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Comparative analysis of inflammatory profiles between COVID-19 patients and healthy controls and its correlation with disease severity

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Abstract

Several reports have highlighted the significant role of inflammation in the pathogenesis of coronavirus Disease-19 (COVID-19). The hyper-inflammatory response triggered by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections is believed to contribute to disease severity and adverse clinical outcomes. Consequently, classical inflammatory markers have been proposed as potential indicators of COVID-19 severity. However, there is still a critical need for extensive analysis of the predictive value of inflammatory biomarkers in large cohorts of patients. The main objective of this study was to evaluate Plasma inflammatory Levels in COVID-19 Patients and Healthy Controls and its association with COVID-19 severity levels.

This study employed cross-sectional and case-control study designs. The study population comprised of SARS-CoV-2 positive patients at Ndola Teaching Hospital (NTH) and Levy Mwanawasa University Teaching Hospital (LMUTH). The laboratory tests involved the assessment of inflammatory profiles in COVID-19 patients compared to control subjects. Furthermore, the study investigated the use of inflammatory profiles in classifying COVID-19 severity in relation to WHO guidelines. Data analysis was conducted using SPSS version 21.

Our study observed elevated plasma levels of inflammatory profiles such as CRP, Ferritin, procalcitonin, Neutrophils, Neutrophil Lymphocyte Ratio (NLR), Erythrocyte Sedimentation Ratio (ESR) and inflammatory cytokines such as IL-1, IL-6,IL-8 and Tumour Necrosis Factor (TNF) in COVID-19 patients compared to the control group. In contrast the mean Lymphocyte count in Covid-19 patients was significantly lower than the control subjects. Additionally, COVID-19 patients exhibited a higher prevalence of hyperinflammation compared to control participants. The study also found that the frequency of hyperinflammation increased with COVID-19 severity. Furthermore, statistically significant differences in mean haemostatic plasma concentration were observed in relation to different levels COVID-19 disease severity.

Inflammatory profile results indicate that COVID-19 patients were more prone to hyper inflammatory state than the Sars-CoV-2 negative individuals and these parameters were observed to correlate with COVID-19 severity levels. The study provides valuable insights into the inflammatory mechanisms of COVID-19 and identifies potential biomarkers for hyperinflammation. Clinicians can utilize this information to identify patients with a poor prognosis and assess disease severity, enabling early intervention.

Keywords: COVID-19; Sars-Cov-2; CRP; IL-6; Hyperinflammation; Cytokine storm

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1. Introduction

Coronavirus disease 2019 (COVID-19) was declared a pandemic by the World Health Organization (WHO) following an outbreak in a seafood market in Wuhan, China, in December 2019. The disease rapidly spread to 187 countries within three months, resulting in significant morbidity and mortality. COVID-19 is caused by a novel coronavirus (CoV) belonging to the Coronaviridae family of single-stranded positive-sense RNA viruses. The viral genome has a size ranging from 26 to 32 kilobases (Gorbalenya et al., 2020).

The COVID-19 pandemic has had a profound impact on global health, with millions of people affected worldwide. Understanding the underlying mechanisms of the disease is crucial for developing effective treatment strategies and improving patient outcomes. Inflammation and hypercoagulability are two key factors that have been implicated in the pathogenesis of COVID-19 and are associated with disease severity.

In this study, we aim to investigate the inflammatory profiles of COVID-19 patients and compare with disease severity. By analyzing a large cohort of patients, we hope to gain insights into the relationship between inflammation and disease progression. This research will contribute to our understanding of the immunological response to SARS-CoV-2 infection and may help identify potential therapeutic targets.

2. Material and methods

This was a hospital-based cross-sectional and case-control study conducted at Ndola Teaching Hospital (NTH) and Levy Mwanawasa University Teaching Hospital (LMUTH) in Zambia. These study designs were chosen because they are cost-effective and time-efficient, which are important considerations for an outbreak investigation. NTH is a tertiary referral hospital for the Copperbelt and Northern regions of Zambia, with a bed capacity of 851. It is located in Ndola, the provincial capital of the Copperbelt province. LMUTH is a provincial hospital with tertiary services, located in Lusaka. It served as a COVID-19 referral centre in Lusaka. The study population consisted of hospitalized or outpatients at NTH and LMUTH who had a confirmed diagnosis of COVID-19 by reverse transcriptase–polymerase chain reaction (RT-PCR) assay on nasopharyngeal swab samples. The control group comprised of non-hospitalized individuals who tested negative FOR COVID-19 by RT-PCR assay.

The study recruited a total number of 340 participants comprising of 87 and 86 SARS-Cov-2 positive patients at NTH and LMUTH respectively while 84 and 83 SARS-Cov-2 negative indiviuals were recruited at NTH and LMUTH respectively. This study adopted the simple random sampling technique to recruit 173 COVID-19 positive patients and 167 COVID-19 negative patients. This type of technique was adopted in this study because it is easy to conduct and when conducted properly, a simple random sample represents an unbiased sample, and therefore is a fair and accurate representation of the population.

2.1. Inclusion Criteria for COVID-19 Patients and Control Participants

This study enrolled individuals who tested positive for COVID-19 by reverse transcription-polymerase chain reaction (RT-PCR) as cases. Control subjects were healthy individuals of both genders aged 18 years or older who tested negative for SARS-CoV-2. Only those who provided informed consent were included in the study.

2.2. Exclusion Criteria for COVID-19 Patients and Control Participants

Participants who had a history of venous thromboembolism or known inherited coagulation disorders, cancer, and hyperthyroidism were excluded from the study. Other exclusion criteria included pregnant individuals, those who had recent surgery, those taking standard anticoagulant treatment, individuals less than 18 years old, and those who did not provide consent.

2.3. Data Collection

Good Laboratory Practice (GLP) principles according to the Ministry of Health laboratory quality manual was observed to ensure uniformity, consistency, reliability and reproducibility of all the laboratory test results in the study. Quality control measures were observed in all the laboratory procedures. Laboratory results of some COVID-19 patients who were once admitted to NTH and LMUTH but have been discharged were retrieved from the laboratory Disalab electronic system.

2.4. Full Blood Count Analysis

The Sysmex XT-2000i is an automated hematology analyzer that was used for full blood count analysis. It employs the electric resistance detecting method (impedance technology) with hydrodynamic focusing to measure various blood parameters such as red blood cells (RBC), platelets (PLT), mean platelet volume (MPV), mean red cell volume (MCV), and hematocrit (HCT). Fluorescence flow cytometry is utilized to measure white blood cells (WBC), differential WBC, optical platelet count, and reticulocyte count. The system incorporates a 633 nm semiconductor laser for flow cytometry analysis for the measurement of the proportional count, expressed as a percentage of the total WBC, of neutrophils (NEUT), lymphocytes (LYMPH), monocytes (MONO), and eosinophils (EOS), white cells are stained with fluorescent dyes that bind to both DNA and RNA1. Side Scatter (SSC) is employed to determine the internal complexity of the cell, including the size, shape, and density of the nucleus and granules1. Fluorescence and scatter measurements are combined to characterize white cell populations1. Basophils (BASO) are measured separately using cell size and SSC properties1. Hemoglobin (HGB) is measured photo colorimetrically using SLS-HGB, a cyanide-free method. In the current study, the full blood count parameters considered were hemoglobin, platelets, neutrophils, and lymphocytes1. The Neutrophil Lymphocyte Ratio (NLR) was calculated by dividing the neutrophil and lymphocyte results obtained from the Sysmex XT-2000i Hematology analyzer.

