Microbes and Infectious Diseases 2024; 5(4): 1253-1263

Microbes and Infectious Diseases

Journal homepage: https://mid.journals.ekb.eg/

Original article

Serum levels of tumor necrosis factor alpha and interferon alpha in COVID-19 patients and their correlations with disease severity

Shorouk Alaa El-deen Ibrahim *[1], Basem Elsayed Eysa [2], Mona A. Khattab [3], Ola Ibrahim Ahmed [3]

1- Medical Microbiology & Immunology, Abbasia Fever hospital

2- Tropical Medicine National Hepatology and Tropical Medicine Research Institute

3- Medical Microbiology & Immunology Faculty of Medicine Ain Shams University

ARTICLE INFO

Article history:

Received 4 May 2024

Received in revised form 24 June 2024

Accepted 4 July 2024

Keywords:

severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)

Tumor necrosis factor alpha (TNF-α)

Interferon type I (IFN)

ABSTRACT

Background: By the end of 2019, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was revealed in China and led to a widespread global outbreak and posed a serious threat to public health. The pathophysiology of COVID-19 is heavily reliant on cytokines either beneficial like type I interferon (IFN), or harmful like tumour necrosis factor alpha (TNF-α), considering the so-called cytokine storm. **Aim of the study:** to assess serum levels of TNF-α & IFN-α in COVID-19 patients and their correlations with disease severity. This might add a new aspect in COVID-19 treatment either cytokine and/or anti-cytokine therapy. **Methodology:** The current study was conducted on 60 patients diagnosed as COVID-19, divided into two groups ICU and non ICU (30 patients each) and 30 apparently healthy individuals. Serum levels of TNF-α and IFN-α were measured by ELISA. **Results:** There was a statistical significant difference between COVID-19 patients and control group as regards TNFα and INFα serum levels (P<0.001, P<0.001) respectively. Significant correlation between TNF -α, INF-α serum levels and patients outcome (p =0.000, p =0.000) respectively. **Conclusion**: Serum level of TNF-α is significantly higher in COVID-19 patients compared to controls while serum level of INF α that is significantly higher in controls compared to COVID-19 patients. Their association with disease severity suggests that they can possibly serve as reliable biomarkers for monitoring disease activity and predicting severity and outcome in COVID-19.

Introduction

Positive-sense RNA viruses known as coronaviruses (CoVs) are enclosed and have a distinct replication method, an abnormally large RNA genome, and club-like spikes which project from their surface. (Fehr and Perlman, 2015).

By the end of 2019, a huge universal outbreak was caused by the rise of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), previously known as 2019 novel coronavirus or 2019-n CoV disease in China. The World Health Organization (WHO) confirmed COVID-19 a public health crisis of universal concern, and on March 11th, the WHO Administrator General mentioned COVID-19 as a pandemic. (Liu et al., 2020).

DOI: 10.21608/MID.2024.286615.1931

* *Corresponding author:* Shorouk Alaa eldeen Ibrahim

E-mail address: *shoroukalaa1993@gmail.com*

Numerous studies had discovered that COVID-19 links states of both immunodeficiency and hyper inflammation which is being expressed by cytokine storm (Jamilloux et al., 2020). Thus, cytokine storm may lead to fatal pneumonia, acute respiratory distress syndrome and multi-organ failure (Del Valle et al., 2020). Among these inflammatory cytokines and chemo-kines: IL-6 ,Tumor necrosis factor alpha (TNF-α), interferon-g-induced protein 10 (IP-10), monocyte chemoattractant protein 1 (MCP-1), chemokine ligand 3 (CCL-3) which were significantly associated with disease severity and particularly observed among cases admitted to ICUs. Apparently, the serum levels of many interleukins have the potential to differentiate between mild and severe illness and probably may be used as prognostic markers (Velavan and Meyer, 2020).

One of the main cytokines causing acute phase reaction, TNF-α is a cell signaling molecule implicated in general inflammation. The main cells that produce it are macrophages and monocytes. It is a key cytokine in viral infections and linked to several autoimmune and chronic inflammatory conditions (Hamilton, 2008). Patients with COVID-19 exhibited high levels of TNF-α, which were directly linked to the severity of the illness. (Chen et al., 2020).

Furthermore, in individuals by active rheumatoid arthritis, the anti-TNF therapy causes a swift drop in IL-6 and IL-1 concentrations. Anti-TNF causes vascular endothelial growth factor and adhesion molecules to decrease, which contributes to capillary leak (Taylor et al., 2000). Thus, anti-TNF-α drugs have been suggested as a possible therapy method for COVID-19 illness management, and a randomised study of Adalimumab has been strongly advised for COVID-19 hospitalised patients. (Maggo et al., 2020).

Anti-TNF therapy should be started as soon as feasible for patients with acute respiratory distress syndrome (ARDS) within an average of two days following hospital admission, according to clinical trials conducted on COVID-19 patients. (Huang et al., 2020).

