions(Ismail, Al-Hashel, Alroughani, & Ahmed, 2021).

1.5 Hypothesis or Research Question

Our inquiry is anchored by the hypothesis: "Variations in IL-6 and the biomarker TNF-α levels correspond with differing intensities of COVID-19 and the pathophysiological evolution of Multiple Sclerosis in Erbil City, KRG, Iraq."

To explore this hypothesis, we pose the research question: "In what ways do IL-6 and cytokine TNF-α levels vary among the virus of COVID-19 patients of different severities as opposed to those in Multiple Sclerosis patients within Erbil City, and what do these variations signify for disease prognosis and the inflammatory process?"

This question is fundamentally tied to a comparative evaluation of IL-6 and cytokine TNF-α levels in various stages of COVID-19 and within the MS context, aiming to elucidate the relationship between cytokine profiles and the clinical expressions of these diseases.

1.6 Study Objectives

The primary aim of our investigation is to decode the relationship between IL-6 and the cytokine TNF-α and the severity of COVID-19 and Multiple Sclerosis (MS) in Erbil City, KRG, Iraq. Our goal is to dissect how these cytokines modulate the inflammatory response and impact disease prognosis. Key objectives encompass a comparative analysis of IL-6 and the cytokine TNF-α across various COVID-19 severities and in MS patients, evaluating their predictive value in disease outcomes. Additionally, we plan to examine the linkage between cytokine levels and the degree of inflammation in both conditions. Generating epidemiological data on these cytokines in Erbil City will provide critical insights to shape local healthcare policies. In addition, this study seeks to examine the potential of IL-6 and the cytokine TNF-α as a biomarker to guiding treatment ways in the controlling of COVID-19 and MS diseases. By considering this way, we want to increase the current knowledge and enhance management of these cases in Erbil Cirt.

Nomenclature and Abbreviations Used

COVID-19	Coronavirus Disease 2019	MRI	Magnetic Resonance Imaging
EDSS	Expanded Disability Status Scale	SD	Standard Deviation
MS	Multiple Sclerosis	ANOVA	Analysis of Variance
IL-6	Interleukin 6	CSF	Cerebrospinal Fluid
PPMS	Primary Progressive Multiple Sclerosis	PCR	Polymerase Chain Reaction
TNF-α:	Tumor Necrosis Factor-alpha	ELISA	Enzyme-Linked Immunosorbent Assay
IFN-β	Interferon-beta	ROC	Receiver Operating

2. Methodology

2.1 Design of Study:

The design of our study was a case-control type, that was done in the hospital in Erbil City. The period of study was from March 2023to September 2023 in Erbil Central Emergency Hospital Lab Department.

2.2 Participants

Our research encompassed 170 participants, comprising 120 patients and 50 apparently healthy controls. The group of patents was 56 females and 64 males. This group is a total of 91 individuals with diagnosed of COVID-19 in different states. In addition to 29 patients were diagnosed with MS. The incorporation of healthy control group acts as foundation of cytokine results for comparison status. The patients were examined by doctors in clinical examination and laboratory tests.

We used the classification procedure admitted by World Health Organization (WHO) clinical progression scale, the separate them to three groups including mild, moderate and severe cases. The patients of MS diseases were classified by using the criteria of Expanded of Disability Status Scale. The control group comprised healthy individuals with no history of COVID-19 or MS, and they were carefully matched with the patient groups in age and gender.

2.3 The Approval of Ethics

The study was granted ethical clearance by the Discussion Committee, Scientific Committee, and Postgraduate Studies Division at the College of Health and Medical Technologies, Middle Technical University. Approval was also obtained from Erbil Central Emergency Hospital, Laboratory Department, and Erbil Health Department to ensure adherence to local ethical standards. Verbal consents were obtained from all participants, ensuring they were fully informed about the study objectives, procedures, risks, and benefits. Confidentiality and anonymity were assured, and participants had the freedom to withdraw at any time. The study followed ethical standards, performed precise and accurate laboratory tests, and aligned with the Declaration of Helsinki for participant protection.

2.4 Inclusion and Exclusion criteria:

This study includes individuals diagnosed with COVID-19 and Multiple Sclerosis, classified by clinical progression and Expanded Disability Status Scale, respectively. The control group is of seemingly healthy individuals, aligned of the patient groups regarding the age and the gender. The exclusion criteria encompass those with concurrent autoimmune disorders, chronic inflammatory conditions, those under immunomodulatory or immunosuppressive treatments, individuals vaccinated against or previously infected with COVID-19, pregnant

individuals, and those unable or unwilling to give informed consent. These exclusions are critical for ensuring precise cytokine level measurements and meaningful comparative analyses.

The study excluded the patients that were with history of important neurological injuries or diseases, such as stroke, brain hemorrhage and tumors of brain and similar conditions. These cases may alter cytokine levels that may complicate the relation between IL-6 and the cytokine level of TNF-α as biomarkers and the diseases that were studied. When excluding these patients we want to confirm that our detection are specifically more related to COVID-19 and MS diseases.

2.5 Data Collection

We planned very carefully to collect the data of our study to confirm it is precise and accurate. Information regarding the age, sex, history of the cases, and the lifestyle by using the research forms. The forms were made to put a detailed information at different factors that affected the values of the biomarker IL-6 and the cytokine TNF-α. To make safety and stop the spread of the virus, nasopharyngeal or oropharyngeal sampling were collected in good work from the patients COVID-19 by using the strict safety works.

The collected swab specimens were directly submerged in a medium designed for virus preservation and then placed in a cool environment. during transportation to the laboratory, where they were preserved at -80°C until PCR testing.

Blood samples were drawn from all participants by trained phlebotomists following standard venipuncture protocols. Approximately 5 ml of blood was collected from each individual using sterile techniques to minimize contamination. Samples were transported to the laboratory in a chilled environment. Upon arrival, Serum was the separated the blood cells using centrifugation and subsequently preserved at a suitable temperature of -80°C to maintain the stability of the cytokines until they could be analyzed.

