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RESEARCH ARTICLE

Analysis of Cytokeratin-18 Levels in COVID-19 Patients

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ABSTRACT

COVID-19, in its early stages, causes severe acute respiratory distress syndrome (ARDS) and acute respiratory symptoms. The administration of Remdesivir in severe COVID-19-infected patients may result in liver damage, also known as Drug-Induced Liver Injury (DILI). Cytokeratin-18 (CK-18) is a potential biomarker for liver inflammation and injury in COVID-19-infected individuals, particularly those receiving antiviral therapies such as Remdesivir. Objective: This study evaluates CK-18 levels as an indicator of DILI and its correlation with the inflammatory marker interleukin-6 (IL-6) in COVID-19-infected individuals. Methods: A retrospective case-control study was carried out on 176 samples, separated into three distinct groups: 63 critical or severe COVID-19-infected patients treated in the intensive care unit (ICU-admitted group), 88 moderate COVID-19-infected patients treated in general hospital wards (non-ICU-admitted group), and 25 healthy individuals serving as the control group. CK-18, IL-6, and liver enzyme levels were measured, and statistical analysis was performed to identify substantial differences and correlations. Results: The CK-18 levels of the patients admitted to the ICU were substantially higher than those who were not and the control group ($p < 0.001$). Post-Remdesivir therapy, CK-18 levels increased significantly ($p < 0.001$). A substantial positive connection was found between CK-18 and IL-6 levels ($p = 0.001$), highlighting their combined role in inflammation and liver injury. Conclusion: CK-18 and IL-6 can serve as predictive biomarkers for liver damage and inflammation in COVID-19 patients, particularly those requiring critical care. These findings underscore the need for further research into CK-18's application in managing severe COVID-19 cases.

INTRODUCTION

SARS-CoV-2, known as COVID-19, was declared a pandemic in March 2020 by the WHO, months after the first case emerged in Wuhan in December 2019. By March 2020, it had spread to more than 180 countries. COVID-19 belongs to the beta-coronavirus family that shares phylogenetic traits with SARS-CoV. Droplet transmission from infected individuals to others in close contact is the primary mode of transmission. Acute respiratory symptoms that occur in the early stages may develop into ARDS and other severe complications, eventually leading to multiple organ failure (Jothimani et al., 2020; Vitiello et al., 2021).

Based on clinical symptoms and laboratory tests (WHO), COVID-19 infections can be divided into critical, severe, moderate/mild, and mild. Approximately 81% of cases are categorized as moderate/mild, while 14% and 5% of cases present severe and critical symptoms, respectively, requiring hospitalization. Nearly 20% of hospitalized patients require intensive care unit (ICU) treatment, and the mortality rate is alarmingly high at 61.5% due to various causes (Licata et al., 2021; Mangalmurti & Hunter, 2020; Pawlotsky, 2020).

There is currently no treatment known to be effective against COVID-19, and supportive care remains the primary approach. However, numerous drug candidates have been proposed for COVID-19 therapy. In Indonesia, Remdesivir is administered for treating severe COVID-19 cases at hospitals such as Dr. Soetomo General Academic Hospital (RSUD Dr. Soetomo), Surabaya (Nogai et al., 2021; Ye et al., 2020).

The administration of certain drugs for treating COVID-19 may result in drug-induced liver injury (DILI), characterized by liver cell death. Research in China by Cai et al. has shown that COVID-19 patients receiving Lopinavir/Ritonavir antiviral therapy had four times higher risk of experiencing liver injury than those who did not receive these drugs. Previous studies have also shown that patients treated with Remdesivir experienced an increase in serum aminotransferase levels (indicative of liver damage) by 15–50%. However, laboratory markers specifically indicating liver damage due to DILI, such as Cytokeratin-18, are rarely reported in these studies (Henry et al., 2022; Nogai et al., 2021; Vitiello et al., 2021).

This study aims to analyze CK-18 levels in COVID-19 patients receiving Remdesivir therapy as a parameter marker for liver damage caused by DILI and to determine the correlation between CK-18 levels and IL-6 as an inflammatory marker. The findings are expected to provide insight into CK-18 as a marker for liver damage due to DILI in COVID-19 patients.

METHODS

This study employed a retrospective case-control design. Data were obtained from 176 samples, of which 151 were confirmed COVID-19 patients at RSUD Dr. Soetomo from October 2022 to November 2022. Ethical clearance was obtained from the Ethics Committee of RSUD Dr. Soetomo No: 0369/KEPK/II/2022, and all of the patient's parents have agreed to give consent form.