Interferon type I belongs to the group of helpful cytokines, in contrast to the detrimental ones. In fact, type I interferons play a serious role in the antiviral response and the removal of viruses (Crow and Ronnblom, 2019). Consequently, poor results have been linked to delayed IFN response. A mismatch between the pro-inflammatory and pro-repair roles of airway macrophages may result from impaired IFN type I production during severe COVID-19. The lungs of SARS-CoV-dead patients have an overabundance of pro-inflammatory macrophages but a shortage of wound-healing macrophages, which secrete IFN type I (Achary et al., 2020). Anti-SARS-CoV-2 medications such as IFN-α and IFN-β have shown promise. (Sallard et al., 2020). Type-I is most frequently used in vivo in conjunction with other antiviral medications, such as ribavirin or lopinavir/ritonavir (Omrani et al., 2014). IFN-α and -β have consistently shown strong activity against coronaviruses in vitro. (Arabi et al., 2020). Numerous investigations revealed that, due to potential tissue damage, type-I IFN must be given as soon as feasible following infection—but not in the latter stages. (Siddiqi and Mehra, 2020).

Aim of the study:

to assess serum levels of TNF-α & IFN-α in COVID-19 patients and their correlations with disease severity. This might add a new aspects in COVID-19 treatment either cytokine and/or anticytokine therapy.

Patients & Methods:

The current study was directed on 60 patients diagnosed as COVID-19 and 30 apparently healthy individuals as controls. SARS-CoV-2 RNA was detected in those patients through nasopharyngeal swabs, confirming their diagnosis. Patients were selected from the National hepatology and tropical medicine research institute & infectious diseases (previously Imbaba fever hospital) from July 2020 to march 2021. An informed consent was taken from all patients. This study was approved by the ethics committee of Ain Shams University's Faculty of Medicine. (Consent number MS 697/2020). Patients were divided into 2 groups: Group Ⅰ: ICU group (30 COVID 19 patients admitted in ICU). Group Ⅱ: Non ICU group (30 COVID 19 patients not hospitalized in ICU). ICU admission was performed according to the protocol of Egyptian ministry of health protocol to patients with respiratory rate more than 30**,** oxygen saturation less than 92 at room air, Po_2/Fio_2 ratio is less than 200 despite oxygen therapy and/or additional oxygen dysfunction. Inclusion criteria: Clinically and radiologically diagnosed COVID-19 patients and laboratory confirmed by positive SARS-CoV-2 RT-PCR testing of respiratory sample (nasopharyngeal swab or invasive respiratory

sample). Exclusion criteria: patients receiving anti-TNFα antibody, corticosteroids, immunosuppressive therapy, chemotherapy, patients with immunological disorders and/ or malignancy.

Blood samples from all patients and controls were collected for TNF α &INF α serum levels measurements. Within 24 hours of admission, samples were collected from each patient. One mL of peripheral venous blood from patients and controls was collected in a serum separation tube under strict aseptic conditions and allowed to clot for 10-20 minutes at room temperature before centrifugation at 2000–3000 rpm for 20 minutes. The levels of INFα and TNFα in the serum were assessed using a sandwich Enzyme Linked Immuno-Sorbent Assay (Andrea B et al., 1997) (Markham R et al. ,1995) by using human Interferon alpha (INFα) ELISA kit (Bioassay Technology Laboratory, Cat.No E0076 Hu,China) and human Tumor Necrosis Factor alpha (TNFα) ELISA kit (Bioassay Technology Laboratory, Cat.No E0082 Hu,China).

A standard curve was created with each assay by plotting the average optical density for each standard on the Y-axis of the curve; measured using Digital and analog systems (das) plate reader, versus the corresponding standard values on the X-axis, obtained by serially diluting the standard stock solution: In INFα 1280 pg/ml to get concentrations of 640 pg/ml, 320 pg/ml, 160 pg/ml, 80pg/ml, and 40 pg/ml. while in TNFα 960ng/L to get concentrations of 480ng/L, 240ng/L, 120ng/L, 60ng/L, and 30ng/L. The concentration of INFα and TNFα in the tested samples was determined by interpolation from the standard curve and expressed in pg/ ml for INFα and in ng/L in TNFα.

Analytical statistics:

All statistical analyses were performed using IBM SPSS (Statistical Package for the Social Sciences). version 26.0. Quantitative variables were presented as medians or mean ± standard deviation (SD), whereas the qualitative variables were described as numbers and percentages. Results were analyzed using unpaired student's t-test; Mann-Whitney U test, Chi square test and correlation coefficient (r) test. For all the used analyses, a probability (p) value of less than 0.05 was considered significant.

RESULTS

The current study included 60 COVID-19 patients (31 men and 29 females), ranging in age from 29 to 78 years, with a mean age of (53.25±11.40). Also thirty obviously healthy controls (15 males and 15 females) were enrolled in the study, ranging in age from 29 to 68 years, with a mean age of (48.90 ± 11.51).

In this study we found that the most common complaint was fever, which was reported by 78.3% of patients, followed by respiratory symptoms such as cough and dyspnea, which were reported by 75.0% of patients.