2.6 Diagnostic Assays and Analysis

2.6.1 Quantitative polymerase chain reaction (qPCR) Protocol for COVID-19 Detection

The (qPCR) procedure for COVID-19 detection commenced with the collection of nasopharyngeal or oropharyngeal swabs from suspected COVID-19 patients. Using the Kit of Nucleic Acid Extraction (the company of Zybio, China), which employs the Magnetic Bead Method, RNA was extracted from the samples. This method ensured rapid and efficient isolation of high-quality nucleic acids.

Following extraction, the RNA served as a template for PCR amplification using the Double Gene of SARS-CoV-2 FAST RT-qPCR kit (Bio-Speedy, Turkey). This kit facilitates the reverse transcription of RNA into cDNA and subsequent amplification of specific genes for SARS-CoV-2 in a single step. The process was advanced due to the kit's "FAST" protocol, reducing the overall reaction time. All procedures were done in strict as with the guidelines specified by the company.

Detection was achieved through the kit's fluorescent probes, which bind to target gene sequences, enabling real-time monitoring of the amplification process. The interpretation of results was based on the presence or absence of fluorescence signals, with a threshold cycle (Ct) value established to distinguish between positive and negative outcomes for SARS-CoV-2.

2.6.2 Cytokine measurements:

Serum values of IL-6 and the cytokine of TNF-α were quantitatively determined using the method of sandwiched Enzyme-linked immunosorbent assay (ELISA) tests with commercially available kits (ELK Biotechnology, China). Serum samples were collected and added to microplates pre-coated with antibodies specific to IL-6 and the cytokine of TNF-α. After incubation, all unreacted materials were rinsed off, and enzyme-conjugated secondary antibodies specific to IL-6 and TNF-α were added. After an additional incubation and washing step to eliminate the enzyme-linked antibodies that unbound, a substrate solution was introduced, leading to a color change indicative of the reaction of substrate of enzyme-.

The intensity of the color change, measured using a spectrophotometer, correlates directly with the IL-6 and the cytokine TNF-α concentrations in the samples. To quantify these cytokines, standard curves were created using known concentrations of the cytokine IL-6 and the cytokine TNF-α. This enabled the determination of cytokine levels in patient serum samples by comparing their absorbance values against the standard curve. All these procedures were meticulously executed in accordance with the guidelines of the company.

2.7 Statistical Methods

Participant characteristics and cytokine levels were elegantly distilled through descriptive statistical techniques, showcasing mean values and SD for the persistent variables, alongside percentage counts for variables that as categories. The Mann-Whitney test served as the instrument of choice for juxtaposing serum biomarker levels across mild and severe COVID-19, MS patients, and healthy cohorts. For exploring quantitative relationships, the independent sample t-test was deployed. In instances where the data diverged from normal distribution patterns, nonparametric methodologies such as the Kruskal-Walli’s test were employed, followed by post-hoc examinations to pinpoint significant disparities in mean serum levels. A p-value threshold of less than 0.05 was established as the beacon of statistical significance. The intricate interplay between IL-6 and the biomarker of TNF-α levels and disease intensity in COVID-19 and MS patients was unraveled using Pearson's Correlation test. All statistical voyages were navigated using the robust toolset of the Statistics software IBM SPSS, version 27.

3. Results

3.1 Distribution of the research Groups

Figure number (1) presents a pie chart illustrating the percentage distribution of the different study groups. The COVID-19 patient group consisted of 91 individuals, further divided into 23 (13.5%) with severe COVID-19, 31 (18.2%) with moderate cases of COVID-19, and 37

(21.8%) with the cases of mild COVID-19. Additionally, the study included 29 (17.1%) individuals diagnosed with Multiple Sclerosis (MS), and 50 (29.4%) healthy controls. The pie chart visually conveys the proportional representation of each subgroup within the total study cohort, offering a clear snapshot of the study's demographic composition.

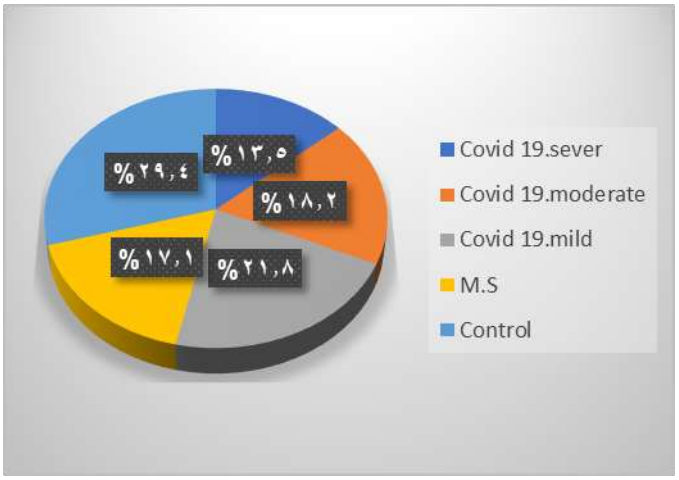

Figure 1: Distribution Percentage of Cases Across Study Groups.

3.2 Demographic Profile of study Groups

In our study, we precisely analyzed the gender and age distribution among the 170 participants. The gender distribution was nearly balanced, with 89 participants (52.4%) identifying as male and 81 participants (47.6%) identifying as female.

The analysis of health data presented in table (1) reveals a gender-differentiated impact of various health conditions, including COVID-19 and Multiple Sclerosis (MS). Females exhibited a higher proportion of mild COVID-19 cases, constituting over a quarter of their total cases (27.16%), compared to males for whom mild cases represented a smaller fraction (16.85%). Conversely, moderate cases of COVID-19 were more prevalent among males, accounting for 22.47% of their cases, versus 13.58% for females. The severe cases of the diseases COVID-19 were slightly more prevalent in males (14.61%) than in females (12.35%). In the context of MS, the data indicated a marginally higher prevalence among males (17.98%) compared to females (16.05%). Control cases were fairly balanced across genders, with females showing a slightly higher percentage (30.86%) relative to males (28.09%). Overall, the total count of cases was higher in males (89) than in females (81), underscoring the necessity for gender-specific considerations in healthcare responses and resource allocation.