2.5. C - Reactive Protein estimation

The C - reactive protein (CRP) is synthesized by the liver in response to interleukin-6 and well known as one of the classical acute-phase reactants and as a marker of inflammation. Ichroma™ II automated equipment manufactured by Boditech Med Incorporated of the Republic of Korea will be used for the analysis of CRP. The test uses a sandwich immunodetection method; the detector antibody in buffer binds to antigen in sample, forming antigen-antibody complexes, and migrates onto nitrocellulose matrix to be captured by the other immobilized-antibody on test strip. The more antigen in sample forms the more antigen-antibody complex and leads to stronger intensity of fluorescence signal on detector antibody, which is processed by instrument for Ichroma™ tests to show CRP concentration in sample. Instrument for Ichroma™ tests calculates the test result automatically and displays CRP concentration of the test sample in terms of mg/L. The cut-off reference value to be used will be 10 mg/L.

2.6. Ferritin analysis

Ferritin, a major iron storage protein, is essential to iron homeostasis and is involved in a wide range of physiologic and pathologic processes. Ichroma™ II automated equipment manufactured by Boditech Med Incorporated of the Republic of Korea was used for the analysis of Ferritin.

The test uses a sandwich immunodetection method; the detector recombinant protein in buffer binds to antibody in sample, forming recombinant protein-antibody complexes, and migrates onto nitrocellulose matrix to be captured by the other immobilized-antigen on test strip.

The more antibody in sample forms the more recombinant protein-antibody complex and leads to stronger intensity of fluorescence signal on detector recombinant protein, which is processed by Instrument for Ichroma™ tests to show ferritin concentration in sample.

Instrument for Ichroma™ calculates the test result automatically and displays ferritin concentration of the test sample in terms of ng/mL. The cut-off (reference range) for Women is 20-250 ng/mL while for men it is 30-350 ng/mL.

2.7. Procalcitonin analysis

Procalcitonin (PCT) is a peptide precursor of the hormone calcitonin. It arises once preprocalcitonin is cleaved by endopeptidase. It is produced by parafollicular cells (C cells) of the thyroid and by the neuroendocrine cells of the lung and the intestine. ichroma™ PCT kit will be used for the analysis of Procalcitonin. is a fluorescence Immunoassay (FIA) for the quantitative determination of Procalcitonin PCT) in human serum /plasma. The test uses a sandwich immunodetection method; the detector antibody in buffer binds to antigen in sample, forming antigen-antibody complexes, and migrates onto nitrocellulose matrix to be captured by the other immobilized-antibody on test strip.

The more antigen in sample forms the more antigen-antibody complex and leads to stronger intensity of fluorescence signal on detector antibody, which is processed by instrument for ichroma™ tests to show PCT concentration in sample.

2.8. Determination of Circulatory Cytokines

The plasma samples for determination of cytokines were frozen upon collection and analyzed later after thawing. The types and quantities of cytokines were detected by flow cytometry using a multiplex assay system that included Becton and Dickinson Cytometric Bead Array (BD CBA) Human Inflammatory Cytokine kit and Becton and Dickinson Fluorescence Activated Cell Sorter (FACS Calibur) flow cytometer (FACS Count System; Plate3.2, BD Biosciences, U.S.A). The BD CBA assays provide a method of capturing a soluble analyte or set of analytes with beads of known size and fluorescence, making it possible to detect analytes using flow cytometry. Each capture bead in the kit has been conjugated with a specific antibody. The detection reagent provided in the kit is a mixture of phycoerythrin (PE)–conjugated antibodies, which provides a fluorescent signal in proportion to the amount of bound analyte. When the capture beads and detector reagent are incubated with an unknown sample containing recognized analytes, sandwich complexes (capture bead + analyte + detection reagent) are formed. Six bead populations with distinct fluorescence intensities have been coated with capture antibodies specific for IL-8, IL-1ß, IL-6, IL-10, TNF, and IL-12 proteins. These complexes can be measured using flow cytometry to identify particles with fluorescence characteristics of both the bead and the detector.

WHO acceptable classification was used for COVID-19 severity classification (Buonsenso et al., 2021) and is as follows;

- **Asymptomatic Infection:** Individuals who test positive for SARS-CoV-2 using a nucleic acid amplification test (NAAT) or an antigen test) but who have no symptoms that are consistent with COVID-19.
- **Mild Illness:** Individuals who have any of the various signs and symptoms of COVID-19 (e.g., fever, cough, sore throat, malaise, headache, muscle pain, nausea, vomiting, diarrhea, loss of taste and smell) but who do not have shortness of breath, dyspnea, or abnormal chest imaging.
- **Moderate Illness:** Individuals who show evidence of lower respiratory disease during clinical assessment or imaging and who have oxygen saturation (SpO2) ≥94% on room air at sea level.
- **Severe Illness:** Individuals with clinical signs of pneumonia (fever, cough, dyspnoea, fast breathing) plus one of the following: respiratory rate > 30 breaths/min; severe respiratory distress; or SpO2 < 90% on room air.
- **Critical Illness:** Individuals who have respiratory failure, septic shock, and/or multiple organ dysfunction.
- We defined hyperinflammation and hypercytokinemia in COVID-19 patients based on the serum levels of CRP and cytokines, respectively. Hyperinflammation was present when CRP was above 14.7 mg/l, and hypercytokinemia was present when any of the following cytokines exceeded the threshold values: IL-6 >11ng/ml, IL-1 >20ng/ml, IL-8>62ng/ml, TNF-α >30ng/ml. We used plasma D-dimer and soluble P-selectin (sP-selectin) as biomarkers of hypercoagulability, which is a condition characterized by an increased tendency to form blood clots. COVID-19 patients were considered hypercoagulable when D-dimer and sP-selectin were above 500 ng/mL and 3.2 ng/ml, respectively. We chose these biomarkers based on the findings of Fenyves et al., (2021), who reported that plasma P-selectin was an early marker of thromboembolism in COVID-19 patients.

2.9. Ethical considerations

The study protocol was approved by the Tropical Diseases Research Centre (TDRC) Ethics Review Committee and National Health Research Authority. We obtained written permission from the Permanent Secretary of the Ministry of Health and the Senior Medical Superintendents of Ndola Teaching Hospital (NTH) and Levy Mwanawasa University Teaching Hospital (LMUTH). We informed the study participants about the study objectives and procedures, and obtained their written informed consent. We ensured the privacy and confidentiality of the participants by using codes instead of names on the data collection forms and storing them in secure cabinets and password-protected computers. Only qualified medical professionals, such as nurses and laboratory staff, who worked in COVID-19 isolation centers were involved in blood sample collection. We followed the public health measures of social distancing and wearing masks to prevent the transmission of COVID-19, and provided free masks to all the participants. We also required all the research assistants to be fully vaccinated against COVID-19 and to undergo one-week training in laboratory safety with a focus on COVID-19.

2.10. Data Analysis

The data was entered and cleaned in Excel sheets before analysis. Data cleaning involved removing duplicates and outliers, correcting inconsistencies, combining data sets, and standardizing data formats. Statistical analysis was performed using SPSS version 21, and the results were summarized in tables and graphs. All statistical tests were performed at a 5% significance level or 95% confidence interval with a p-value of less than 0.05 to determine statistical significance. The distribution of the data was analyzed using the Kolmogoroff-Smirnoff test. Normally distributed results were reported as the mean +/- standard deviation. The independent T-test was used to compare the mean inflammatory

parameters of COVID-19 patients and correlated with COVID-19 disease severity. Analysis of Variance (ANOVA) was used to determine the average levels of inflammatory profiles in relation to COVID-19 disease severity. The Tukey post-hoc test was employed to ascertain the significant pairwise differences in inflammatory profile concentration among different COVID-19 severity levels.