66.7% of patients had hypertension, 58.3% diabetes, 30.0% cardiovascular disease, 15.0% renal problems, and 15.0% hepatic disorders.

Table (2) shows that there was statistically increase in CRP & Lymphoctes percentage in ICU patients than non ICU & control group with p-value < 0.01, while there is no statistical difference between ICU patients, Non-ICU patients and control group as regards ALT and AST and there is no statistical difference between ICU patients and Non-ICU patients as regards creatinine.

Table (3) shows that 43.3% of Non-ICU patients recovered & clinically improved, while 26.7% of them were transferred to the ICU. However, all ICU patients died as they arrived to the hospital in very late condition and almost all of them were complaining of respiratory failure on their ICU admission.

Figures (1 and 2) show a significant difference between COVID-19 patients and control groups as regards TNFα in COVID-19 patients (median, IQR= 126, 103-210) and healthy controls (median, IQR=60, 45-75) (P<0.001).INFα in COVID-19 patients (median, IQR= 70, 40-97.5) and healthy controls (median, IQR=220, 200-300) (P<0.001).

Figures (3 and 4): show statistically significant difference between ICU &Non-ICU COVID-19 patients and control group as regards:

TNFα: ICU COVID-19 patients (median, IQR= 210, 175-240), Non-ICU COVID-19 patients (median, IQR= 105, 95-115) and healthy controls (median, IQR=60, 45-75) (P<0.001).

INFα: ICU COVID-19 patients (median, IQR= 40, 15-60), Non-ICU COVID-19 patients (median, IQR= 90, 80-100) and healthy controls (median, IQR=220, 200-300) (P<0.001).

Table (4) reveals no significant correlation between TNF α and ALT and AST , while shows highly significant correlation between TNF α, CRP, lymphocytes and creatinine (r =0.717, p

=0.000, r =0.717**,** p =0.000, r =0.717**,** p =0.000) in all patients respectively. While in ICU patients there is a significant correlation between TNF α, lymphocytes and CRP (r =0.669**,** p =0.000, r=0.433, p=0.017) respectively**,** while in Non ICU patients there is a significant correlation between TNF α and lymphocytes only (r=0.399, p=0.039).

The table reveals no statistical significant correlation between INF α and ALT and AST, while shows high statistical significant correlation between INF α and CRP (r =0.632**,** p =0.000) ,lymphocytes (r =0.647**,** p =0.000) and creatinine (r =0.288**,** p =0.039) in all patients.

Table (5) shows a highly statistical significant correlation between TNF α and outcome (p =0.000), as increasing serum levels of TNF α is correlated with bad outcome (death and ICU entery) more than recovery.

The table shows a highly statistically significant correlation between INF α and outcome (p =0.000), as increasing serum levels of INF α is correlated with recovery more than bad outcome (death and ICU entery).

Table 1. Demographic data of patients

Demographic and clinical data	
Sex *Males, n (%); Females, n (%)*	31 (52%); 29 (48%)
Age Mean ± SD	53.25±11.40
Co-morbidities within cases *Diabetes mellitus; Hypertension; Chronic heart dseases; Renal disorders; hepatic disorders*	35 (58.3%),40 (66.7%), 18 (30.0%),9 (15.0%),9 (15.0%),

Table 2. Comparison between Non-ICU patients , ICU patients and control group as regarding laboratory data:

		Control group	Non-ICU patients	ICU patients	Test value	P-value	Sig.
		No. = 30	No. = 30	No. = 30			
CRP latex	Median (IQR) Range	6 (6 – 6) 6 – 24	24 (12 – 24) 6 – 96	96 (48 – 192) 12 – 384	66.313‡‡	0.000	HS
ALT	Median (IQR) Range	125 (22 – 305) 15 – 375	45 (35 – 55) 20 – 355	45 (40 – 70) 25 – 450	1.236‡‡	0.539	NS
AST	Median (IQR) Range	105 (20 – 225) 10 – 325	35 (20 – 45) 10 – 215	37.5 (25 – 55) 10 – 330	1.353‡‡	0.508	NS
Creatinine	Median (IQR) Range	No available data	0.75 (0.65 – 1.1) 0.6 – 2.1	1.1 (0.75 – 1.8) 0.6 – 6.1	-1.898‡	0.058	NS
Lymphocytes %	Median (IQR) Range	33 (30 – 37) 28 – 45	16.5 (15.2 – 18.4) 11 – 33	6.9 (5.6 – 10.5) 5.2 – 22.5	58.622‡‡	0.000	HS

P-value >0.05: Non significant (NS); P-value <0.05: Significant (S); P-value< 0.01: highly significant (HS)
‡: Mann Whitney test; ‡‡: Kruskal Wallis test
CRP C reactive proteins; LDH lactate dehydrogenase; ALT alanine aminotransferase; AST aspartate aminotransferase.

Table 3. Clinical outcome of both ICU & Non-ICU COVID 19 patients:

Outcome	Non-ICU patients		ICU patients		Test value	P-value	Sig.
	No.	%	No.	%			
Recovery	13	43.3%	0	0.0%	60.000	0.000	HS
ICU	8	26.7%	0	0.0%			
Death	0	0.0%	30	100.0%			
NA	9	30.0%	0	0.0%			

Table 4. Correlation for TNF α and INF α with variable laboratory parameters.