Table 1: Gender-Specific Distribution of COVID-19 Severity and MS Cases

Case Type	Female No. (%)	Male No. (%)
COVID 19. Severe	10 (12.35%)	13 (14.61%)
COVID 19 Moderate	11 (13.58%)	20 (22.47%)
COVID 19 Mild	22 (27.16%)	15 (16.85%)
M.S	13 (16.05%)	16 (17.98%)
Controls	25 (30.86%)	25 (28.09%)
Total	**81 (100.00%)**	**89 (100.00%)**

The P-value from the Kruskal-Wallis H test comparing the distribution of cases between males and females is approximately 0.462 (>0.05). This finding indicates that, at the conventional alpha level of 0.05, there is no observation of significant statistically variation in the distribution of COVID-19 severity and MS cases across genders.

Upon analyzing the age distribution, it was observed that the average age of individuals in the COVID-19 cohort was 45.3 years, with a variation of 12.7 years. Meanwhile, the age average of members in the control group was 52.3 years, with a variation of 12.4 years. However, this difference in mean age between the COVID-19 and control groups was not statistically significant, P = 0.316 (>0.05), indicating that age was comparably distributed across both groups.

An insightful examination of the provided health data, as presented in Table (2), discloses key trends in the distribution of COVID-19 severity, MS incidence, and control group representation across different demographics. Notably, males reported a higher incidence of moderate COVID-19 cases (22.47%) compared to females (13.58%), suggesting a possible gender-based disparity in disease experience. Females were more frequently affected by mild COVID-19 cases, which comprised 27.16% of their total cases, emphasizing the variance in disease severity between genders.

The oldest age group (>60 years) exhibited a heightened severity in COVID-19 cases, with 30% experiencing severe symptoms, the highest among all age groups. This underscores the increased vulnerability of the elderly to severe manifestations of the disease.

The proportion of MS cases rose with age, peaking in the 41-60 age group (22.67%), which suggests an age-related increase in MS prevalence. The control group was fairly evenly distributed across the age groups, barring those under 20, indicating a balanced representation of age in the non-affected cohort.

Overall, these findings highlight the critical need for age and gender stratification in public health surveillance and intervention strategies, particularly in the context of COVID-19 severity and MS management. The data further emphasize the amplified intensity of COVID-19 symptoms in the elderly, justifying the need for dedicated protective actions for this population segment.

Table 2: Comparative Analysis of COVID-19 Severity, MS Incidence, and Control Distribution by Gender and Age Group

Gender and Age Groups	COVID-19 No. (%)			MS No. (%)	Controls No. (%)
	Sever	Moderate	Mild		
Males	13 (14.61%)	20 (22.47%)	15 (16.85%)	16 (17.98%)	25 (28.09%)
Females	10 (12.35%)	11 (13.58%)	22 (27.16%)	13 (16.05%)	25 (30.86%)
<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%)

The p-value from the Kruskal-Wallis H test for the age groups is approximately 0.00138 (<0.05). This result indicates that there is important difference in the distribution of case types (COVID-19 severity, MS cases, and control cases) across the different age groups at the typical alpha level of 0.05.

Figure (2) displays a breakdown of COVID-19 case severity within four separate age brackets. It is particularly evident that severe instances are most prevalent among those aged 60 and above, with an alarming 30% of cases in this group being classified as severe. This highlights the heightened vulnerability and consequences of COVID-19 among the elderly demographic. The under-20 age group shows a significant absence of moderate and mild cases, yet it is noteworthy that the few cases present (25%) are severe, which could indicate a low infection rate but a high risk of severe outcomes when infection occurs in this age group.

The age groups 21-40 and 41-60 demonstrate a more balanced distribution across severity levels, with mild cases being slightly more prevalent in the 21-40 age group. This could reflect a better immune response or less exposure to risk factors for more severe disease manifestations among these age demographics.

Overall, the graph presents a clear visual representation of how COVID-19 severity is not uniformly distributed across ages, which may be pivotal for healthcare providers and policymakers in tailoring public health measures and resources to protect the most vulnerable groups effectively.

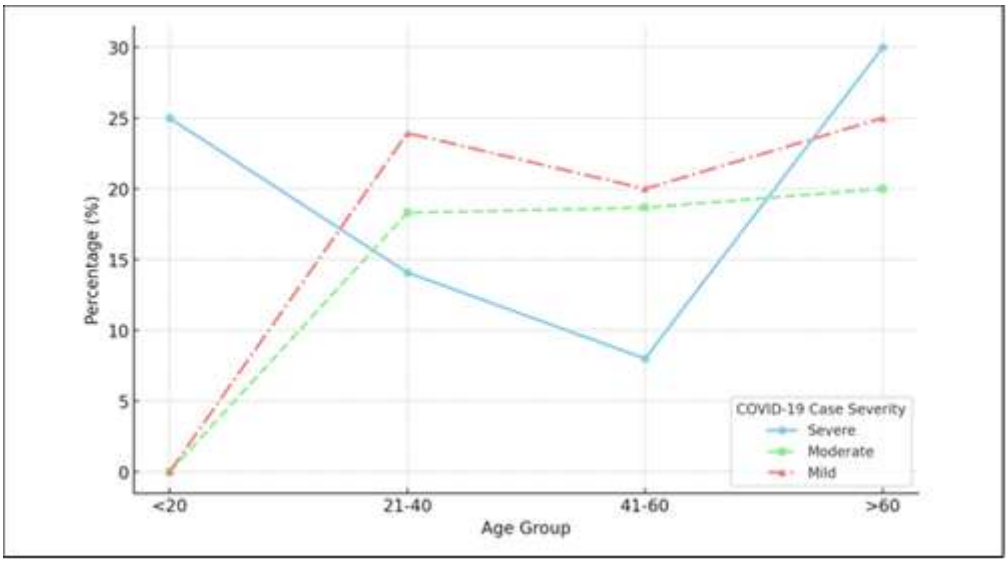

Figure 2: Age-Group Distribution of COVID-19 Case Severity

3.3 Quantity of the IL-6 and cytokine of TNF-α in Serum

The table (3) provides a side-by-side comparison of these biomarkers in COVID-19 patients, those with MS, and a control group. The data reveal that the average IL-6 level in COVID-19 patients is significantly elevated at 22.45 pg/mL, a stark contrast to the control group's 1.74 pg/mL, as evidenced by a statistically significant p-value below 0.001. This indicates a clear relation between high IL-6 values and the infection of COVID-19. Similarly, individuals with MS have higher IL-6 concentrations, averaging 18.20 pg/mL, which is also significantly greater than that of the group of the control, with a p-value below 0.001.