3. Results

Table 1 Independent T-test for analysis of inflammatory profiles in Sars-CoV-2 Positive individuals and control Subjects

Biomarker	SARS-COV-2 Positive	Controls	t-value	P-Value
CRP	59.5±42.2 mg/l	6.0±3.6	8.4	0.000
Ferritin	399.7±224.7 ng/ml	122.6±72.9	10.7	0.000
Procalcitonin	0.82±0.79 ng/ml	0.06±0.09	4.9	0.000
Lymphocytes	1.6±1.0 x10^9/l	2.5±0.5	10.6	0.000
Neutrophils	6.2±3.6 x10^9/l	5.0±1.0	4.1	0.000
NLR	5.68±3.38	1.98±0.22	7.5	0.000
ESR	41.5±34.6 mm/hr	7.0±4.6	34.5	0.000
IL-1	42.85±30.48 ng/l	14.46±8.81	8.86	0.004
IL-6	35.33±30.21 ng/l	4.45±2.67	9.90	0.000
IL-8	78.90±62.11 ng/l	37.82±23.38	8.02	0.000
TNF	44.5±37.1 ng/l	21.0±11.5	7.82	0.000

Table 1 shows results of an independent-sample t-test to compare the inflammatory profiles in Sars-CoV-2 Patients and control participants. The table reveals that mean Plasma Concentration of C-Reactive Protein (CRP) in Sars-Cov-2 patients was significantly increased (59.5±42.2 mg/l) than in the control subjects (6.0±3.6 mg/l), t=8.4; P<0.05. The table further shows that mean Ferritin plasma levels in Sars-CoV-2 patients was significantly higher (399.7±224.7 ng/ml) than in the control subjects (122.6±72.9ng/ml), t = 10.7; P<0.05. Similarly the mean plasma concentration of Procalcitonin was significantly increased in Sars-CoV-2 patients (0.82±0.79 ng/ml) than the control subjects (0.06±0.09 ng/ml), t = 4.9, P<0.05.

Table 1 further shows that Lymphocyte count in Sars-Cov-2 patients was significantly lower (1.6±1.0 x 109 /l) than the control subjects (2.5±0.5 x 109 /l), t = 10.6, P<0.05.The results further indicate that mean Neutrophil count for the Sars-CoV-2 patients was significantly higher (6.2±3.6 x 109 /l) than the control subjects with the mean Neutrophil count of (5.0±1.0 x 109 /l), t =4.1, P<0.05. The mean Neutrophil Lymphocyte Ratio (NLR) was significantly increased in Sars-Cov-2 patients (5.68±3.38) than in the control subjects (1.98±0.22), t = 7.5, P<0.05. Table 1 further shows that mean Erythrocyte Sedimentation Rate (ESR) in Sar-Cov-2 patients (41.5±34.6 mm/hr) was significantly increased than in the control subjects (7.0±4.6 mm/hr), t = 34.5, P<0.05. Table 1 reveals that the mean concentration of Interleukin 1 (IL-1) in Sars-Cov-2 patients was higher (42.85±30.48 ng//) than in the control subjects (14.46±8.81 ng/l) and this differences in the concentration was significant, t = 8.86, P<0.05.

The table additionally shows that the mean circulatory levels of Interleukin 6 (IL-6) was increased in Sars-CoV-2 patients (35.33±30.21 ng/l) than the control subjects (4.45±2.67 ng) and the differences in the mean comcentration of IL-6 between the Sars-CoV-2 patients and the control subjects was significant, t = 9.90, P<0.05. The mean Interleukin-8 (IL-8) circulatory levels in Sars-Cov-2 patients was significantly higher (78.90±62.11 ng/l) than the control subjects (37.82±23.38 ng/l), t =8.02, P<0.05.

Similary, table 4.6 shows that mean circulatory Tumour Necrosis Factor (TNF) in Sars-Cov-2 patients was significantly increased (44.5±37.1 ng/l) than in the control subjects (21.0±11.5 ng/l), t =7.82, P<0.05.

3.1.1. Frequency of hyperinflammation in SARS-Cov-2 patients and control participants.

Table 2 Comparison of hyperinflammation in SARS-Cov-2 patients and control participants

		Hyperinflammation (+)	**Hyperinflammation (-)**
		CRP(>14.7mg/l) IL-6>(11ng/l)	**CRP (≤14.7mg/l) IL-6≤(11ng/l)**
		P value = 0.000	
	Total	**N (%)**	**N (%)**
Status			
Control	167	8(4.8)	159(95.2)
Covid 19	173	107(61.8)	66(38.2)

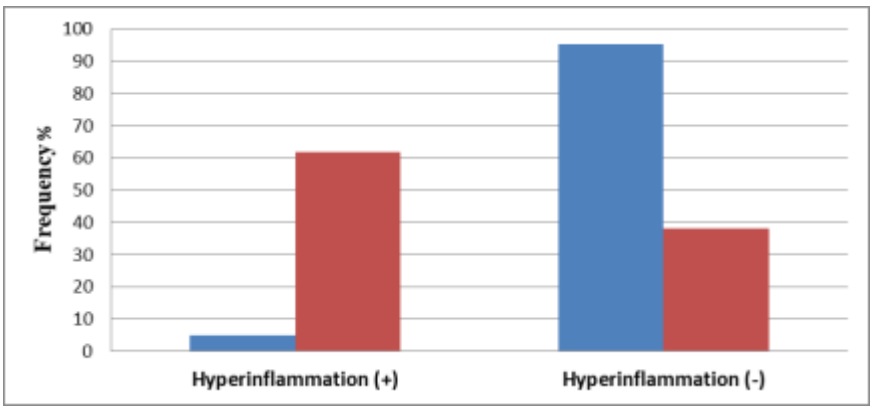

Figure 1 Frequency of hyperinflammation in SARS-CoV-2 Patients and Control Subjects

CRP and IL-6 plasma concentrations, which were continuous variable in SPSS, were recoded so as to categorise the results into two categories; those who had CRP and IL-6 plasma concentration of greater than 14.7mg/l and 11ng/l respectively were categorized as hyperinflammatory and those whose CRP and IL-6 results were ≤ 14.7mg/l and 11ng/l respectively were regarded as normal. Thereafter a chi-square test of independence was done to determine the proportion of hyperinflammation in Sars-CoV-2 patients and control subjects. Table 2 and Figure 1 reveals that Sars-CoV-2 patients had higher prevalence of hyperinflammation [107(61.8%)] than control participants [8(4.8%)]. The difference was significance P=0.00.

Table 3 Frequency of hyperinflammation according to disease severity

		Hyperinflammation (+)	**Hyperinflammation (-)**
		CRP (>14.7mg/l) IL-6 (>11ng/l)	CRP (≤14.7mg/l) IL-6 (≤11ng/l)
		P value =0.001	
Disease Severity	**Total**	**N (%)**	**N (%)**
Asymptomatic	26	7(26.9)	19(73.1)
Mild	32	13(40.6)	19(59.4)
Moderate	61	31(50.8)	30(49.2)
Severe	39	34(87.2)	5(12.8)
Critical	15	14(93.3)	1(6.7)