	TNF α						INF α					
	All patients		Non-ICU patients		ICU patients		All patients		Non-ICU patients		ICU patients	
	r	P-value	r	P-value	r	P-value	r	P-value	r	P-value	r	P-value
CRP latex	0.717**	0.000	-0.080	0.674	0.433*	0.017	-0.632**	0.000	0.333	0.072	-0.323	0.082
ALT	0.214	0.118	-0.104	0.620	0.172	0.364	-0.247	0.069	0.096	0.648	-0.330	0.075
AST	0.160	0.243	-0.125	0.551	0.140	0.462	-0.193	0.158	0.128	0.542	-0.297	0.111
Creatinine	0.294*	0.034	0.060	0.790	0.169	0.373	-0.288*	0.039	0.178	0.429	-0.280	0.133
Lymphocytes %	-0.717**	0.000	0.399*	0.039	-0.669**	0.000	0.647**	0.000	-0.134	0.506	0.260	0.166

Table 5. Correlation for TNF α with outcome of patients:

All patients		TNF α					INF α				
		Median (IQR)	Range	Test value	P-value	Sig.	Median (IQR)	Range	Test value	P-value	Sig.
Outcome	Recovery	110 (103 – 122)	85 – 130	29.773‡‡	0.000	HS	95 (75 – 100)	70 – 110	26.963 ‡‡	0.000	HS
	ICU	107.5 (99 – 110)	90 – 122				92.5 (82.5 – 102.5)	70 – 110			
	Death	210 (175 – 240)	40 – 285				40 (15 – 60)	5 – 320			
	NA	92 (88 – 95)	80 – 135				85 (80 – 95)	20 – 110			

P-value >0.05: Non significant (NS); P-value <0.05: Significant (S); P-value< 0.01: highly significant (HS)
‡: Mann Whitney test; ‡‡: Kruskal Wallis test

Figure 1. Comparison between control and patients groups as regards TNFα.

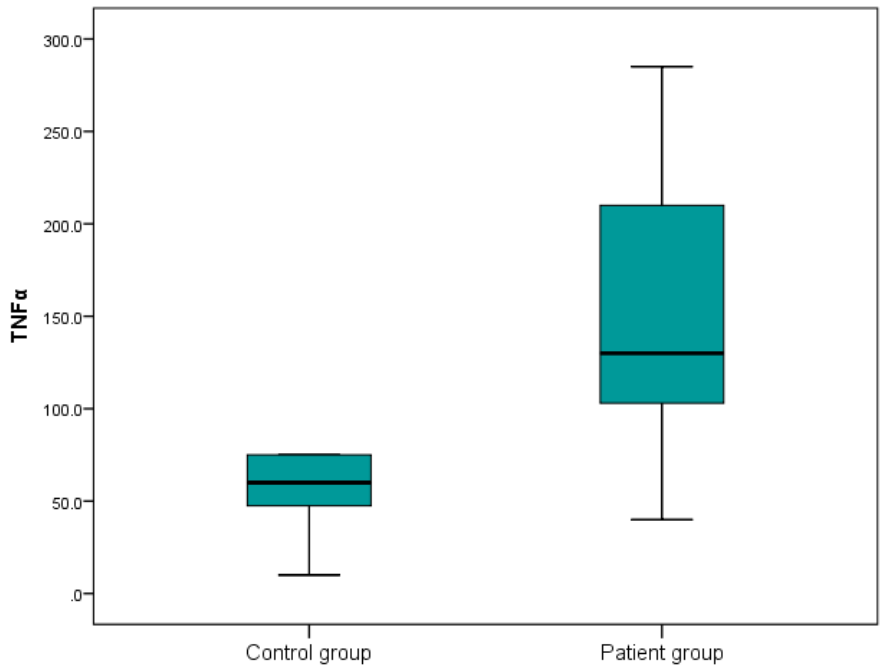


Figure 2. Comparison between control and patients group as regards INFα.