The evaluation of serum TNFα levels reveals a significant elevation in patients with COVID-19 (mean 17.808 pg/mL) when compared to the control group (mean 1.3198 pg/mL), as shown by the highly significant p-value of less than 0.001. This suggests a significant inflammatory response in COVID-19 patients as reflected by TNFα levels. MS patients also displayed elevated TNFα levels (mean 18.144 pg/mL), as designated by the highly significant p-value of less than 0.001. The standard deviation indicates considerable variability among MS patients, suggesting a diverse inflammatory response within this group. The data underscores the role of TNFα as an important marker for inflammation in both COVID-19 and MS, with implications for understanding disease mechanisms and therapeutic targets.

Table 3: Comparative Analysis of IL-6 and the cytokine TNF- α values Across COVID-19, MS, and Control Groups

Parameters	Group	No. of Cases	Levels of Mean (pg/mL)	SD	P-value
IL-6	COVID-19	91	22.45	13.81	

	MS	29	18.20	16.14	<0.001
	Control	50	1.74	2.40	
TNF- α	COVID-19	91	17.808	5.6787	
	MS	29	18.144	13.9841	
	Control	50	1.3198	2.2186	

The Kruskal-Wallis H test yields a p-value of approximately $2.00\times10^{-37}2.00\times10^{-37}$, which is extremely small. This result indicates that there are statistically significant differences in the IL-6 within the group of COVID-19, MS, and control groups, with a strong statistical significance.

Given the magnitude of the p-value, it can be concluded that the median IL-6 values across these groups are not the same, and the observed differences are unlikely to be due to chance. The significantly higher values of IL-6 in the group of COVID-19 infections relative to both the control group and MS patients are particularly striking, pointing to IL-6 as a potential key indicator of inflammation in COVID-19.

The Kruskal-Wallis H test for the TNFα levels across the COVID-19, MS, and control groups yields a p-value of approximately 2.00×10^{-37} 2.00×10^{-37}which is extremely small. This discovery underscores the presence of differences which were significant by using statistical approach in the concentration of the biomarker TNFα among the three study groups, with each distinction holding a high degree of importance.

The elevated TNFα levels in the COVID-19 infected persons compared to the control group, as well as the elevated levels in MS patients, suggest that TNFα is a significant biomarker of the inflammatory response associated with these conditions.

Table (4) represents serum values of the cytokine IL-6 and cytokine TNF across varying severities of COVID-19. While IL-6 levels appear highest in severe cases, the differences are not statistically significant ($p > 0.05$), indicating no clear trend in IL-6 levels as COVID-19 severity increases. TNF levels display a comparable trend, with the highest mean concentrations noted in severe COVID-19 cases. This observation reveals that the variations in TNFα levels between the three groups are not statistically significant, as reflected by a p-value exceeding 0.05. This indicates that any apparent trend towards increased cytokine levels with escalating severity of the disease does not reach statistical significance, suggesting that the variability within each severity group is substantial. Consequently, the observed variations in TNF levels across the severity spectrum cannot be reliably attributed to the severity of the disease alone. The wide confidence intervals reinforce the presence of this substantial variability within the groups.

Table 4: Distribution of Cytokine IL-6 and Cytokine TNF Values by COVID-19 Severity.

Severity Level	No. of Cases	Mean IL-6 Level	SD	Mean TNFα Level	SD	P-value

Severe	23	24.08 ± 12.25	2.56	20.17 ± 6.23	1.30	>0.05
Moderate	31	23.64 ± 13.73	2.47	17.27 ± 4.92	0.88	
Mild	37	20.44 ± 14.86	2.44	16.79 ± 5.64	0.93	
Total	91	22.45 ± 13.81	1.45	17.81 ± 5.68	0.60	

3.4 Pearson correlation coefficients between IL6 & TNF

Table (5) reveals a moderate positive correlation (Pearson correlation coefficient = 0.479) between IL6 and the cytokine level of TNF in COVID-19 patients. The correlation is statistically significant, suggesting that as IL6 levels increase, TNF levels tend to increase as well. Although the reasons cannot be confirmed by relation only, this correlation may have indication for knowing the inflammatory process in COVID patients. So, other research is needed to know the mechanism behind this relation.

Table 5: Pearson Correlation Coefficients between IL6 & TNF

Parameter	IL6	TNF	Sig. (2-tailed)
IL6	1	0.479*	0.01
TNF	0.479*	1	0.01

Discussion:

Our study analyzed IL-6 and the cytokine TNF-α levels in 91 patients with the disease COVID-19, 29 MS patients, and 50 healthy individuals, with balanced gender distribution. Findings showed females had milder COVID-19 cases, while males had more moderate and severe cases and MS. Elderly individuals were more susceptible to severe COVID-19, with a higher MS occurrence in those aged 41-60. Both COVID-19 and MS patients had elevated IL-6 and the cytokine TNF-α values, indicating significant inflammation. However, COVID-19 severity didn't alter cytokine levels, suggesting other factors at play. A notable link in values of the IL-6 and TNF-α was recorded, underscoring their importance in the pathophysiology of the disease.

Numerous research efforts have highlighted gender-specific disparities in the severity of COVID-19. Based on the clinical classification of severity, suggests that men are experiencing severe forms of the disease compared to women(Jin et al., 2020; Mukherjee & Pahan, 2021). A meta-analysis encompassing various studies indicates that being male may forecast a greater severity in COVID-19 infections, yet it does not correlate with an increased risk of death(Ueyama et al., 2020). The comparatively milder impact in females infected with o COVID-19 could be attributed to influences of the gender on how the virus enters cells, the modulation of immune and inflammatory responses during infection, and differences in endothelial and vascular function(Raimondi et al., 2021). Our findings reveal a slight male

predominance in severe COVID-19 cases, in partial agreement with existing studies that suggest men generally experience greater severity. However, this does not extend to mortality, necessitating further research to understand the underlying mechanisms.