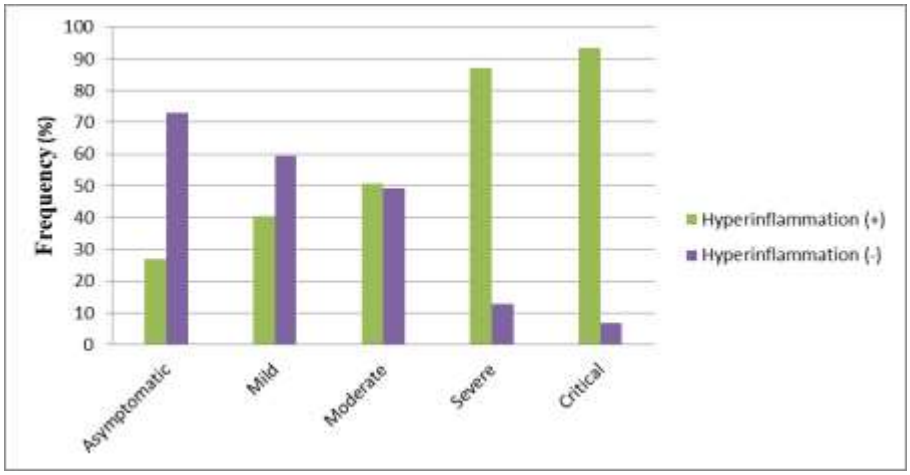

Figure 2 Frequency of hyperinflammation according to Disease severity

Table 3 and Figure 2 show results of frequency of hyperinflammation according to Sars-CoV-2 disease severity. The results indicate that frequency of hyperinflammation was higher in the critical Sars-CoV-2 patients 14(93.3%) than the Asymptomatic Sars-CoV-2 patients 7(26.9), Mild 13(40.6%), Moderate 31(50.8%) and Severe 34(87.2). These differences in the frequency of hyperinflammation according to disease severity was significant, $P<0.05$.

3.2. Inflammatory profiles of Covid-19 patients according to disease severity

Table 4 Analysis of variance for the means of inflammatory profiles in Sars-CoV-2 Positive individuals according to disease Severity

Blood Parameter	**Sars-Cov-2 Asymptomatic (N = 26)**	**Sars-Cov-2 Mild Cases (N=32)**	**Sars-CoV-2 Moderate cases (N=61)**	**Sars-CoV-2 Severe (N=39)**	**Sars-CoV-2 Critical (N=15)**		
Haemostatic Profiles	**Mean**	**Mean**	**Mean**	**Mean**	**Mean**	**Anova (F)**	**P-value**
CRP (mg/l)	19.6	26.8	54.0	97.5	122.1	8.347	0.000*
Ferritin (ng/ml)	236.6	199.3	329.4	597.4	882.3	28.260	0.000*
PCT (ng/ml)	0.07	0.14	0.63	1.44	2.73	7.386	0.000*
WBC ($X10^9$/l	8.7	10.6	10.9	12.1	12.2	4.353	0.061*
Lymphocytes ($x10^9$/l)	1.6	2.1	1.7	1.3	1.2	3.4	0.071
Neutrophils ($x10^9$/l)	4.2	5.2	5.5	7.7	11.1	15.4	0.000*
NLR	2.91	3.55	4.05	9.63	11.33	12.4	0.000*
ESR	14.7	17.4	35.7	63.8	105.1	58.186	0.000*
IL-1(ng/l)	16.32	22.88	33.14	66.37	109.83	33.801	0.000*
IL-6 (ng/l)	9.53	20.76	23.39	55.87	106.27	34.143	0.000*
IL-8 (ng/l)	44.75	54.41	60.45	110.28	183.80	29.493	0.000*
TNF (ng/l)	21.92	29.34	36.18	69.56	84.53	18.887	0.000*

*= Significant at $p<0.05$.

Table 4 presents the results of an Analysis of Variance (ANOVA) for the means of inflammatory profiles in Sars-CoV-2 patients based on Covid-19 disease severity. The study found statistically-significant differences in mean C-Reactive Proteins (CRP) plasma concentration with respect to Covid-19 disease severity ($F=8.347$, $p<0.05$). Critical patients had

the highest average CRP concentration of 122.1 mg/l. Tukey post-hoc test revealed significant pairwise CRP concentration differences between Critical Sars-CoV-2 patients and Asymptomatic, Mild and Moderate Covid-19 patients, with an average difference of 102.5, 95.3 and 68.1 mg/l CRP concentration respectively ($p<0.05$). Furthermore, statistically-significant differences in average CRP plasma concentration were found between Severe and Asymptomatic including mild to moderate Covid-19 patients with an average difference of 77.9, 70.7 and 43.5 mg/l CRP concentration.

Table 4 further presents the results of an Analysis of Variance (ANOVA) for the means of inflammatory profiles in Sars-CoV-2 patients based on Covid-19 disease severity. The study found statistically-significant differences in mean Ferritin plasma concentration with respect to Covid-19 disease severity ($F=28.260$, $p<0.05$). Critical patients had the highest average Ferritin concentration of 882.3 mg/l. Tukey post-hoc test revealed significant pairwise ferritin concentration differences between Critical Sars-CoV-2 patients and Asymptomatic, Mild and Moderate Covid-19 patients, with an average difference of 645.7, 682.9, 552.9 and 284.8 ng/ml ferritin concentration respectively ($p<0.05$). Furthermore, significant pairwise ferritin concentration differences between Severe Sars-CoV-2 patients and Asymptomatic, mild and moderate Sars-CoV-2 patients were obtained with an average difference of 360.8, 398.1 and 268.0 ng/ml ferritin concentration respectively ($p<0.05$).

Table 4 presents the results of an Analysis of Variance (ANOVA) for the means of inflammatory profiles in Sars-CoV-2 patients based on Covid-19 disease severity. The study found statistically-significant differences in mean Procalcitonin (PCT) plasma concentration with respect to Covid-19 disease severity ($F=7.386$, $p<0.05$). Critical patients had the highest average PCT concentration of 2.73 ng/ml. The Tukey post-hoc test revealed significant pairwise PCT concentration differences between Critical Sars-CoV-2 patients and Asymptomatic, Mild, and Moderate Sars-CoV-2 patients, with an average difference of 2.66, 2.59, and 2.10 ng/ml PCT concentration respectively ($p<0.05$). Furthermore, significant pairwise ferritin concentration differences between Severe Sars-CoV-2 patients and Asymptomatic and Mild Sars-CoV-2 patients with an average difference of 0.47 and 0.44 ng/ml PCT concentration respectively were reported ($p<0.05$).

We further reported statistically-significant differences in mean Neutrophil count in relation to Covid-19 disease severity ($F=15.4$, $p<0.05$). Critical Sars-CoV-2 patients had the highest average Neutrophil count of 15.4×10^9 /l. The Tukey post-hoc test revealed significant pairwise Neutrophil count differences between Critical Sars-CoV-2 patients and Asymptomatic, Mild, Moderate, and Severe Covid-19 patients, with an average difference of 6.8, 5.9, 5.5, and 3.4×10^9 /l respectively ($p<0.05$). Furthermore, we obtained significant pairwise Neutrophil count differences between Severe Sars-CoV-2 patients and Asymptomatic, mild, and moderate Sars-CoV-2 patients with an average difference of 3.4, 2.5, and 2.1×10^9 /l respectively ($p<0.05$).

Table 4 presents the results of an Analysis of Variance (ANOVA) for the means of inflammatory profiles in Sars-CoV-2 patients based on Covid-19 disease severity. The study found statistically-significant differences in mean Neutrophil Lymphocyte Ratio (NLR) with respect to Covid-19 disease severity ($F=12.4$, $p<0.05$). Critical Sars-CoV-2 patients had the highest average NLR of 11.33. The Tukey post-hoc test revealed significant pairwise NLR differences between Critical Sars-CoV-2 patients and Asymptomatic, Mild, and Moderate Covid-19 patients, with an average difference of 8.42, 7.78, and 7.28 ($p<0.05$). Furthermore, significant pairwise NLR differences between Severe Sars-CoV-2 patients and Asymptomatic, mild, and moderate Sars-CoV-2 patients were obtained with an average difference of 6.71, 6.08, and 5.57 respectively ($p<0.05$).

