Immunological and Biochemistry Comparative Studies of COVID-19, Vaccinated, and Healthy Individuals

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ABSTRACT

Insufficient information on the immune response to the Severe Acute Respiratory Syndrome Coronavirus 2(SARS-CoV-2), an essential component in developing effective vaccines and therapies, is a critical issue. Consequently, establishing clinical methods for evaluating immune responses to the SARS-CoV-2 virus and validating the direct participation of the parameters in the development of adaptive immune responses are necessary. Furthermore, a comprehensive guide to the diseases caused by the novel SARS-CoV-2 coronavirus is critical. Employing universal and non-specific early cytokines, specifically, interferon-gamma (IFN-γ) in immune clinics during immune response assessments, presents potential in several respects. IFN-gamma promotes the production of other antiviral molecules and stimulates immune system responses against the virus. Nonetheless, reports indicated that some individuals with severe COVID-19 exhibited impaired IFN-gamma production, which might contribute to the severity of the illness. Enhancing IFN-gamma synthesis or exogenous IFN-gamma therapy has demonstrated potential as a COVID-19 treatment approach. Significant IL-6 levels are associated with cytokine storm progression, which denotes excessive and uncontrolled immune responses that could lead to tissue damage. Targeting IL-6 with specific inhibitors, such as monoclonal antibodies, could reduce COVID-19 severity in certain patients. The results obtained in this study indicated that blood electrolytes and immune variable levels (interleukin 6 and IFN-gamma) were significantly different among the infected, vaccinated, and control groups.

1. Introduction

Coronaviruses (CoVs) are found globally and infect various animals. The viruses impart conditions spanning gastrointestinal disturbances, brain inflammation, and nerve damage, with potentially fatal outcomes. Traditionally, human CoVs (hCoVs) were primarily linked to mild upper respiratory and gastrointestinal illnesses. Nevertheless, hCoVs have recently demonstrated the capacity to trigger severe lower respiratory infections, including bronchitis, pneumonia, and the life-threatening acute respiratory distress syndrome (ARDS) (Graham et al., 2013, Santacroce et al., 2021).

The severity of SARS ranges from symptom-free infections to deadly acute ARDS, with exceedingly rare asymptomatic or mild cases. The initial symptoms of the disease are vague and resemble the typical flu. Typically, individuals exposed to the virus exhibit early signs within one to two days post-exposure, such as fever, headache, chills, shivering, general discomfort, and muscle aches (Pormohammad et al., 2020)

Interferon-gamma (IFN-γ) and interleukin 6 (IL-6) are cytokines, which are signalling molecules that promote cellular communication. The cytokines are involved in host defence and tissue repair during viral infections. Nonetheless, excessive and sustained release of the chemicals could result in diseases or death of the infected hosts due to exhaustion of tissue cells and energy utilised for repair. The harmful phenomenon is termed a cytokine storm (Li et al., 2022, Kaur and Ghorai, 2022).

Coronavirus infection could lead to the detrimental release of IFN-γ and IL-6 in some hosts. Consequently, regulating the cytokines during treatments is required to mitigate initiating cytokine storms (Copaescu et al., 2020). Recent research indicated that multiple dietary supplements, including black seeds, oranges, omega-3 and -6 fatty acids, vitamins, such as A, B complex, C, D, and E, and essential minerals, such as copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), sodium (Na), selenium (Se), and zinc (Zn), possess antiviral properties. The supplements demonstrated effectiveness against various respiratory viruses, including CoVs linked to severe acute respiratory syndrome(Islam

et al., 2021, Omer et al., 2022).

Early COVID-19 reports suggested that infected patients exhibited electrolyte irregularities upon admission, such as sodium, potassium, chloride, and calcium. Some studies also indicated that severe COVID-19 cases had greater initial hypokalaemia prevalence than their milder counterparts. Accordingly, electrolyte disruptions are crucial indicators during patient care and for uncovering potential disease mechanisms that could lead to innovative treatment strategies (Huang et al., 2020, Chen et al., 2020).

2. Methodology

Sample collection

The present study involved 35 COVID-19 patients and 35 COVID-19-vaccinated individuals. Another 35 individuals were also employed as the control group. The COVID-19 patients were selected after they had undergone clinical examination by their consultant physicians. They also provided consent. The respondents were between 25 and 55 years old. This study was conducted at general teaching hospitals in Ramadi from December 2023 to February 2024.

Immunological assay

Human IL-6 ELISA kit catalogue number (Cat. No.) EZHIL6/Sigma-Aldrich

Human IFN-γ Cat. No. 430107/BioLegend

Chemicals and materials

No.	Material	Company and origin
1	Electrolyte kit (Ca, K, Na, Mg)	AGAPPE, India

3. Result and Discussion

The distribution of immune variables according to study groups

Based on the results, the INF-γ immune variables recorded by the study groups were notably different ($P < 0.001$). Table 1 summarises the significant variations between the infected, vaccinated, and control groups evaluated.