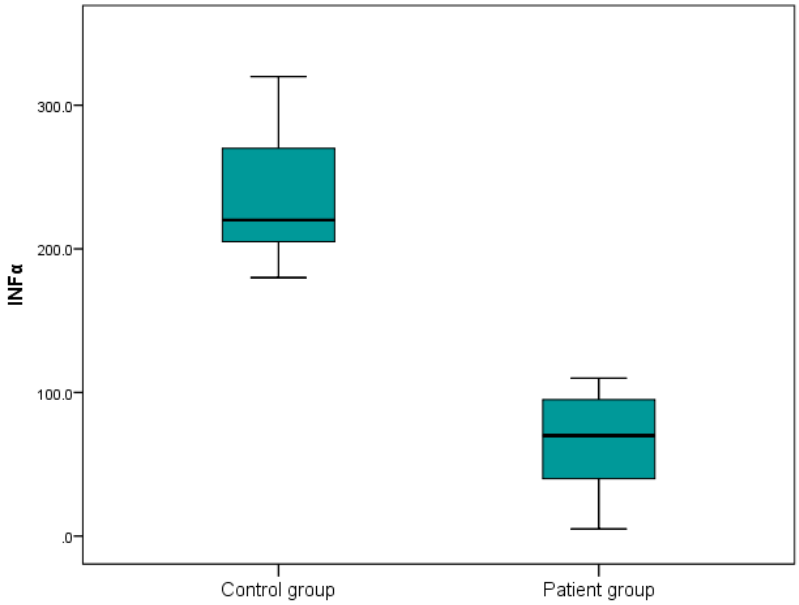


Figure 3. Comparison between control, ICU and Non-ICU patients groups as regards TNFα.

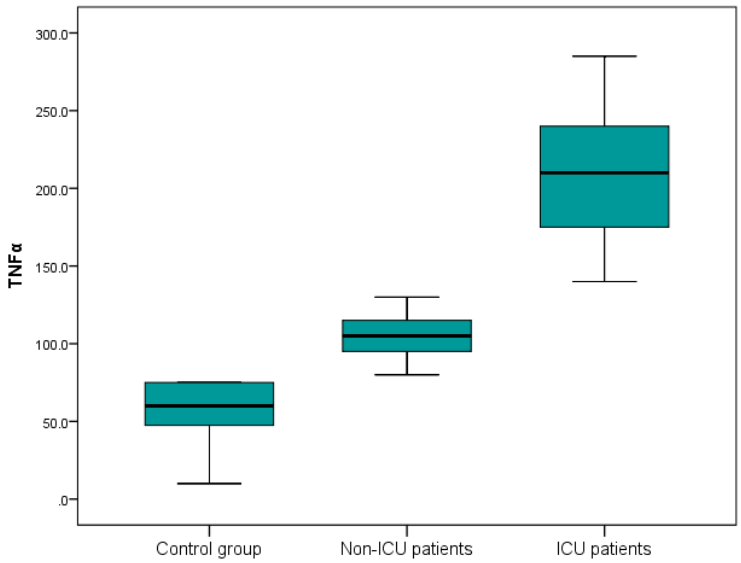
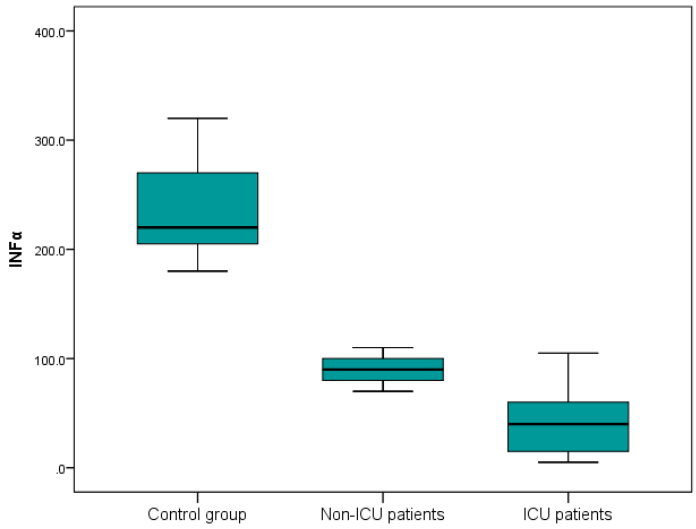


Figure 4. Comparison between control, ICU and Non-ICU patients groups as regards INFα.



Discussion

This study aims to assess serum levels of TNF-α & IFN-α in COVID-19 patients and their correlations with disease severity. This might add a new aspects in COVID-19 treatment either cytokine and/or anti-cytokine therapy.

The current study revealed that there was statistically increase in mean age of ICU patients than non ICU with p-value < 0.001 while there was no statistically significant variance between groups regarding gender of subjects with p-value = 0.957.

In a study by ***Nachtigall et al. (2021),*** a group of 23,235 patients from 83 hospitals with PCR-confirmed infection with SARS-CoV-2 between 4 February 2020 and 22 March 2021 were included. On average, males were about 2.2 years younger than females (mean ± SD = males 67.9 ± 17.4, females 70.1 ± 19.8, *p*-value < 0.0001).

The current study reported that there was statistically increase in number of patients complaining with fever, chest symptoms and GIT symptoms in ICU patients than non ICU. 70.0% of Non-ICU patients had fever while 86.7% of ICU patients had fever, 50.0% of Non-ICU patients had chest symptoms while 100.0% of ICU patients had chest symptoms and 26.7% of Non-ICU patients had GIT symptoms while 33.3% of ICU patients had GIT symptoms.