We further found statistically-significant differences in mean Erythrocyte Sedimentation Rate (ESR) with respect to Covid-19 disease severity ($F=58.186$, $p<0.05$). Critical Sars-CoV-2 patients had the highest average ESR of 105.1 mm/hour. The Tukey post-hoc test revealed significant pairwise ESR differences between Critical Sars-CoV-2 patients and Asymptomatic, Mild, Moderate, and Severe Covid-19 patients, with an average difference of 90.4, 87.7, 69.4, and 41.2 mm/hour ESR respectively ($p<0.05$). Furthermore, significant pairwise ESR differences between Severe Sars-CoV-2 patients and Asymptomatic, mild, and moderate Sars-CoV-2 patients were obtained with an average difference of 49.2, 46.4, and 28.2 mm/hour ESR respectively ($p<0.05$). In addition, significant pairwise ESR differences were obtained between Covid-19 patients in moderate state and asymptomatic Sars-Cov-2 patients with an average difference of 21.0 mm/hour ($p<0.05$).

We further found a statistically-significant differences in mean Interleukin (IL-1) in relation to Covid-19 disease severity ($F) =33.801$, $p < 0.05$). Critical Sars-CoV-2 patients patients had the highest average plasma IL-1 of 109.83 ng/l. Tukey post-hoc test revealed significant pairwise IL-1 plasma concentration differences between Critical Sars-CoV-2 patients and Asymptomatic, Mild, Moderate and Severe Covid-19 patients, with an average difference of 93.51, 86.95, 76.70 and 43.47 ng/l IL-1 concentration respectively ($p < 0.05$). Furthermore, we recorded significant pairwise IL-1 differences

between Severe Sars-CoV-2 patients and Asymptomatic, mild and moderate Sars-CoV-2 patients with an average difference of 50.04, 3.48 and 33.23 ng/l respectively ($p < 0.05$).

The results further show statistically-significant differences in the average interleukin-6 (IL-6) plasma concentration in relation to Covid-19 disease severity ($F = 34.143$, $p < 0.05$). Critical patients had the highest average IL-6 plama concentration of 106.27 ng/l. Appendix 26 report results of a Tukey post-hoc test which revealed significant pairwise IL-6 concentration differences between Critical Sars-CoV-2 patients and Asymptomatic, Mild, Moderate and Severe Sars-CoV-2 patients, with an average difference of 97.74, 85.50, 82.87, and 50.40 ng/l IL-6 concentration respectively ($p < 0.05$). Furthermore, significant pairwise IL-6 concentration differences were obtained between Severe Sars-CoV-2 patients and Asymptomatic, Mild and moderate Sars-CoV-2 patients with an average difference of 46.34, 35.10, and 32.47 ng/l concentration respectively ($p < 0.05$).

Table 4 presents the results of an Analysis of Variance (ANOVA) for the means of inflammatory profiles in Sars-CoV-2 patients based on Covid-19 disease severity. The study found statistically-significant differences in mean Interleukin-8 (IL-8) plasma concentration with respect to Covid-19 disease severity ($F=29.493$, $p<0.05$). Sars-CoV-2 patients in a critical state had the highest IL-8 plasma concentration of 183.80 ng/l. The Tukey post-hoc test revealed significant pairwise IL-8 concentration differences between Critical Sars-CoV-2 patients and Asymptomatic, Mild, Moderate, and Severe Covid-19 patients, with an average difference of 139.05, 129.39, 123.35, and 73.5 ng/l IL-8 plasma concentration respectively ($p<0.05$). Furthermore, statistically-significant differences in the average IL-8 concentration were found between severe and asymptomatic mild and moderate Covid-19 patients with an average difference of 65.53, 55.87, and 49.83 ng/l IL-8 plasma concentration respectively ($p<0.05$).

We further found a statistically-significant differences in mean Tumour Necrosis Factor (TNF) in relation to Covid-19 disease severity ($F = 18.887$, $p < 0.05$). Critical Sars-CoV-2 patients patients had the highest average TNF of 84.53 ng/l. Tukey post-hoc test revealed significant pairwise TNF plasma concentration differences between Critical Sars-CoV-2 patients and Asymptomatic, Mild and Moderate Covid-19 patients, with an average difference of 62.61, 55.20, 48.36 ng/l TNF plasma concentration respectively ($p < 0.05$). Furthermore, we recorded significant pairwise TNF differences between Severe Sars-CoV-2 patients and Asymptomatic, mild and moderate Sars-CoV-2 patients with an average difference of 47.64, 40.23 and 33.38 ng/l TNF plasma concentration respectively ($p < 0.05$).

4. Discussion

Hyperinflammation plays an important role in severe and critical COVID-19 (Hasan et al., 2022). It involves activation of multiple inflammatory pathways leading to hyperinflammation and cytokine storm, resulting in tissue damage, acute respiratory distress syndrome (ARDS), and multi-organ failure (Tan, et al.,2021). COVID-19–associated hyperinflammatory syndrome begins with the failure of the regulatory immune response to SARS-CoV-2, including abnormal interferon (INF) production that drives macrophage hyperactivation. This study reports higher proportion of COVID-19 patients who had hyper inflammation (61.8%) than the control individuals (4.8%). Furthermore severe and critically ill COVID-19 patients had the highest proportion of individuals in hyperimflammatory state. This results accords a study titled "Correlation analysis between disease severity and inflammation-related parameters in patients with COVID-19: a retrospective study" which found significant associations between disease severity and various inflammation-related parameters, including age, Interleukin-2 receptor (IL2R), Interleukin-6 (IL-6), Interleukin-8 (IL-8), Interleukin-10 (IL-10), Tumor necrosis factor α (TNFα), C-reactive protein (CRP), Ferroprotein, rocalcitonin, White cell counts (WBC), Lymphocyte counts (LC), Neutrophil count (Gong, et al.,2020).

Our study found that COVID-19 patients had elevated mean inflammatory profile levels compared to healthy control subjects, and these levels were consistent with the severity of COVID-19. For instance, the mean concentration of C-reactive protein (CRP) was higher in COVID-19 patients than in control individuals, which is in line with the findings of Ikeagwulonu et al. (2021) . Furthermore, our study revealed that CRP levels were increased in severe and critically ill COVID-19 patients compared to asymptomatic and mildly ill patients. CRP is an acute-phase pentameric protein synthesized in the liver, primarily induced by interleukin-6 in response to inflammation. It plays a crucial role in the recognition and clearance of foreign pathogens, exhibiting both pro- and anti-inflammatory properties.

A comprehensive literature review conducted by Tjendra et al. (2020) on multiple biomarkers associated with predicting COVID-19 severity demonstrated that elevated inflammatory markers, including elevated CRP, were associated with a higher risk of disease progression to critical illness, a severe disease course, an increased risk of developing sepsis with rapid progression, and ultimately a higher risk of intubation and in-hospital mortality.

In the current study mean ferritin concentration was higher in COVID-19 patients than in the control individuals. This accords the results obtained in a study done by Patil et al.,(2022) in which the authors reported increased ferritin levels in COVID-19 patients. Our study further revealed that the plasma levels of ferritin were significantly higher in the severe and critically ill COVID-19 patients than the asymptomatic, Mild or moderate disease. This is consistent with a study by Vargas-Vargas et al (2020) who reviewed the evidence supporting the hypothesis that ferritin levels might be a crucial factor influencing the severity of COVID-19. They reported that fatal outcomes by COVID-19 are accompanied by cytokine storm s Circulation ferritin level increases during viral infections and can be a marker of viral replication (Cheng et al., 2020). Increased levels of ferritin due to cytokine storm have also been reported in severe COVID-19 patients (Cheng et al., 2020). During the cytokine storm in COVID-19, many inflammatory cytokines are rapidly produced, including IL-6, TNF-α, IL-1β, IL-12, and IFN-γ, which stimulate hepatocytes, Kupffer cells, and macrophages to secrete ferritin (Cheng et al., 2020). The uncontrolled and dysfunctional immune response associated with macrophage activation, hyperferritinemic syndrome, and thrombotic storm finally leads to multiple organ damage. Notably, ferritin is not only the result of excessive inflammation, but also plays a pathogenic role in the inflammation process through its binding with the T-cell immunoglobulin and mucin domain 2 (TIM-2) by promoting the expression of multiple pro-inflammatory mediators (Kernan et al.,2017).