In our study participants, Multiple Sclerosis (MS) was identified in 17.1% of individuals, presenting an opportunity to explore its interplay with COVID-19 severity. The distribution of MS across gender and age groups mirrored broader epidemiological trends, with a peak prevalence in the 41-60 age bracket. It was shown that MS itself does not raise the chances of getting COVID-19, but certain factors affect how one responds to the virus. These include taking certain disease-modifying therapies (DMT), having another medical condition, being older than 60, and not being able to move around much. Caregivers and family members of people with MS should also take steps to lower the chances of getting and passing on COVID-19, including taking the vaccine if possible and washing hands often(Arnaldez et al., 2020).

Our research uncovers trends in COVID-19 severity that increase with age, consistent with findings reported in the existing scientific literature(Kang, Jung, & chemotherapy, 2020; Y. Liu et al., 2020). Specifically, we observed that the elevated severity of COVID-19 symptoms was observed in the >60 years of age, with 30% suffering from severe manifestations of the disease, indicating a pronounced vulnerability in this demographic(Unim et al., 2021). According to various studies, there are notable age-related trends in COVID-19 disease severity The highest percentage of COVID-19 disease was observed in persons their ages occur between 20-29 group; wo constituted more than 20% of all verified cases(Boehmer & report, 2020).

While our study did not capture an important number of cases in the 20-29 years age group, our findings indicate the dangerous of non-mild COVID-19 forms increases with age. This is evidenced by the highest severity of COVID-10 symptoms found in individuals over than 60 years of age, with 30% suffering from severe manifestations of the illness. According to our study, it was shown that older individuals have a higher susceptibility to severe cases of COVID(Mueller, McNamara, & Sinclair, 2020).

Our data also detect an increase of MS prevalence in the 41-60 age group. This may suggest an age-related increase in MS incidence, which warrants further investigation to understand its complication(Wandall-Holm, Andersen, Buron, & Magyari, 2022).

Our research results align with the wider body of research on age and COVID-19 severity, which consistently shows that older age is linked to a higher likelihood of severe illness(Pijls et al., 2021). These conclusions reinforce the idea that age is a factor for the severity of disease and highlights the importance of age-specific public health approaches. Such strategies should include focused screening and giving priority to medical interventions for the elderly(Romero Starke et al., 2020).

The results of this study reveal a significant elevation in serum values of IL-6 and the cytokine TNF-α among the patients' cases of COVID-19 compared to the control healthy group, indicating their detectable role in the appearance and appearance of cases(Darif et al., 2021). Our results show that the average of IL-5 level in the COVID-19 cases detected at 22.45 pg/mL,

and TNF-α at 17.808 pg/mL. These figures are considerably higher than those in the healthy group of control, corroborating prior research that noted distinct disparities in these cytokines between COVID-19 affected persons and those that were not with disease(del Valle-Mendoza et al., 2022). As a result, the high values of the IL-6 and the cytokine TNF-α may act as important markers for the intensity of the disease and may play significance role in leading the treatment ways(B. M. Liu, Martins, Peterson, & Hill, 2021).

Multiple Sclerosis cases demonstrated an increase in interleukin-6 (IL-6) levels, averaging 18.20 pg/mL, significantly exceeding the levels in the control group ($p < 0.001$). This elevation in IL-6 among MS patients is consistent with existing literature, emphasizing the pivotal role of cytokines in MS pathology. Cytokines are key players in the MS disease course, from the differentiation of pathogenic T-cells in the periphery to their activity within the central nervous system (CNS)(Palle, Monaghan, Milne, & Wan, 2017). It was detected that IL-6 which is a proinflammatory biomarker, is indicated for its role in activating different protiens occur in the responsiveness of inflammation(Vani, Chitra, & Journal, 2022). Additionally, high serum and cerebrospinal fluid (CSF) levels of TNF-α and IFN-β are linked to the result of the MS exacerbations. The advance of the MS is also marked by changes in cytokine levels(Kallaur et al., 2013).

The Pearson correlation coefficient of 0.479 in our research points to a moderate positive correlation, implying that increases in IL-6 levels are generally accompanied by rises in TNF levels in COVID-19 and MS patients. This coefficient measures the degree of linear relationship between two variables on a scale ranging from -1 to 1, where zero show no correlation, and 1 or -1 denotes an accurate positive or negative linear correlation, respectively(Gogtay & Thatte, 2017). However, it's crucial to recognize that while correlation can reveal associations, it does not confirm causality. Further research is required to explore any causal links between these variables. Using a partial correlation approach could offer a more nuanced understanding by accounting for the effects of additional variables.

The robust correlation observed between COVID-19 and MS in relation to cytokines underscores the significant role these molecules play in driving inflammation. The concurrent elevation in cytokine levels, particularly IL-6 and TNF alpha, indicates a collaborative effort in amplifying the inflammatory response, especially in severe instances of both COVID-19 and MS(Fernandes de Souza, Fonseca, & Sartori, 2023). There is growing evidence suggesting a synergistic interaction between increases in IL-6 and TNF alpha, intensifying inflammation in severe cases of these diseases. Higher levels of IL-6 and TNF alpha have been linked with increased severity and mortality in COVID-19(Del Valle et al., 2020; Halim et al., 2022; Schultheiß et al., 2022). A comprehensive systematic review and meta-analysis encompassing 22 studies highlighted that elevated IL-6 levels are a hallmark of prolonged COVID-19, a condition known for persistent inflammation, autoimmune reactions, and even cancer(Yin et al., 2023). The concept of the IL-6 amplifier, a positive feedback loop between IL-6 and the cytokine TNF alpha, is central to the local initiation model. This model suggests that diseases commence locally through interactions between non-immune and immune cells, triggered by various factors such as infection, injury, obesity, senescence, stressors, and smoking(Hirano, 2021).