Patients were diagnosed based on WHO guideline criteria and confirmed by RT-PCR. The 176 samples were separated into three distinct groups: 63 critical or severe COVID-19-infected patients treated in the intensive care unit (ICU-admitted group), 88 moderate COVID-19-infected patients treated in general hospital wards (non-ICU-admitted group), and 25 healthy individuals serving as the control group.

The samples were included based on these criteria: age over 21 years, positive COVID-19 PCR results, treatment in either the ICU or non-ICU, and an increase in ALT and AST levels exceeding three times the normal range, and without a history of liver disease. Patients who required ICU treatment but did not receive Remdesivir therapy and those who died within five days of Remdesivir administration were excluded from the study. Patients with negative RT-PCR results, no COVID-19 symptoms, no history of liver disease, and no use of drugs that could increase liver enzyme function were selected as controls.

Peripheral blood samples were collected from each participant for further examination of AST, ALT, IL-6, and CK-18 parameters using the ELISA method (Elabscience). Sample processing was performed by competent personnel specifically assigned to this task and in accordance with the reagent instructions for each examination.

All statistical analyses were conducted utilizing IBM SPSS version 25.0. The AST, ALT, IL-6, and CK-18 examination results were reported as mean ± standard deviation (SD). Clinical chemistry parameter results between the groups were compared utilizing the Mann-Whitney U test and presented as interquartile ranges (IQRs). Receiver operating characteristic (ROC) curve analysis and area under the curve (AUC) were employed to investigate possible degrees of severity and to determine the optimal cutoff for predicting severity in COVID-19. Statistical significance was defined as a p-value of less than 0.05.

RESULTS

Patient characteristics

The total sample size for this study was 176 patients, all meeting the inclusion criteria. The mean age of the 63 ICU-admitted patients (24 female and 39 male) was 51.05 years (SD 12.10 years). The mean age of the 88 non-ICU-admitted patients (41 female and 47 male) was 52.8 (SD 15.91). Meanwhile, the 25 healthy control patients (10 female and 15 male) had a mean age of 53.28 (SD 13.32). The p-

values for age and gender among the three groups were 0.383 and 0.310, respectively, indicating no substantial differences among the groups. Table 1 displays the characteristics of the COVID-19-infected patients.

Table 1: Characteristic data

Parameter	Control (25)	Non-ICU-Admitted (88)	ICU-Admitted (63)	p-value
Age	53.28	52.80	51.80	0.383[a]
Sex				0.310[b]
Female	10	41	24	
Male	15	47	39	

[a] Unpaired T-Test, [b]Chi-square Test

Result analysis

The laboratory results for AST, ALT, and CK-18 parameters are presented in Table 2 and Figures 1–3. The AST parameters of the patients receiving ICU care and those who did not were substantially higher (111.69 and 88.56, respectively) than those of the healthy control group (29.86) ($p < 0.005$). Meanwhile, the AST parameters of the ICU-admitted, non-ICU-admitted, and healthy control groups did not differ substantially ($p = 0.1771$).

The CK-18 parameters of the ICU-admitted group (137.65) were substantially higher than those of the healthy control group (39.18) but not exceeding those of the non-ICU-admitted group (67.32) ($p < 0.001$).

Table 2: Clinical chemistry parameter data

Parameter	Control (25)	Non-ICU-Admitted (88)	ICU-Admitted (63)	p-value
AST (U/L)	29.86	29.86	111.69	0.005 [a]
ALT (U/L)	44.08	44.08	102.76	0.171 [a]
CK-18 (U/L)	39.18	39.18	137.65	0.000[a]

[a] Mann-Whitney test

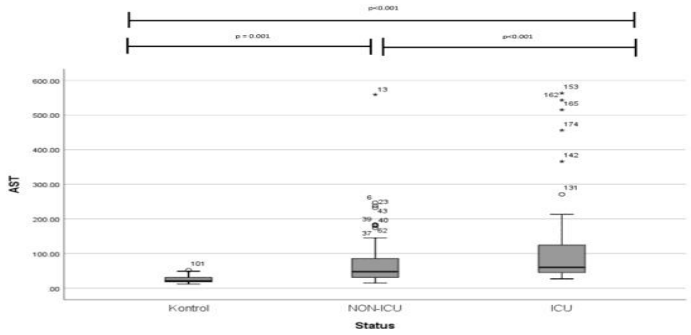

Figure 1: AST Value Comparison: The AST levels of the patients receiving ICU care and those who did not exceed those of the healthy control patients (p less than 0.001).

