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Association of Ferritin Levels and Biochemical Markers with COVID-19 Severity

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Abstract

Objective: To investigate the association between ferritin levels and other biochemical markers (such as total bilirubin, LDH, AST, and ALT) with the severity of COVID-19 and to identify potential predictive markers that could help in assessing disease severity, improving patient management, and reducing mortality rates.

Methodology: This retrospective observational study was conducted between June 2020 to December 2020 admitted to various hospitals in twin cities, comprised 420 confirmed cases of COVID-19 detected through PCR testing. Blood samples collected upon admission from patients in various hospitals across Islamabad and Rawalpindi were analyzed for Lactate Dehydrogenase (LDH), Aspartate Aminotransferase (AST), Alanine Transaminase (ALT), Total Bilirubin and ferritin levels . The diagnosis was made using Reverse Transcription-Polymerase Chain Reaction (RT-PCR) on nasal and pharyngeal swab specimens

The data was statistically analyzed by using SPSS ver 25. The Kruskal-Wallace test was used to compare severe, moderate, and mild patient COVID-19 groups

Results: The mean age of the deceased was significantly high 76.09± 4.94 years vs 54.34 ± 13.03 years compared to those recovered. Deceased had ten times higher median levels of ferritin 1000 ng/ml vs. 120ng/ml. In multivariate analysis, advanced age (AOR 1.673 C.I (1.372,2.040) p = 0.0001)and a raised level of ferritin (AOR 2.938 C.I. (1.244,6.941) p = 0.014) showed significant association with mortality.

Conclusion: Ferritin and age can be used to predict the severity of COVID-19. This study can help physicians to lower the mortality rate and number of admissions in the ICU.

Keywords: Ferritin, Coronavirus, Covid -19, risk factors, inflammatory marker.

Introduction

The COVID-19 illness is caused by a novel type of Coronavirus, later named as Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) which was first detected and isolated in December 2019 in Wuhan City, located in Hubei Province, China.[1] The virus resembles genetically to SARS-CoV and MERS-CoV.[2] Due to a significant rise in cases and fatalities, a global health emergency was declared by the World Health Organization (WHO)on January 30, 2020.[3] This pandemic caused not just a health crisis but also had serious impacts on the economy and society. Infected individuals exhibited a spectrum of symptoms ranging from asymptomatic to symptomatic, with common manifestations including shortness of breath, dry cough, tachypnea, and fever.[4]

Numerous studies, carried out Worldwide, have reported raised concentrations of different clinical chemistry parameters, particularly ferritin, following COVID-19 infection.[5,6] Ferritin is an iron-storage protein found in body cells. Its levels may rise in response to infection or inflammation.[7] Inflammation is characterized as a nonspecific immune reaction elicited against potential or acute infections. The immune system mounts two responses: innate and adaptive, both crucial in fighting the infection[8]Inflammation causes the release of several cytokines, but some of the more common ones include Interleukin 1 beta (IL-1β), Interleukin-17 (IL-17), Interleukin-6 (IL-6) and Tumour Necrosis Factor-Alpha (TNF-α).[9] Ferritin is an integral mediator in immune dysregulation, which culminates in a cytokine storm.[10] Its concentration increases in various diseases and cancers.[11]

The RNA molecules of SARS-CoV-2 can potentially serve as pathogen-associated molecular patterns by binding and activating the Toll-like receptors. This activation subsequently stimulates the innate immune response, resulting in the generation of pro-inflammatory agents such as ferritin. Upon endocytosis, ferritin stimulates the synthesis of key cytokines such as IFN-γ, IL-6, and IL-

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1β.[12] This study aims to investigate routine chemistry parameters, including total bilirubin, Aspartate Aminotransferase (AST), Lactate Dehydrogenase (LDH), Ferritin, and Alanine Transaminase (ALT) as inflammatory markers for predicting the severity and mortality rate in case of OVID-19.

Methodology

The clinical chemistry data of COVID-19 patients with moderate illness as well as those admitted to various hospitals in twin cities was retrieved from the hospital management information system. The World Health Organization (WHO) guidelines were followed to categorize the patients into three groups, depending on the severity of their disease. The diagnosis was made using Reverse Transcription-Polymerase Chain Reaction (RT-PCR) on nasal and pharyngeal swab specimens. The study excluded patients with chronic inflammatory diseases or secondary infections during COVID-19. Clinical chemistry parameters including LDH, ferritin, total bilirubin, AST, and ALT were assessed using the automated Chemistry Analyzer Beckman Coulter, and ferritin was tested using Immulite Special Chemistry Analyzer.