Nachtigall et al. (2021) showed that at hospital admission, more COVID-19 symptoms were documented for male than female patients i.e., fever (34.9% vs. 26.5%; OR = 1.55, 95% CI = 1.45–1.65), cough (26.1% vs. 22.5%, OR = 1.24, 95% CI = 1.16–1.33) or dyspnoea (29.9% vs. 22.9%, OR = 1.47, 95% = 1.37–1.57). Patients admitted to the ICU at any time during their hospital stay presented more often with fever (34.5% vs. 29.5%; OR = 1.29; 95% CI = 1.20–1.39) or dyspnoea (35.8% vs. 23.1%; OR = 2.02; 95% CI = 1.88–2.18) at admission compared to non-ICU-patients, again with the same male preponderance.

This study illustrated that there was statistically increase in number of patients complaining with hypertension & cardiovascular diseases in ICU patients than non ICU with p-value < 0.01, while there is statistically increase in number of patients had diabetes mellitus in ICU patients than non ICU with p-value < 0.05, while there is no statistically difference between ICU patients and Non-ICU patients regarding renal and hepatic disorders.

In a study by **Nachtigall et al. (2021),** males had a higher prevalence of comorbidities as measured by the Elixhauser Comorbidity Index (mean ± SD = males 11.7 ± 11.8, females 10.9 ± 11.3, *p*-value < 0.0001). The comorbidity index was higher in ICU than in non-ICU patients (16.0 ± 12.3 vs. 9.6 ± 10.8, $p < 0.0001$) and highest in ventilated ICU patients (17.0 ± 12.3, $p < 0.0001$).

The current study showed that there was statistically increase in CRP & lymphocytes percentage in ICU patients than non ICU & control group with p-value < 0.01, while there is no statistically difference between ICU patients, Non-ICU patients and control group regarding ALT, AST &creatinine.

Saleh et al. (2022) revealed which the absolute leucocytic counts (soft loving care) D-dimer levels are fundamentally higher in the extreme gathering contrasted with the mild direct ones (p-values=0.022 zero.017, one by one). The lymphocytic counts, serum ferritin CRP middle ranges showed genuinely magnificent contrasts between the 3 gatherings.

The current study reported that 43.3% of Non-ICU patients recovered & clinically improved, while 26.7% of them were transferred to the ICU. Whatever all ICU patients died as they arrived to our hospital in very late condition and almost all of them were complaining of respiratory failure on their ICU admittion.

In total, **Nachtigall et al. (2021)** reported that 21.1% of all patients admitted to a hospital with SARS-CoV-2 died, 47.0% of them had been treated in an ICU. The risk of death was higher for males than females (OR = 1.31, 95% CI = 1.22–1.40); higher risk for males was also observed among ICU and non-ICU-patients. Mortality was highest among ICU patients who had been ventilated (58.4%) compared to all ICU patients (41.1%, $p < 0.0001$) and to non-ICU patients (14.8%, $p < 0.0001$) with no significant difference between sexes in the ventilated group.

The current study demonstrated statistical significant difference between COVID-19 patients and control group as regarding: TNFα: COVID-19 patients (median, IQR= 126, 103-210) and healthy controls (median, IQR=60, 45-75) (P<0.001). This study showed statistical significant difference between ICU &Non-ICU COVID-19 patients and control group as regarding: TNFα (median, IQR=210 ,175 – 240, =105 (95 – 115) respectively.

Saleh et al. (2022) aimed to evaluate the serum levels of TNF-α in COVID-19 patients and to explore their potential relation with disease severity. In COVID-19 patients, the median serum levels of TNF-α (77.95pg/mL) were significantly higher compared to controls (0.40 pg/mL) (p-values <0.001). and were found to be generally higher with more disease severity.

In a study by ***del Valle-Mendoza et al. (2022),*** a total of 35 COVID-19 patients and 10 healthy subjects were recruited from each study site. It can be observed that the mean levels of IL-6 and TNF-α were significantly different among the study groups. A comparison of the inflammatory cytokines in healthy subjects from the two study sites, showed significant differences only in levels of TNF-α.

Alabd et al. (2021) aimed to investigate the association between TNF-α serum levels and mild COVID-19 infection. Their study reveals that serum TNF-α levels for mild COVID-19 patients and healthy control people were a nonsignificant p-value of 0.1191 between the two groups.

Zheng et al. found no statistical differences in TNF-α and IL-6 plasma levels among the three groups of their study, which was also divided according to severity. They also reported decreased cases in IFN-γ serum levels in severe cases

Mortaz et al. detected an association between mortality of COVID-19 patients in the ICU and elevated serum levels of soluble TNF-α receptors.

Merza et al. reported that the mean TNF-_ levels were not significantly higher in patients with severe COVID-19 compared with non-severe COVID-19 patients

Saleh et al. (2022) reported that as for TNF-α, it was significantly higher in patients with severe illness compared to the mild group (p-value <0.001) and although levels were still higher in severe disease compared to the moderate group and in the moderate compared to the mild but values were statistically insignificant (p values=0.299 and 0.062, respectively).

Contoli et al. (2021) aimed to evaluate the interferon (IFN)-α levels in a cohort of COVID-19 patients in relation to severity, evolution of the clinical manifestations and immune/inflammatory profile. Fifty-four COVD-19 and 11 control patients matched for severity were enrolled. At recruitment, lower levels of blood IFN-α were found in COVID-19 patients compared to controls (3.8-fold difference, $p < 0.01$).