The targeted of therapies that done on the cytokines can be a good way to deal with the cytokine storm that detected in severe cases of COVID-19 and to deal the inflammation in the Multiple Sclerosis (MS)(Morgulchik, Athanasopoulou, Chu, Lam, & Kamaly, 2021). The storm cytokine, which is an increased the immune reaction, that occurred in some of COVID-19 patients, that lead to important inflammation and destruction to the tissues (Quirch, Lee, & Rehman, 2020).Various of treatment methods that suggested to control on this storm cases. These include ways that attack directly the virus, reduced the inflammation that it causing, and suppressed the excessive cytokine reactions (Yang et al., 2021). Potential treatments to make regulation of the cytokine levels involved the corticosteroids, devices that taken cytokines, intravenous immunoglobulin, drugs of antimalaria, and IL-6 inhibitors (Tang et al., 2020).A thorough understanding of the cytokine storm in COVID-19 is important to develop the more effective ways to make and controlling the immune-related facts of this and other diseases (Rabaan et al., 2021).

Our research examining the relationship between IL-6, the cytokine TNF, and the severity of COVID-19 and MS. We found that an increased in the cytokine levels corresponded of the patterns of the inflammatory responses in two conditions. Gender and age analyses suggest differentiated disease management, with significant IL-6 and TNF correlations hinting at their joint impact on severity. These findings reinforce the cytokine's potential as therapeutic targets in infectious and autoimmune disorders, underscoring the importance of personalized medicine. Our robust methodological approach strengthens the study's validity, although generalizability is limited by sample-specific characteristics, highlighting the need for further diverse cohort studies.

Conclusions:

Our study elucidates the intricate association between cytokine levels and the intensity of COVID-19 and MS, underscoring the importance of IL-6 and the TNF as biomarkers for inflammatory response. Despite no clear trend in cytokine levels with increasing COVID-19 severity, their correlation suggests a synchronized immune response. These findings hold significant implications for clinical practice, highlighting the need for targeted therapeutic strategies. They also pave the way for policy-making focused on cytokine modulation in patient management. Future research should explore these biomarkers' prognostic utility and their role in personalized medicine for inflammatory diseases.

Recommendations:

Future research should delve into the longitudinal tracking of IL-6 and TNF levels to decipher their prognostic value in COVID-19 and MS progression. There is a need to understand the mechanisms driving cytokine response variations among individuals. Unanswered questions pertain to cytokine roles in long COVID and MS remission. Studies could investigate if modulating these biomarkers alters disease outcomes or if they could guide personalized treatment protocols. Additionally, the impact of demographic factors on cytokine levels warrants deeper exploration to enhance the precision of disease management strategies.

Interest Conflict:

We proclaim that we have no conflicts of interest to divulge.

Financial Disclosure:

No funding was received for this study.

Acknowledgements:

We would like to express our heartfelt appreciation to the dedicated staff of the Laboratory Department at Erbil Central Emergency Hospital for their invaluable support and collaboration in facilitating this study. Additionally, our profound gratitude goes out to the study participants, whose generous contribution of samples made this research possible.

References

1. Abd El Ghaffar, A. S., Mohamed, H. S., Zidan, A. A., Alhadi, W. Y., & Emad, E. M. J. J. o. P. N. R. (2023). General Overview About Multiple Sclerosis. 3607-3613.
2. Al-Malkey, M. K., Al-Sammak, M. A. J. T. m., & disease, i. (2020). Incidence of the COVID-19 in Iraq–Implications for travellers. 38, 101739.
3. Al-Naseria, M. A. S., Salmanb, E. D., & Ad'hiahc, A. H. J. I. (2017). The association between multiple sclerosis and genetic variations in tumor necrosis factor alpha and interleukin-6 genes in a sample of Iraqi patients. 18.
4. Al-Sarray, A. A. M., & Shareef, R. H. J. J. o. T. (2022). Awareness of Healthcare Providers About COVID-19 Vaccinations in Karbala City. 4(Special Issue), 91-96.
5. Arnaldez, F. I., O'Day, S. J., Drake, C. G., Fox, B. A., Fu, B., Urba, W. J., . . . Wigginton, J. M. J. J. f. i. o. c. (2020). The Society for Immunotherapy of Cancer perspective on regulation of interleukin-6 signaling in COVID-19-related systemic inflammatory response. 8(1).
6. Barzinji, A. A., Jaff, H. H., & Ismael, S. A. J. Z. J. o. M. S. (2022). Epidemiology and clinical characteristics of patients after recovery from COVID-19 infection in Erbil government, Iraq. 26(2), 127-136.
7. Beig Parikhani, A., Bazaz, M., Bamehr, H., Fereshteh, S., Amiri, S., Salehi-Vaziri, M., . . . Azadmanesh, K. J. C. m. (2021). The inclusive review on SARS-CoV-2 biology, epidemiology, diagnosis, and potential management options. 78, 1099-1114.
8. Bijoux Leist, J., Leist, T. P. J. H. P., & Medicine, B. (2022). Multiple sclerosis: relationship between locus of control and quality of life in persons with low versus high disability. 10(1), 316-334.
9. Boehmer, T. K. J. M. M., & report, m. w. (2020). Changing age distribution of the COVID-19 pandemic—United States, May–August 2020. 69.
10. Bordon, Y. J. N. R. I. (2023). Hypoxia and IL-24 drive a sterile wound healing pathway. 23(6), 344-344.
11. Cashion, J. M., Young, K. M., & Sutherland, B. A. J. N. o. D. (2023). How does neurovascular unit dysfunction contribute to multiple sclerosis? , 178, 106028.
12. Correale, J., Marrodan, M., & Ysrraelit, M. C. J. B. (2019). Mechanisms of neurodegeneration and axonal dysfunction in progressive multiple sclerosis. 7(1), 14.
13. Darif, D., Hammi, I., Kihel, A., Saik, I. E. I., Guessous, F., & Akarid, K. J. M. p. (2021). The pro-inflammatory cytokines in COVID-19 pathogenesis: What goes wrong? , 153, 104799.
14. del Valle-Mendoza, J., Tarazona-Castro, Y., Merino-Luna, A., Carrillo-Ng, H., Kym, S., Aguilar-Luis, M. A., . . . Peña-Tuesta, I. J. B. i. d. (2022). Comparison of cytokines levels among COVID-19 patients living at sea level and high altitude. 22(1), 96.
15. Del Valle, D. M., Kim-Schulze, S., Huang, H.-H., Beckmann, N. D., Nirenberg, S., Wang, B., . . . Stock, A. J. N. m. (2020). An inflammatory cytokine signature predicts COVID-19 severity and survival. 26(10), 1636-1643.
16. Ellul, M. A., Benjamin, L., Singh, B., Lant, S., Michael, B. D., Easton, A., . . . Solomon, T. J. T. L. N. (2020). Neurological associations of COVID-19. 19(9), 767-783.
17. Fernandes de Souza, W. D., Fonseca, D. M. d., & Sartori, A. J. C. (2023). COVID-19 and Multiple Sclerosis: A Complex Relationship Possibly Aggravated by Low Vitamin D Levels. 12(5), 684.
18. Gogtay, N. J., & Thatte, U. M. J. J. o. t. A. o. P. o. I. (2017). Principles of correlation analysis. 65(3), 78-81.