Table 1: The comparison between the IFN-γ and IL-6 parameters within the study groups

Group	No.	IFN-γ (IU/L)		IL-6 (pg/mL)	
		Mean	SD	Mean	SD
Infected	35	150	5.10	142.62	2.57
Vaccinated	35	110	2.52	82.70	2.71
Normal	35	0.55	2.66	1.76	2.06
P-value		P < 0.001**		P < 0.001**	

The distribution of biochemical variables according to study groups

Table 2 lists the biochemical readings of the infected, the vaccinated, and the control individuals involved in this study. The results indicated statistical differences ($P < 0.001$) between the study groups.

Table 2: The ion parameter comparison between the study groups

Group	No.	Ca (mg/dL)		Na (mg/dL)		K (mg/dL)		Mg (mg/dL)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Infected	35	5.82	0.20	112.40	4.08	2.69	0.30	1.07	0.97
Vaccinated	35	7.98	0.76	147.76	11.33	5.45	0.26	2.95	0.36

Normal	35	9.72	1.66	150.30	10.09	5.86	0.68	3.98	0.76
P-value		P < 0.001***		P < 0.001***		P < 0.001***		P < 0.001***	

Discussion

Several reports recorded electrolyte imbalances among COVID-19 patients. Previous studies also documented that a majority of patients diagnosed with COVID-19 had irregular electrolyte concentrations, including Na, potassium (K), chloride (Cl), and calcium (Ca)(ELHABIBY et al., 2023, Alfano et al., 2021). Electrolyte disturbances are essential indicators for managing COVID-19-affected individuals. The parameters could also aid in uncovering potential pathophysiological mechanisms of the disease, paving the way for innovative therapeutic strategies (Richardson et al., 2020).Consequently, the current study aimed to establish serum electrolyte levels, including calcium (Ca^{2+}), magnesium (Mg^{2+}), potassium (K^{+}), and sodium (Na^{+}), of individuals diagnosed with COVID-19 prior to receiving treatments (Pourfridoni et al., 2021).

Ca is essential for viral fusions in enveloped viruses, including SARS-CoV, MERS-CoV, and Ebola. The chemical engages directly with the fusion peptides of the viruses, facilitating their replication (Cheungpasitporn et al., 2018). Numerous studies have investigated COVID-19 patients' clinical and laboratory features, including inflammation biomarkers and organ injuries. Although hypocalcemia has been documented in several COVID-19 cases at the initial presentation, comprehensive population data on Ca levels in infected individuals are unavailable (Wang et al., 2020, Puig-Domingo et al., 2020, Richardson et al., 2020).Nevertheless, multiple reports have demonstrated a link between hypocalcemia and increased mortality rates and adverse clinical outcomes in critically ill hospitalised patients(Minasi et al., 2023).

During the early stages of the COVID-19 epidemic in Wuhan, substantial hypocalcemia incidences were reported among the critically ill hospitalised patients. The observations suggested that reduced serum Ca levels could be associated with the severity and prognosis of the infection(Torres et al., 2021). In a significant SARS patient cohort in North America, hypocalcemia was detected in 60% and 70% of the patients at admission and during their hospital stay, respectively. A similar trend was reported in Ebola-infected individuals in the United States and Europe(Sanaie et al., 2018, Sun et al., 2020).

Severe and acute hypocalcemia can adversely affect cardiac functions and might be fatal. Ca levels should be routinely evaluated upon initial hospital assessments, considering the notable hypocalcemia incidences in COVID-19 patients, as the information could indicate the severity of the illness and the necessity for hospitalisation. Consequently, monitoring and sustaining appropriate Ca levels in all hospitalised patients are necessary (24).

A potential explanation for hypocalcemia in COVID-19-infected individuals might be the elevated levels of unbound and unsaturated fatty acids detected(Mohammadi et al., 2023). The fatty acids bind to Ca with a significantly favourable enthalpy of −20 kJ/mol, resulting in acute and substantial hypocalcemia(Khatua et al., 2020). Moreover, unbound and unsaturated fatty acids could trigger a cytokine storm, contributing to multiorgan system failure. In severe cases of COVID-19, unsaturated fatty acids might also lead to hypoalbuminemia(Cartin-Ceba et al., 2022, di Filippo et al., 2022).

Data on Mg levels in COVID-19 patients is limited. Nevertheless, an inverse relationship between hypomagnesemia and elevated inflammatory markers, such as IL-6, tumour necrosis factor-α, soluble intracellular adhesion molecule, soluble vascular cell adhesion molecule 1, and C-reactive protein, which are also heightened in COVID-19 patients, might exist. Co-supplementation with magnesium has also been demonstrated to suppress the genes that regulate IL-1 and tumour necrosis factor-α expressions (Costello et al., 2016, Afshar Ebrahimi et al., 2018).