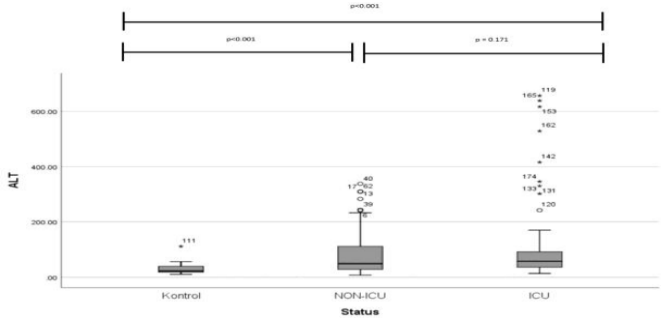

Figure 2: ALT Value Comparison: The ALT levels of all the groups did not differ substantially (p less than 0.171).

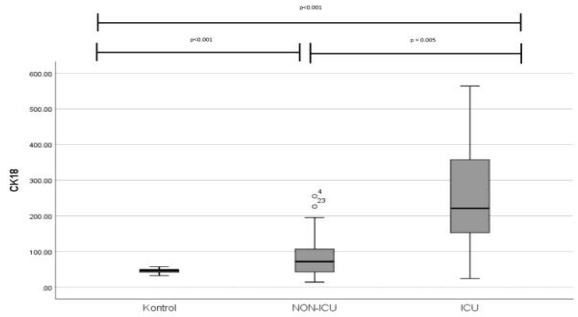

Figure 3: CK-18 Value Comparison: The CK-18 levels of the patients receiving ICU care and those who did not were substantially different (p less than 0.001).

Table 3 and Figure 4 compare CK-18 levels before and after five days of Remdesivir therapy. CK-18 levels after therapy were substantially exceeded (220.99) CK-18 levels before therapy (57.54) (p less than 0.001).

Table 3: CK-18 Data of the ICU-admitted patients before and after Remdesivir therapy

Parameter	Pre-Therapy	Post-Therapy	p-value
CK-18 (U/L)	57.54	220.99	< 0.001[c]

[c] *Wilcoxon*

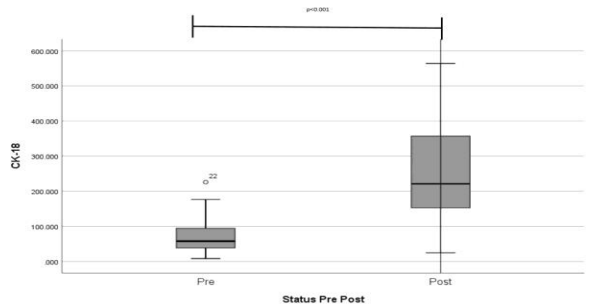

Figure 4: Comparison of CK-18 levels before and after remdesivir therapy: The CK-18 levels of the ICU-admitted patients were significantly higher after therapy (p < 0.001).

In this study, IL-6 levels were also analyzed to determine the relationship between pro-inflammatory mediators and intensive care requirements in COVID-19 patients. Using Spearman correlation analysis, a significant relationship was found between IL-6 parameters and the need for intensive care (p = 0.001).

DISCUSSION

Among COVID-19-infected patients, males (57%) outnumbered females (43%), and the same was true for ICU-admitted patients, where 31% were male and 27% were female. Older age and male sex are epidemiologically connected to the prevalence of severe COVID-19 symptoms. Other research has confirmed that male sex and increasing age are associated with worse disease severity. Advanced age may increase the likelihood of worsening COVID-19 infection, likely due to a decline in T cell and B cell function, which leads to a general weakening of the immune system (Bertolini et al., 2020; Costela-Ruiz et al., 2020; Gao et al., 2021; Ye et al., 2020).

The mean AST levels of the patients receiving ICU care and those who did not, as well as the healthy control group, were substantially different. The mean ALT levels of the patients receiving ICU care and those who did not, as well as the healthy control group, were not substantially different. However, the average ALT level of the ICU-admitted group exceeded that of the healthy control group. This finding aligns with a study by Grein et al. (2020), demonstrating that 22% of COVID-19 patients receiving Remdesivir experienced liver enzyme increases. The increase in ALT and AST levels in patients treated with Remdesivir was not excessive (less than five times the normal value). Remdesivir therapy administered for 5 to 14 days was associated with minor increases in serum aminotransferase levels (less than five times the normal upper limit) (Bertolini et al., 2020; Gao et al., 2021; Marjot et al., 2021).

Based on research conducted by Gilead and the FDA, Remdesivir administration is not recommended in COVID-19-infected patients with increased AST/ALT levels exceeding five times the normal value (Jothimani et al., 2020).