19. Haidar, M. A., Shakkour, Z., Reslan, M. A., Al-Haj, N., Chamoun, P., Habashy, K., . . . Shaito, A. J. N. R. R. (2022). SARS-CoV-2 involvement in central nervous system tissue damage. 17(6), 1228-1239.
20. Halim, C., Mirza, A. F., & Sari, M. I. J. P. (2022). The association between TNF-α, IL-6, and vitamin D levels and COVID-19 severity and mortality: a systematic review and meta-analysis. 11(2), 195.
21. Han, H., Ma, Q., Li, C., Liu, R., Zhao, L., Wang, W., . . . infections. (2020). Profiling serum cytokines in COVID-19 patients reveals IL-6 and IL-10 are disease severity predictors. 9(1), 1123-1130.
22. Hirano, T. J. I. i. (2021). IL-6 in inflammation, autoimmunity and cancer. 33(3), 127-148.
23. Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y., . . . Gu, X. J. T. l. (2020). Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. 395(10223), 497-506.
24. Ismail, I. I., Al-Hashel, J., Alroughani, R., & Ahmed, S. F. J. N. R. (2021). A case report of multiple sclerosis after COVID-19 infection: causality or coincidence? , 1, 100008.
25. Jin, J.-M., Bai, P., He, W., Wu, F., Liu, X.-F., Han, D.-M., . . . Yang, J.-K. J. F. i. p. h. (2020). Gender differences in patients with COVID-19: focus on severity and mortality. 8, 545030.
26. Kallaur, A. P., Oliveira, S. R., Delicato de Almeida, E. R., Kaminami Morimoto, H., Lopes, J., de Carvalho Jennings Pereira, W. L., . . . Kaimen-Maciel, D. R. J. M. M. R. (2013). Cytokine profile in relapsing-remitting multiple sclerosis patients and the association between progression and activity of the disease. 7(3), 1010-1020.
27. Kang, S.-J., Jung, S. I. J. I., & chemotherapy. (2020). Age-related morbidity and mortality among patients with COVID-19. 52(2), 154.
28. Lai, C.-C., Shih, T.-P., Ko, W.-C., Tang, H.-J., & Hsueh, P.-R. J. I. j. o. a. a. (2020). Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and coronavirus disease-2019 (COVID-19): The epidemic and the challenges. 55(3), 105924.
29. Li, Y. C., Bai, W. Z., & Hashikawa, T. J. J. o. m. v. (2020). The neuroinvasive potential of SARS-CoV2 may play a role in the respiratory failure of COVID-19 patients. 92(6), 552-555.
30. Lima, M., Siokas, V., Aloizou, A.-M., Liampas, I., Mentis, A.-F. A., Tsouris, Z., . . . Bogdanos, D. P. J. C. t. o. i. n. (2020). Unraveling the possible routes of SARS-COV-2 invasion into the central nervous system. 22, 1-15.
31. Liu, B. M., Martins, T. B., Peterson, L. K., & Hill, H. R. J. C. (2021). Clinical significance of measuring serum cytokine levels as inflammatory biomarkers in adult and pediatric COVID-19 cases: A review. 142, 155478.
32. Liu, Y., Mao, B., Liang, S., Yang, J.-W., Lu, H.-W., Chai, Y.-H., . . . Zhao, L. J. E. R. J. (2020). Association between age and clinical characteristics and outcomes of COVID-19. 55(5).
33. MacDougall, M., El-Hajj Sleiman, J., Beauchemin, P., & Rangachari, M. J. F. i. I. (2022). SARS-CoV-2 and multiple sclerosis: potential for disease exacerbation. 13, 871276.
34. Montazersaheb, S., Hosseiniyan Khatibi, S. M., Hejazi, M. S., Tarhriz, V., Farjami, A., Ghasemian Sorbeni, F., . . . Ghasemnejad, T. J. V. j. (2022). COVID-19 infection: an overview on cytokine storm and related interventions. 19(1), 92.
35. Morgulchik, N., Athanasopoulou, F., Chu, E., Lam, Y., & Kamaly, N. J. I. F. (2021). Potential therapeutic approaches for targeted inhibition of inflammatory cytokines following COVID-19 infection-induced cytokine storm. 12(1), 20210006.
36. Mueller, A. L., McNamara, M. S., & Sinclair, D. A. J. A. (2020). Why does COVID-19 disproportionately affect older people? , 12(10), 9959.
37. Mukherjee, S., & Pahan, K. J. J. o. N. P. (2021). Is COVID-19 gender-sensitive? , 16, 38-47.
38. Nath, A., & Smith, B. J. N. L. (2021). Neurological issues during COVID-19: An overview. 742, 135533.
39. Nazzal, A. G., & Sabbar, A. G. J. J. o. T. (2022). Estimation of some biomarkers in recovered COVID19 patients. 4(Special Issue), 21-27.
40. Organization, W. H. (2023). COVID-19 weekly epidemiological update, edition 150, 6 July 2023.
41. Palle, P., Monaghan, K. L., Milne, S. M., & Wan, E. C. J. M. s. (2017). Cytokine signaling in multiple sclerosis and its therapeutic applications. 5(4), 23.
42. Pattanaik, A., Bhandarkar B, S., Lodha, L., & Marate, S. J. A. o. V. (2023). SARS-CoV-2 and the nervous system: current perspectives. 168(6), 171.
43. Pijls, B. G., Jolani, S., Atherley, A., Derckx, R. T., Dijkstra, J. I., Franssen, G. H., . . . Zalpuri, S. J. B. o. (2021). Demographic risk factors for COVID-19 infection, severity, ICU admission and death: a meta-analysis of 59 studies. 11(1), e044640.