Mg is a Ca-channel blocker, which impedes Ca influx into immunocompetent cells. The action prevents nuclear factor-κB activation, cytokine production, particularly interleukin-6, and systemic inflammation. Consequently, reduced Mg levels due to COVID-19 could result in intensified inflammation triggered by the virus and a cytokine storm (Iotti et al., 2020, Sugimoto et al., 2012).

COVID-19 patients have been documented with hyponatraemia. The condition arises from the increased Angiotensin-Converting Enzyme 2 (ACE2) expression in the proximal tubule. In severe hyponatremia cases, SARS-CoV-2 reportedly induced irregular antidiuretic hormone secretion, resulting in symptoms associated with low sodium levels (Habib et al., 2020).

Osteoporosis is not correlated with an increased COVID-19 infection risk or a more severe form of the disease post-infection. Consequently, osteoporotic individuals are not of higher priority for COVID-19 vaccination than their non-osteoporotic counterparts(Sapra et al., 2022, Creecy et al., 2024). Moreover, osteoporosis treatments do not affect the efficacy or side effects of COVID-19 vaccines and, thus should not be discontinued or postponed indefinitely due to vaccination. Nonetheless, a specific osteoporosis drug requires slight administration timing modifications concerning the COVID-19 vaccination schedule (Tsourdi et al., 2020).

IFN-γ is involved in regulating adaptive immune responses and maintaining immune homeostasis. The cytokine is essential in balancing pro- and anti-inflammatory processes, ensuring effective immune responses without causing excessive tissue damage(Janakiram et al., 2021). Moreover, IFN-γ is crucial in viral clearance due to its potent antiviral activities. Conversely, dysregulated IFN-γ signalling has been established in various autoimmune and inflammatory disorder pathogenesis. Comprehending the dual role of IFN-γ in immune responses is essential to develop targeted therapeutic interventions and immunomodulatory strategies (Green et al., 2017, Baxter and Griffin, 2016).

Current data indicates that in post-viral infections, compromised cells facilitate copious pro-inflammatory cytokines release. The excessive immunologic reaction, termed cytokine storm, induces lung epithelial and microvascular endothelial cell damage. The phenomenon also triggers numerous clinical complications, including tissue ischemia, hypoxia, pneumonia, pulmonary fibrosis, widespread inflammation, elevated blood ferritin levels, and unstable blood flow and pressure, culminating in potential multi-organ failure (Murdaca et al., 2021, Chen and Pan, 2021, Cao, 2020). Nonetheless, varied methodologies employed in in vitro and in vivo investigations might result in inconsistencies. For instance, initial in vitro data on SARS-CoV viruses indicated that interferons (IFNs) could serve as potent external viral replication regulators (King and Sprent, 2021, Ortiz-Prado et al., 2020).

CD4+ T cells can differentiate into Th1 and Tfh cells. Th1 cells produce IFN-γ, while Tfh cells, activate B cells and are crucial in properly functioning neutralising antibodies. Moreover, CD4+ T cells aid CD8+ T cells in infection responses. A notable concentration of specific infected cell-destroying CD8+ T cells has also been associated with a better prognosis for COVID-19 patients (Sette and Crotty, 2021)

COVID-19 patients have been reported with excessive NF-kB activation, which is triggered by the binding of the coronavirus spike protein S to alveolar epithelial cells(Zhao et al., 2023). The reaction up-regulates IL-6 and facilitates its systemic circulation. The process results in alveolar damage and extrapulmonary injuries. Consequently, IL-6 can be employed in assessing respiratory failure and identifying asymptomatic patients as these individuals require vigilant monitoring and early hospital transfer(Qureshi, 2008, Motes et al., 2023).

A range of biomarkers, specifically inflammatory markers, such as C-reactive protein (CRP), ferritin, fibrinogen, D-dimer, and Interleukin 6 (IL-6), are linked to the progression of COVID-19(Hong et al., 2021). Nonetheless, evidence suggests that IL-6 surpasses CRP and other inflammation markers in its ability to predict respiratory failure in COVID-19 patients. Determining necessary antibodies and immune system response duration, including cellular response, is required in further research aiming to improve comprehension of the protective mechanisms, pathogenesis, and prognostic factors of COVID-19. The knowledge is vital for developing effective treatments and vaccines (Grifoni et al., 2020, Dan et al., 2021)

4. Conclusion and future scope

Electrolyte imbalances, including abnormal levels of Na, K, Mg, and Ca, are common among COVID-19 patients and can be crucial indicators for managing the disease,Monitoring and maintaining

appropriate ,Calcium plays a role in the fusion process of enveloped viruses like SARS-CoV-2, and hypocalcemia has been observed in critically ill COVID-19 patients, potentially indicating disease severity. Hypocalcemia in COVID-19 patients may be linked to elevated levels of unbound and unsaturated fatty acids, which can also trigger a cytokine storm and contribute to multiorgan system failure.

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