The current study reported 10-fold increased mean procalcitonin concentration in COVID-19 patients than the healthy control individuals and critically ill patients had higher levels of Procalcitonin than the asymptomatic or mildly ill COVID-19 patients. Procalcitonin is a protein that is produced by the body in response to bacterial infections. It can be used as a biomarker to help diagnose bacterial infections and guide antibiotic therapy. However, procalcitonin levels can also be elevated in some viral infections, such as COVID-19, which is caused by the novel coronavirus SARS-CoV-2. Several studies have investigated the association between procalcitonin levels and COVID-19 severity and outcomes. A systematic review and meta-analysis by Kumar et al (2022) included 32 studies with 13,154 patients and found that procalcitonin had good discriminatory power for predicting mortality and disease severity in COVID-19 patients. Another study by Mazaheri et al (2022) compared cytokine levels and procalcitonin in COVID-19 patients who required intensive care unit (ICU) admission versus those who did not. The study found that pro-inflammatory cytokines (IL-6, IL-8, TNFα) and procalcitonin were higher in ICU patients than non-ICU patients, despite similar levels of C-reactive protein (CRP), which is another marker of inflammation. These studies suggest that procalcitonin may reflect the degree of systemic inflammation and tissue damage in COVID-19 patients, rather than the presence of bacterial infection. Therefore, procalcitonin measurement may help identify potentially severe cases and thus decrease mortality by offering early aggressive treatment. However, procalcitonin should not be used as the sole criterion for initiating or withholding antibiotic therapy in COVID-19 patients, as it may not accurately differentiate between viral and bacterial infections. Higher procalcitonin levels in COVID-19 could be due to Some COVID-19 patients who might have developed bacterial co-infections or superinfections, which can stimulate the production of procalcitonin by the cells. Bacterial co-infections or superinfections are more common in patients with severe or critical COVID-19, especially those who require mechanical ventilation or intensive care unit admission (Powell et al., 2021). The cytokine storm and the tissue damage caused by COVID-19 can also induce the production of procalcitonin by the cells, as part of the acute-phase response. The acute-phase response is a general reaction of the body to inflammation or infection, which involves the release of various proteins, such as procalcitonin, C-reactive protein (CRP), and ferritin (Kumar et la., 2022). Therefore, procalcitonin levels can reflect the presence and severity of bacterial infections, as well as the degree of inflammation and tissue damage in COVID-19 patients. However, procalcitonin levels are not specific for COVID-19 and can be influenced by many other factors, such as age, gender, obesity, smoking, diabetes, pregnancy, and other infections or inflammatory conditions. Therefore, procalcitonin levels should not be used alone to diagnose or monitor COVID-19, but rather in combination with other clinical and laboratory parameters.

Our study revealed that Sars-CoV-2 patients had lower mean total Lymphocyte count than the control healthy subjects. This result accords that of many previously studies (Huang et al., 2020; Fathi & Rezaei 2020. Our results further indicate that Lymphocyte count was correlated with disease severity. Critically ill COVID-19 patients had lower mean lymphocyte count than the asymptomatic and mildly ill COVID-19 patients. A study by Gu et al., (2021) analyzed the characteristics of lymphocyte subset alterations in COVID-19 patients with different levels of disease severity. They found that patients with critical COVID-19 infection exhibited an overall decline in lymphocytes including CD4+ T cells, CD8+ T cells, total T cells, B cells, and natural killer (NK) cells compared to mild and severe patients. However, some lymphocyte subsets, such as CD21 low CD38 low B cells, effector T4 cells, and PD1+ depleted T8 cells, were moderately increased in critical COVID-19 patients compared to mild cases. These studies show that lymphocyte count is an important factor in the pathogenesis and outcome of COVID-19. Lymphocyte count can reflect the immune status and inflammatory response of COVID-19 patients, and can help clinicians identify and distinguish patients with different disease severity and mortality risk.

There are several possible reasons for the reduction of lymphocytes in COVID-19 patients, according to literature. Both MERS-CoV and SARS-CoV directly infect human primary T cells and induce massive apoptosis and lymphopenia, while the viral expansion in these cells is abortive. The MHV-3, a murine coronavirus, also infects and destroys lymphocytes, thus facilitating viral replication and persistence (Jafarzadeh et al.,2021).Higher expression of p53, a key pro-apoptosis gene, was measured in Peripheral Blood Mononuclear Cells (PBMCs) collected from COVID-19 patients, suggesting that lymphopenia may be partly due to apoptosis(Xiong et al.,2020). The activation of caspase-1 as an effector element of inflammasome promotes IL-1β production and induces pyroptosis (Mendonça-Gomes et al., 2021). It was reported that SARS-CoV-related Viroporin 3a activates the NLRP3 inflammasome and induces the secretion of IL-1β, which indicate that the SARS-CoV infection can cause cell pyroptosis(da Costa et al., 2019). The Viroporin 3a has also been identified on the genome of SARS-CoV-2, which represents that SARS-CoV-2 may cause NLRP3 inflammasome activation (Mousavizadeh et al.,2021). SARS-CoV-2 can induce pyroptosis, particularly in lymphocytes, via the induction of NLRP3 inflammasome. Elevated serum levels IL-1β in COVID-19 patients also indicate the occurrence of pyroptosis, because IL-1β release is a downstream process of cell pyroptosis (Mousavizadeh et al., 2021)

Our study found increased mean Neutrophil count in Sars-Cov-2 patients than in the control subjects and increased Neutrophil count correlated with COVID-19 severity. Our results are in agreement with the results obtained by Wang et al., (2020), who reported elevated neutrophil count in COVID-19 patients. Neutrophils are a type of white blood cell that play an important role in the immune system. They are among the first cells to respond to an infection and can kill pathogens by various mechanisms, such as phagocytosis, degranulation, and the release of neutrophil extracellular traps (NETs). Neutrophils can increase in COVID-19, the disease caused by the novel coronavirus SARS-CoV-2, for several reasons; The viral infection itself can stimulate the production of neutrophils by the bone marrow and their release into the bloodstream. This is a normal response of the body to fight the infection and clear the virus. The cytokine storm, which is a dysregulated immune response that causes excessive inflammation and tissue damage, can also induce the production and activation of neutrophils. The lung injury and fibrosis caused by COVID-19 can also raise the neutrophil levels by triggering the release of more inflammatory mediators and activating the coagulation system, which can lead to blood clots and impaired oxygen delivery. Therefore, neutrophil levels can reflect the extent of inflammation and tissue damage in COVID-19 patients, as well as their risk of developing complications such as blood clots, acute kidney injury, respiratory failure, and death.

Recent studies have observed a significant rise in neutrophil count among patients with COVID-19 (Pastorek et al., 2022; Janiuk et al., 2021). Chen et al. (2021) reported that severe or critical COVID-19 patients had notably higher neutrophil counts upon admission than mild/moderate COVID-19 patients. In addition, elevated neutrophil count has been associated with increased disease severity and a poorer prognosis. Similarly, Li et al.(2021) observed that a significantly elevated neutrophil count could serve as an indicator to assess disease severity, consistent with the findings of previous reports (Chiang et al.,2020).