44. Qin, C., Zhou, L., Hu, Z., Zhang, S., Yang, S., Tao, Y., . . . Wang, W. J. C. i. d. (2020). Dysregulation of immune response in patients with coronavirus 2019 (COVID-19) in Wuhan, China. 71(15), 762-768.
45. Qudus, M. S., Tian, M., Sirajuddin, S., Liu, S., Afaq, U., Wali, M., . . . Zhang, Q. J. J. o. M. V. (2023). The roles of critical pro-inflammatory cytokines in the drive of cytokine storm during SARS-CoV-2 infection. 95(4), e28751.
46. Quirch, M., Lee, J., & Rehman, S. J. J. o. m. I. r. (2020). Hazards of the cytokine storm and cytokine-targeted therapy in patients with COVID-19. 22(8), e20193.
47. Rabaan, A. A., Al-Ahmed, S. H., Muhammad, J., Khan, A., Sule, A. A., Tirupathi, R., . . . Dhawan, M. J. V. (2021). Role of inflammatory cytokines in COVID-19 patients: A review on molecular mechanisms, immune functions, immunopathology and immunomodulatory drugs to counter cytokine storm. 9(5), 436.
48. Raimondi, F., Novelli, L., Ghirardi, A., Russo, F. M., Pellegrini, D., Biza, R., . . . Amoroso, M. J. B. p. m. (2021). Covid-19 and gender: lower rate but same mortality of severe disease in women—an observational study. 21, 1-11.
49. Romero Starke, K., Petereit-Haack, G., Schubert, M., Kämpf, D., Schliebner, A., Hegewald, J., . . . health, p. (2020). The age-related risk of severe outcomes due to COVID-19 infection: a rapid review, meta-analysis, and meta-regression. 17(16), 5974.
50. Schultheiß, C., Willscher, E., Paschold, L., Gottschick, C., Klee, B., Henkes, S.-S., . . . Frese, T. J. C. R. M. (2022). The IL-1β, IL-6, and TNF cytokine triad is associated with post-acute sequelae of COVID-19. 3(6).
51. Shah, A., Panchal, V., Patel, K., Alimohamed, Z., Kaka, N., Sethi, Y., & Patel, N. J. D.-a.-M. (2023). Pathogenesis and management of multiple sclerosis revisited. 69(9), 101497.
52. Simões, J. L. B., de Araujo, J. B., & Bagatini, M. D. J. M. N. (2021). Anti-inflammatory therapy by cholinergic and purinergic modulation in multiple sclerosis associated with SARS-CoV-2 infection. 58, 5090-5111.
53. Singh, H., Dahiya, N., Yadav, M., Sehrawat, N. J. C. J. o. I. D., & Microbiology, M. (2022). Emergence of SARS-CoV-2 new variants and their clinical significance. 2022.
54. Šiško Markoš, I., Franceschi, M., Vidranski, V., Markoš, P., Jukić, T., Fröbe, A., & Bašić Kes, V. J. A. c. C. (2023). The Concentration of Interleukin 6 and Tumor Necrosis Factor Alpha in Saliva and Blood of Patients with Inactive Multiple Sclerosis And Coexisting Hashimoto's Thyroiditis. 62(2), 339-344.
55. Tang, Y., Liu, J., Zhang, D., Xu, Z., Ji, J., & Wen, C. J. F. i. i. (2020). Cytokine storm in COVID-19: the current evidence and treatment strategies. 11, 1708.
56. Tanzadehpanah, H., Lotfian, E., Avan, A., Saki, S., Nobari, S., Mahmoodian, R., . . . Pharmacotherapy. (2023). Role of SARS-COV-2 and ACE2 in the pathophysiology of peripheral vascular diseases. 166, 115321.
57. Ueyama, H., Kuno, T., Takagi, H., Krishnamoorthy, P., Vengrenyuk, Y., Sharma, S. K., . . . Lerakis, S. J. C. c. e. (2020). Gender difference is associated with severity of coronavirus disease 2019 infection: an insight from a meta-analysis. 2(6), e0148.
58. Unim, B., Palmieri, L., Lo Noce, C., Brusaferro, S., Onder, G. J. A. c., & research, e. (2021). Prevalence of COVID-19-related symptoms by age group. 33, 1145-1147.
59. Vani, P., Chitra, V. J. B., & Journal, P. (2022). The Role of the Proinflammatory and Anti-inflammatory Cytokines in Multiple Sclerosis. 15(1), 137-146.
60. Wandall-Holm, M. F., Andersen, M. A., Buron, M. D., & Magyari, M. J. F. i. N. (2022). Aging with multiple sclerosis: age-related factors and socioeconomic risks. 13, 818652.
61. Yang, L., Xie, X., Tu, Z., Fu, J., Xu, D., Zhou, Y. J. S. t., & therapy, t. (2021). The signal pathways and treatment of cytokine storm in COVID-19. 6(1), 255.
62. Yin, J.-X., Agbana, Y. L., Sun, Z.-S., Fei, S.-W., Zhao, H.-Q., Zhou, X.-N., . . . Kassegne, K. J. I. D. o. P. (2023). Increased interleukin-6 is associated with long COVID-19: a systematic review and meta-analysis. 12(1), 43.