The current study revealed that there is no statistically significant correlation between TNF α and ALT and AST**,** while shows highly statistically significant between TNF α and CRP and lymphocytes in all patients and between TNF α and lymphocytes only in ICU patients**,** while shows statistically significance between TNF α and creatinine in all patients, lymphocytes in Non-ICU patients and CRP in ICU patients. This study revealed no statistically significant correlation of TNF α with symptoms except chest symptoms that revealed highly statistically significance and shows no statistically significance between TNF α and various comorbidities, while shows a highly statistically significant correlation between TNF α and outcome.

Saleh et al. (2022) reported that significant positive correlations between TNF-α levels with serum ferritin (p=0.008) and CRP (p=0.009) and inversely with lymphocytic counts (p=0.001) were observed. No significant relations were observed with the following parameters: TLC (p=0.166), D dimer (p=0.09), LDH (p=0.132), ALT (p=0.827) and PCT (p=0.757).

Han et al. (2020) showed that CRP was significantly positively correlated with IL-10 while ***Taghiloo et al. (2020)*** reported that CRP levels positively correlated with the levels of TNF-α but not IL-10. They also reported inverse correlation of TNF-α with the lymphocyte counts in COVID19 patients. It is noteworthy that no significant correlations were detected with either TNF-α or IL-10 with the TLC, LDH and PCT levels in COVID-19 patients in the present study.

The current study revealed that there is no statistically significant correlation between INF α and ALT and AST**,** while shows highly statistically significant between INF α and CRP and lymphocytes in all patients while shows statistically significance between INF α and creatinine in all patients. This study revealed no statistically significant correlation of INF α with symptoms and shows no statistically significance between INF α and various comorbidities except hypertension that revealed statistically significance while shows a highly statistically significant correlation between INF α and outcome.

Conclusion

Serum level of TNF-α is significantly higher in COVID-19 patients compared to controls while serum level of INF α that is significantly higher in controls compared to COVID-19 patients. Their association with disease severity suggests that they can possibly serve as reliable biomarkers for monitoring disease activity and predicting severity and outcome in COVID-19.

The current study highlights the role played by TNF-α and INF α in the immune pathogenesis of COVID-19 and the development of the COVID-19 associated cytokine storm. Better understanding of the cytokine response pattern in COVID-19 would contribute to the improved development of more effective immunomodulatory therapies for COVID-19 in the future.

Conflict of interests

The authors report no conflicts of interest.

Funding

The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

References

1- Acharya D., Liu G.Q., Goak M.U (2020): Dysregulation of type I interferon responses in COVID-19. Nature reviews | Immunology;20: 396-397.

2- Alabd et al. (2021) aimed to investigate the association between TNF-α serum levels and mild COVID-19 infection. Their study reveals that serum TNF-α levels for mild COVID-19 patients and healthy control people were a nonsignificant p-value of 0.1191 between the two groups.

3- Arabi Y.M., Shalhoub S., Mandourah Y., Al-Hameed F., Omari A., Al Qasim E., et al. (2020): Ribavirin and interferon therapy for critically ill patients with Middle East Respiratory Syndrome :a multicenter observational study. Clin Infect Dis.70:1837–1844..

4- Chen G., Wu D., Guo W., Cao Y., Huang D., Wang H., et al. (2020): Clinical and immunologic features in severe and moderate Coronavirus Disease 2019 . J Clin Invest.130 (5):2620-2629.

5- Contoli M, Papi A, Tomassetti L, Rizzo P, Vieceli Dalla Sega F, Fortini F, Torsani F, Morandi L, Ronzoni L, Zucchetti O, Pavasini R, Fogagnolo A, Volta CA, Bartlett NW, Johnston SL, Spadaro S, Campo G. Blood Interferon-α Levels and Severity, Outcomes, and Inflammatory Profiles in Hospitalized COVID-19 Patients. Front Immunol. 2021 Mar 9;12:648004. doi: 10.3389/fimmu.2021.648004. PMID: 33767713; PMCID: PMC7985458.

6- Crow M. K and Ronnblom L. (2019): Type I interferons in host defense and inflammatory diseases. Lupus Sci Med 6(1): 1-10.

7- Del Valle D.M., Schulze S.K., Huang H.H., et al. (2020): An inflammatory cytokine signature helps predict COVID-19 severity and death. medRxiv.1: 1-5.

8- del Valle-Mendoza J, Tarazona-Castro Y, Merino-Luna A, Carrillo-Ng H, Kym S, Aguilar-Luis MA, Del Valle LJ, Aquino-Ortega R, Martins-Luna J, Peña-Tuesta I, Silva-Caso W. Comparison of cytokines levels among COVID-19 patients living at sea level and high altitude. BMC infectious diseases. 2022 Jan 28;22(1):96. doi: 10.1055/s-0040-1712187.

9- Fehr A.R and Perlman S. (2015): Coronaviruses: an overview of their replication and pathogenesis. Methods Mol. Biol. 1282: 1–23.