Neutrophils can release web-like structures called NETs, which capture and immobilize pathogenic microorganisms and produce elevated concentrations of myeloperoxidase (MPO) and defensins to resist exogenous infections (Papayannopoulos et al., 2018). NETs contain a combination of cell-free DNA, citrullinated histones and neutrophil granular proteins. Initially, the formation of NETs was discovered as a response of neutrophils to the presence of bacteria. Interestingly, NETs also possess antiviral defense effects (Thierry et al., 2020). Their role in combating viral infections has been observed in various diseases, including respiratory syncytial virus (RSV) (Muraro et al., 2018), dengue virus, influenza virus, and even human immunodeficiency virus (HIV) (Mutua et al., 2021; Saitoh et al., 2012.

Studies have demonstrated that NETs can promote the formation of thrombosis in a platelet-dependent manner through mechanisms such as platelet adhesion and activation, binding of cells to fibrinogen and von Willebrand Factor (vWF), and direct activation of the coagulation cascade (Behzadifard & Soleimani, 2022). Furthermore, NETs can initiate thrombosis by activating the extrinsic pathway through tissue factor (TF) production and the contact pathway via the activation of coagulation factor XII (FXII) (Behzadifard & Soleimani, 2022). Ammollo et al. discovered that the excess extracellular histones associated with NETs have prothrombotic activity by inhibiting thrombin-dependent protein C activation, leading to increased thrombin production (Ammollo et al., 2011).

Our study further recorded increased mean Neutrophil/Lymphocyte ratio (NLR) in COVID-19 patients than in the healthy control subjects. In addition NLR Correlated with COVID-19 severity. Severely and Critically ill COVID-19 patients had increased NLR than the asymptomatic and mildly ill patients. This result accords that of Chan et al., (2020) who reported increased NLR in COVID-19. The combination of an increased neutrophil count and a decreased lymphocyte count in COVID-19 patients leads to an elevated neutrophil-to-lymphocyte ratio (NLR) (Gujar et al., 2021; LA Torre et al., 2022). Sun et al. (2020) compared COVID-19 patients admitted to the intensive care unit (ICU) with non-ICU admitted COVID-19 patients, and found that COVID-19 patients admitted to ICU had the lowest lymphocyte

count, the highest neutrophil count and NLR, and the study showed that NLR was an independent predictor of disease severity in patients with COVID-19. Recent studies have identified NLR as an independent predictive marker of disease severity in COVID-19 patients (Wang et al., 2021). Zeng et al. (2021) found that the NLR remained significantly higher in non-survivors compared to survivors from admission to the end of hospitalization, further supporting the use of NLR as a reliable prognostic biomarker for early-stage COVID-19.

In COVID-19, the NLR may increase for several reasons. One possible reason is that the virus can infect and destroy lymphocytes, especially T cells, which are essential for eliminating infected cells and preventing viral replication. This can lead to a decrease in the number of lymphocytes and a relative increase in the number of neutrophils. Another possible reason is that the virus can trigger a cytokine storm, which is a hyperactive inflammatory response that releases large amounts of pro-inflammatory molecules, such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-alpha). These cytokines can stimulate the production and activation of neutrophils. Therefore, the NLR may be a useful biomarker for predicting the clinical outcome and guiding the treatment of COVID-19 patients. However, the NLR may also be affected by other factors, such as age, gender, comorbidities, medications, and infections. Therefore, the NLR should be interpreted with caution and in combination with other clinical parameters.

The mean Erythrocyte Sedimentation Rate (ESR) was elevated in COVID-19 patients than the healthy control subjects and ESR correlated well with COVID-19 severity. A review by Xie et al. (2020), including the data of 16,526 COVID-19 patients, evaluated the characteristics that predict progression and reported elevated ESR in 72.2% of the patients. In the meta-analysis of Zeng et al.(2020), CRP, procalcitonin, interleukin-6 (IL-6), ESR, and ferritin were found to be higher in severe COVID-19 patients. In a systematic review and meta-analysis of Mahat et al. (2021), CRP, ESR, procalcitonin, IL-6, IL-10, IL-2R, serum amyloid A, and the neutrophil-to-lymphocyte ratio were found to be significantly higher in severe COVID-19 patients than the asymptomatic and mildly ill COVID-19 patients.

A study done by Liu et al. (2020) in which they compared the clinical characteristics of COVID-19 patients with rapid or normal ESR and found that rapid ESR was associated with older age, male gender, and higher severity of illness. Also found that hemoglobin and C-reactive protein (A study by Wang et al. (2020) who evaluated the correlation between ESR and other inflammatory markers, such as neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), and eosinophils, with the severity of chest CT lesions in COVID-19 patients found that ESR correlated poditively with NLR and PLR .

Kaya et al. (2021) investigated the diagnostic utility of ESR as a prognostic factor for the disease severity and mortality in patients with COVID-19. The study found that ESR was significantly higher in patients who died or required mechanical ventilation than in those who survived or did not need mechanical ventilation. The study also found that ESR was an independent predictor of mortality after adjusting for other factors.

COVID-19 as a viral infection that can trigger an inflammatory response in the body, especially in the lungs and other organs can result in high ESR in some patients with COVID-19. However, the exact mechanism of how COVID-19 causes an increase in ESR is not fully understood. The virus may directly damage the red blood cells, making them more prone to clumping and settling (Zeng et al.,2020). The virus may stimulate the production of cytokines, which are inflammatory molecules that can affect the shape and function of red blood cells.

Erythrocyte Sedimentation Rate (ESR) is a simple, cheap, and rapid laboratory test that measures the rate at which red blood cells (erythrocytes) settle to the bottom of a test tube. The ESR test can indicate the presence of inflammation in the body, which may be caused by various conditions such as infections, autoimmune diseases, cancers, and others. However, the ESR test is not very specific, meaning that it cannot pinpoint the exact cause of inflammation or diagnose a particular disease. Moreover, the ESR test can be affected by several factors that are not related to inflammation, such as age, sex, pregnancy, anemia, obesity, and chronic diseases. These factors can increase or decrease the normal range of ESR values for different individuals. One way to account for these factors is to use formulas that adjust the normal ESR values according to age and sex. There are different formulas that have been proposed by various researchers, but one of the most widely used ones is the Miller formula (Miller, 1983) which was derived from a study of about 1000 individuals over the age of 20. According to this formula, the normal values of ESR in men is age (in years) divided by 2; for women, the normal value is age (in years) plus 10, divided by 2. For example, a 40-year-old man would have a normal ESR value of 20 mm/h (40/2), while a 40-year-old woman would have a normal ESR value of 25 mm/h ((40+10)/2). However, these values are only approximate and may vary depending on other factors such as ethnicity, health status, and laboratory methods.

In the current study inflammatory cytokines were significantly higher in COVID-19 patients than the control subjects. Furthermore, most inflammatory cytokines correlated very well with the disease severity. The severely and Critically

ill COVID-19 patients had increased mean cytokines profiles than the Asymptomatic,mild and moderately ill patients. Similar results have been obtained in previous studies (Rokni et al.,2020; Han et al.,2020; Kunnumakkara et al., 2021; Liu et al.,2020)

Cytokine release syndrome (CRS) is a life-threatening systemic inflammatory syndrome. Also known as a cytokine storm, CRS is characterized by elevated levels of circulating cytokines and immune cell hyperactivation. CRS can be triggered by various pathogens, cancers, autoimmune conditions, and T cell–based therapies (Kadura & Raghu, 2020). COVID-19 can trigger a cytokine storm, which is a condition where the body produces excessive amounts of inflammatory cytokines, such as interleukin-6 (IL-6), interleukin-1 (IL-1), interleukin-8 (IL-8), tumor necrosis factor-alpha (TNF-α), and interferon-induced protein-10 (IP-10). These cytokines can cause severe inflammation in the lungs and other organs, leading to acute respiratory distress syndrome (ARDS) and multiple organ dysfunction syndrome (MODS), which are the main causes of death in COVID-19 patients (Montazersaheb et al.,2022)

IL-6 is considered a key component of the immune response to SARS-CoV-2 (37). IL-6 has pleiotropic functions involved in acute infections due to its role in regulating the acute phase response (Rincon et al., 2012). It is produced by macrophages, monocytes, dendritic cells, mast cells, lymphocytes, and other non-lymphocytic cells, such as fibroblasts, endothelial cells, and keratinocytes. IL-6 expression is enhanced by IL-1β and TNF. Patients with severe COVID-19 have high serum levels of IL-6, which are associated with pulmonary inflammation and extensive lung damage (Schultheib et al., 2022).