The mean CK-18 levels of the patients receiving ICU care and those who did not, as well as the healthy control group, were not substantially different. Several observational studies have demonstrated a substantial increase in CK-18 levels in critically ill patients with ARDS, including those with COVID-19. This demonstrates the potential of the CK-18 test in risk stratification for COVID-19-infected patients, as well as its possible use as a quantitative biomarker for cell death to assess the effectiveness of treatments in the early phases of clinical trials (Korver et al., 2021; Nogai et al., 2021).

In the correlation test, a substantial correlation was found between increased CK-18 levels and elevated IL-6 levels in the ICU-admitted patients. Interleukin-6 serves as a predictor of an uncontrolled inflammatory state, making it a valuable prognostic marker for confirmed COVID-19-infected patients. This condition is associated with hypercytokinemia. Increased cell death rates may also be influenced by TNF-α, IL-6, and IL-1, which are capable of inducing caspase-mediated cell death. The observed positive correlation between CK-18 levels and inflammatory markers in this study supports this finding (Costela-Ruiz et al., 2020; Gao et al., 2021; Sun et al., 2020).

This research faces multiple limitations. Firstly, it was conducted in only one health center. Secondly, the sampling was inconsistent regarding the length of hospital stay. Future research should consider uniform sampling methods to account for variations in the length of stay.

CONCLUSIONS

This study analyzed 176 samples, including 63 ICU-admitted patients and 88 non-ICU-admitted patients. A substantial difference was found between the AST and CK-18 levels of the patients receiving ICU care and those who did not. Additionally, a unidirectional correlation was observed between the need for ICU care and IL-6 levels in COVID-19-infected patients.

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REFERENCES

Bertolini A, IP van de Peppel, F Bodewes, H Moshage, A Fantin, F Farinati, et al., 2020. Abnormal Liver Function Tests in Patients With COVID-19: Relevance and Potential Pathogenesis. Hepatology, 72: 1864-1872.

Costela-Ruiz VJ, R Illescas-Montes, JM Puerta-Puerta, C Ruiz and L Melguizo-Rodríguez, 2020. SARS-CoV-2 infection: The role of cytokines in COVID-19 disease. Cytokine Growth Factor Rev, 54: 62-75.

Gao YM, G Xu, B Wang and BC Liu, 2021. Cytokine storm syndrome in coronavirus disease 2019: A narrative review. Journal of Internal Medicine, 289: 147-161.

Henry BM, I Cheruiyot, SW Benoit, F Sanchis-Gomar, G Lippi and J Benoit, 2022. Cytokeratin 18 cell death assays as biomarkers for quantification of apoptosis and necrosis in COVID-19: a prospective, observational study. Journal of Clinical Pathology, 75: 410-415.

Jothimani D, R Venugopal, MF Abedin, I Kaliamoorthy and M Rela, 2020. COVID-19 and the liver. Journal of Hepatology, 73: 1231-1240.

Korver S, J Bowen, K Pearson, RJ Gonzalez, N French, K Park, et al., 2021. The application of cytokeratin-18 as a biomarker for drug-induced liver injury. Archives of Toxicology, 95: 3435-3448.

Licata A, MG Minissale, M Distefano and G Montalto, 2021. Liver injury, SARS-COV-2 infection and COVID-19: What physicians should really know? GastroHep, 3: 121-130.

Mangalmurti N and CA Hunter, 2020. Cytokine Storms: Understanding COVID-19. Immunity, 53: 19-25.

Marjot T, GJ Webb, ASt Barritt, AM Moon, Z Stamataki, VW Wong, et al., 2021. COVID-19 and liver disease: mechanistic and clinical perspectives. Nature Reviews: Gastroenterology & Hepatology, 18: 348-364.

Nogai L, K Singh and R Bohra, 2021. Dealing with SARS COV-2 pandemic: perusing remdesivir and hydroxychloroquine as therapeutic alternative. European Journal of Molecular & Clinical Medicine, 7: 2132-2148.

Pawlotsky J-M, 2020. COVID-19 and the liver-related deaths to come. Nature Reviews Gastroenterology & hepatology, 17: 523-525.

Sun X, T Wang, D Cai, Z Hu, J Chen, H Liao, et al., 2020. Cytokine storm intervention in the early stages of COVID-19 pneumonia. Cytokine Growth Factor Rev, 53: 38-42.

Vitiello A, R La Porta, V D'Aiuto and F Ferrara, 2021. The risks of liver injury in COVID-19 patients and pharmacological management to reduce or prevent the damage induced. Egypt Liver J, 11: 11.

Ye Q, B Wang and J Mao, 2020. The pathogenesis and treatment of the `Cytokine Storm' in COVID-19. Journal of Infection, 80: 607-613.