10- Hamilton J.A. (2008): Colony stimulating factors in inflammation and autoimmunity, Nat. Rev. Immunol.8: 533–544.

11- Huang C., Wang Y., Li X., Ren L., Zhao J., Hu Y., Zhang L., Fan G., Xu J., Gu X., Cheng Z., Yu T., Xia J., Wei Y., Wu W., Xie X., Yin W., Li H ., Liu M., Xiao Y., Gao H., Guo L., Xie J., Wang G., Jiang R., Gao Z., Jin Q., Wang J., Cao B. (2020): Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China, Lancet 395 :497–506.

12- Jamilloux Y.V., Henry T., Belotb A, Vielb S., Fautera M ., El Jammala T, Walzer T., Françoise B., Sèvea P. (2020): Should we stimulate or suppress immune responses in COVID-19? Cytokine and anti-cytokine interventions. Autoimmunity reviews.19 (7):1-14.

13- Ketenci S, Saraçoğlu İ, Duranay R, Elgörmüş ÇS, Aynacıoğlu AŞ. Retrospective analysis of biochemical markers in COVID-19 intensive care unit patients. Egypt J Bronchol. 2022;16(1):27. doi: 10.1186/s43168-022-00129-7. Epub 2022 May 13. PMCID: PMC9100315.

14- Liu Y., Gayle A.A., Wilder-Smith A., Rocklöv J. (2020): The reproductive number of COVID-19 is higher compared to SARS coronavirus, J.TravelMed.27(2).1-4.

15- Liu Y., Zhang C., Huang F., Yang Y., Wang F., Yuan J., Zhang Z., Qin Y., Li X., Zhao D., Li S., Tan S., Wang Z., Li J., Shen C., Li J., Peng L., Wu W., Cao M., Xing L., Xu Z., Chen L., Zhou C., Liu WJ., Liu L., Jiang C.(2020): Elevated plasma level of selective cytokines in COVID-19 patients reflect viral load and lung injury. National Science Review.6:1003-1011.

16- Maggo S., Dhull P., Dubey A.P., Brashier D., Karan A., Singh N.K., Josh K. (2020): Cytokine Storm Syndrome in COVID-19: Diagnosis and Management Strategies. International Journal of Health Sciences and Research 6:140-149.

17- Merza MY, Hwaiz RA, Hamad BK, Mohammad KA, Hama HA, Karim AY. Analysis of cytokines in SARS-CoV-2 or COVID-19 patients in Erbil city, Kurdistan Region of Iraq. Plos one. 2021 Apr 29;16(4):e0250330.

18- Nachtigall I, Bonsignore M, Thürmann P, Hohenstein S, Jóźwiak K, Hauptmann M, Eifert S, Dengler J, Bollmann A, Groesdonk HV, Kuhlen R, Meier-Hellmann A. Sex Differences in Clinical Course and Intensive Care Unit Admission in a National Cohort of Hospitalized Patients with COVID-19. J Clin Med. 2021 Oct 26;10(21):4954.

19- Omrani A.S., Saad M. M., Baig K., Bahloul A., Abdul-Matin M., Alaidaroos A.Y., et al. (2014): Ribavirin and interferon alfa 2 for severe Middle East respiratory syndrome corona virus infection: a retrospective cohort study. Lancet Infect Dis: 14:71066-71070.

20- SALEH MA, TAMIM HH, MARAWAN M, SAMEH A, SELIM M. TNF-a and IL-10 Serum Levels in COVID-19 Patients and their Relation to Disease Severity. The Medical Journal of Cairo University. 2022 Sep 1;90(9):1459-67.

21- Sallard E., Lescure F .X., Yazdanpanah Y., Mentre F., Peiffer-Smadja N. (2020): Type I interferons as a potential treatment against COVID-19.AntiviralRes;178:1-4.

22- Siddiqi H.K and Mehra M.R. (2020): COVID 19 illness in native and immunosuppressed States: a clinical therapeutics tagging proposal .J Heart Lung Transplant. 39(5): 405–407.

23- Taylor P.C., Peters A.M., Paleolog E., Chapman P.T., Elliott M.J., McCloskey R., et al. (2000): Reduction of chemokine levels and leukocyte traffic to joints by tumor necrosis factor alpha blockade in patients with

rheumatoid arthritis .Arthritis Rheum;43:38–47.

24- Velavan T.P and Meyer C.G. (2020): The COVID 19 epidemic. Insights immunology;25: 278-280.

25- Zheng HY, Zhang M, Yang CX, Zhang N, Wang XC, Yang XP, Dong XQ, Zheng YT. Elevated exhaustion levels and reduced functional diversity of T cells in peripheral blood may predict severe progression in COVID-19 patients. Cellular & molecular immunology. 2020 May;17(5):541-3

Ibrahim S, Essa B, khattab M, Ahmed O. Serum levels of tumor necrosis factor alpha and interferon alpha in COVID-19 patients and their correlations with disease severity. Microbes Infect Dis 2024; 5(4): 1253-1263.