Elevated IL-6 levels have been found in patients with COVID-19 and related to a poor prognosis (Dong et al., 2020). Wan et al.(2020) detected elevated IL-6 levels in one-third of patients with mild symptoms and three-quarters of those with severe symptoms, concluding that IL-6, alongside IL-10, may be of prognostic value in patients with COVID-19. A study of 452 patients infected with SARS-CoV-2 also reported that the elevation of IL-6 levels was more marked with more severe symptoms (Qin et al., 2020). IL-6 levels were also found to be markedly higher in patients who died from COVID-19 than in those who recovered (Zhou et al., 2020). It has been demonstrated that a high expression of IL-6 in patients with COVID-19 can accelerate the inflammatory process, contributing to the cytokine storm and worsening the prognosis (Zhou et al., 2020).

Liu et al. (2020) found elevated IL-1α levels in patients with severe COVID-19, and these were strongly associated with lung injury. IL-1 levels are related to the virulence of the process, and significantly higher serum levels have been observed in SARS-CoV-2 cases with severe symptoms than in mild cases or in those infected with the 2003 SARS-CoV or 2012 MERS coronavirus(Qin et al.,2020). Most COVID-19 patients with severe symptoms have elevated levels of IL-1β, which has been associated with SARS, hypercoagulation, and disseminated intravascular coagulation (Zhang et al., 2020). For this reason, some therapeutic strategies have used the inhibition of IL-1 in an attempt to avoid the cytokine storm (Tanaka et al., 2016).

TNF-α is produced by various cell types, such as monocytes, macrophages, and T cells, among others. This cytokine has been related to proinflammatory responses mediated by IL-1β and IL-6. Alongside other cytokines, TNF-α is involved in the regulation of inflammatory processes, infectious diseases, and malignant tumors (Pasquereau et al., 2017).

It has been observed that serum TNF-α levels are elevated in patients with COVID-19 and are higher with more severe disease (Qin et al., 2020). Diao et al.(2020) reported similar results in a sample of 522 patients with COVID-19 and found an inverse relationship between TNF-α levels and T-cell counts . In contrast, Wan et al. described normal TNF-α levels in patients with COVID-19 (Wan et al., 2020). TNF-α was one of the cytokines whose overproduction was related to a poor prognosis in patients with SARS-CoV and MERS (Costela-Ruiz et al., 2020). Zhang et al. proposed that the administration of certolizumab, an anti-TNF-α antibody, might have beneficial effects on patients with COVID-19 (Zhang et al., 2020).

Our study reveals that Interleukin 8 (IL-8) was elevated in COVID-19 Patients than in the control and covid-19 disease severity correlated with the levels of Interlekin 8 is a pro-inflammatory cytokine that plays a role in lung inflammation and injury, especially in the context of COVID-19. According to some recent studies, IL-8 may have potential as a biomarker for disease prognosis and a therapeutic target for COVID-19 patients. IL-8 is one of the main chemokines responsible for recruitment, activation, and accumulation of neutrophils in the lungs of patients with severe COVID-19

IL-8 levels correlated better than IL-6 levels with the overall clinical disease scores at different stages of the same COVID-19 patients. IL-6 and IL-8 can be respectively used as biomarkers for severe COVID-19 patients and for COVID-19 disease prognosis (Li et al., 2020).

A meta-analysis of 14 studies involving 1,462 COVID-19 patients found that serum IL-8 levels were significantly higher in severe cases than in non-severe cases, with a pooled mean difference of 16.67 pg/mL (95% CI 9.01 to 24.33). The authors concluded that IL-8 could be a potential biomarker for disease severity and prognosis of COVID-19 (Ma et al., 2021). IL-8 has demonstrated to be significantly higher in non-survivors compared to survivors of COVID-19, and the dynamic change of the serum IL-8 levels has been correlated with the severity of the disease (Nagant et al., 2020; Li et al., 2021). Similarly, within 1,484 COVID-19 patients, IL-8 was associated with decreased survival even after controlling for covariates including patient demographics and comorbidities. Furthermore, within 663 COVID-19 patients, IL-8 levels were shown to be associated with worse survival after controlling for covariates including Sequential Organ Failure Assessment (SOFA) severity scale scores (HR: 1.6, $p = 0.04$) (Del Valle et al., 2020). IL-8 serum levels have also been shown to correlate better than IL-6 levels with overall clinical disease scores (Li et al., 2020; Nagant et al., 2020). Scoring cytokine storm by levels of MCP-3 and IL-8, accurately can stratify COVID-19 patients for high risk of mortality (Chen et al., 2020). Thus, supporting the possibility of using IL-8 as prognostic biomarker.

The management of the cytokine storm in COVID-19 patients is challenging and requires a multidisciplinary approach. There is no specific treatment for COVID-19 yet, but some strategies may help to reduce the cytokine storm. Antiviral agents, such as remdesivir, favipiravir, lopinavir/ritonavir, umifenovir, ribavirin, and interferons. These agents may inhibit the replication of the virus and prevent further infection and inflammation. Corticosteroids, such as dexamethasone, methylprednisolone, and hydrocortisone. These agents may suppress the immune system and reduce the production of cytokines. However, they may also increase the risk of secondary infections and other adverse effects. Specific inhibitors of cytokines or their receptors, such as tocilizumab (anti-IL-6 receptor antibody), anakinra (recombinant IL-1 receptor antagonist), sarilumab (anti-IL-6 receptor antibody), siltuximab (anti-IL-6 antibody), canakinumab (anti-IL-1β antibody), etanercept (TNF receptor fusion protein), infliximab (anti-TNF antibody), adalimumab (anti-TNF antibody), baricitinib (JAK inhibitor), ruxolitinib (JAK inhibitor), and eculizumab (complement inhibitor). These agents may block the signaling pathways of cytokines and prevent their deleterious effects. However, they may also impair the immune response against the virus and other pathogens, and cause serious side effects such as infections, hypersensitivity reactions, and cytopenias. Immunomodulatory agents, such as thalidomide, lenalidomide, and mesenchyme stem cells (MSCs). These agents may modulate the immune system and restore the balance between pro-inflammatory and anti-inflammatory cytokines.

5. Conclusion

This study provides enough evidence that inflammatory markers are associated with the severity and prognosis of COVID-19. Inflammatory markers are, therefore, necessary important assays in the management of COVID-19 patients. Patients with elevated inflammatory markers should be given adequate attention and proper management to avert deterioration and cost effective markers like Neutrophil-Lymphocyte ratio may be used especially in resource constrained settings.

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Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

The study was conducted under a protocol that was reviewed and approved by the Tropical Diseases Research Centre (TDRC) Ethics Review Committee and National Health Research Authority (NHRA). Informed consent was obtained from all individual participants included in the study.

Statement of informed consent

We informed the study participants about the study objectives and procedures, and obtained their written informed consent. We ensured the privacy and confidentiality of the participants by using codes instead of names on the data collection forms and storing them in secure cabinets and password-protected computers.

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