The data was statistically analyzed by using SPSS (version 25). The Kolmogorov-Smirnov and Shapiro-Wilk tests were used for evaluating normality. The values of non-parametric clinical chemistry variables data were described as the median, minimum, and maximum. The Kruskal-Wallace test was used to compare severe, moderate, and mild patient COVID-19 groups. The Mann-Whitney U test was used to analyze the data on gender and survivors against non-survivors. The Binary logistic regression analysis was performed to check the association of different variables with COVID-19 severity. The Receiver Operator Curve (ROC)was drawn to find the cut-off values of significantly associated variables.

Results

Majority of participants in this study were males218 (51.9%) with a mean age of 55.6±15.32. The most affected age group was 41-60 (41.9%) followed by 61-80 (33.6%). Among those enrolled, 49 (11.7%) died during hospitalization. The laboratory findings of all of the patients are shown in Table I. The concentrations of ALT, AST, total bilirubin, LDH, and ferritin were elevated in severeCOVID-19 infection (Table I). Demographic and biochemical parameters of study participants at presentation

ALT and total Bilirubin concentrations were significantly different between males and females, p = 0.0001 and p = 0.015, respectively. No significant differences were observed for Age, AST, LDH, and Ferritin between the two groups (Table II).

Table II: Comparison of biochemical parameters between female and male COVID-19 patients.

Parameters	Males (218)	Females (202)	p-value
	Mean ± SD		
Age (years)	55.59 ± 15.32	52.98 ± 16.66	0.165
ALT (U/L)	54.62 ± 43.29	45.80 ± 46.05	0.0001
AST (U/L)	74.62 ± 81.24	76.51 ± 11.89	0.465
LDH (U/L)	728.84 ± 640.43	655.82 ± 507.76	0.829
Total Bilirubin (mg/dl)	0.81 ± 0.56	0.87 ± 1.98	0.015
Ferritin (ng/ml)	371.52 ± 447.12	305.81 ± 425.19	0.071

Table III: Comparison of study variable between Recovered and Deceased.

Parameters	Recovered (371)	Died (49)	p-value
	Mean ± SD		
Age (years)	51.35 ± 14.51	76.09 ± 4.94	0.0001
ALT (U/L)	51.07 ± 44.85	45.14 ± 44.55	0.126
AST (U/L)	71.53 ± 96.78	105.76 ± 125.7	0.013
LDH (U/L)	645.33 ± 483.26	1060.1 ± 995.07	0.0001
Total Bilirubin (mg/dl)	0.84 ± 1.51	0.81 ± 0.54	0.737
Ferritin ng/ml)	248.06 ± 346.30	1035.3 ± 432.41	0.0001

Table I: Comparison of study variables between male and females.

Variables	Mild (n= 123)	Moderate (n= 183)	Severe (n= 114)	Total (n= 420)	p-value
Age(years)	46.5±17.6	50.5±11.4	68.8±10.3	54(16-86)	0.0001
Sex(male)	55(25.2%)	101(46.3%)	62(28.0%)	218 (51.9%)	0.164
ALT (U/L)	34.1(12-250.3)	36.4(12-312.5)	38.8(12-301.8)	37.2(12-312.5)	0.0583
AST (U/L)	46.4(15.6-279.1)	51.0(12.1-335.0)	55.3(20.6-1441.0)	50.4(1441.0)	0.093
TB (mg /dl)	0.6(0.3-8.0)	0.6(0.3-26.8)	0.6(0.3-2.8)	0.6(0.3-26.8	0.114
LDH (U/L)	521.8 (106.5-3864.0)	440.2 (126.8-3864.0)	727.6(185.0-3864.0)	523.7(106.5-3864.0)	0.0001
Ferritin (ng/ml)	17.2(6.3-416.0)	121.0(6.3-797.0)	1000.0(6.30-1500.0)	127 (6.3-1500.0)	0.0001
Mortality	1(2%)	1(2%)	47(95.9%)	49(11.7%)	0.0001

The deceased subjects were significantly older (76.09 years) compared to those who recovered (51.35 years). Regarding biochemical parameters, Ferritin levels were markedly higher in deceased patients (1035.3 ng/ml) compared to recovered patients (248.06 ng/ml), p-value = **0.0001**. Although ALT levels are slightly lower in deceased patients (45.14 U/L) than in recovered patients (51.07 U/L), the difference is not statistically significant (**p = 0.126**), indicating that ALT may not be a strong predictor of COVID-19 outcomes in this cohort. AST and LDH concentration were also significantly high in deceased subject. Figure 1 and Table III

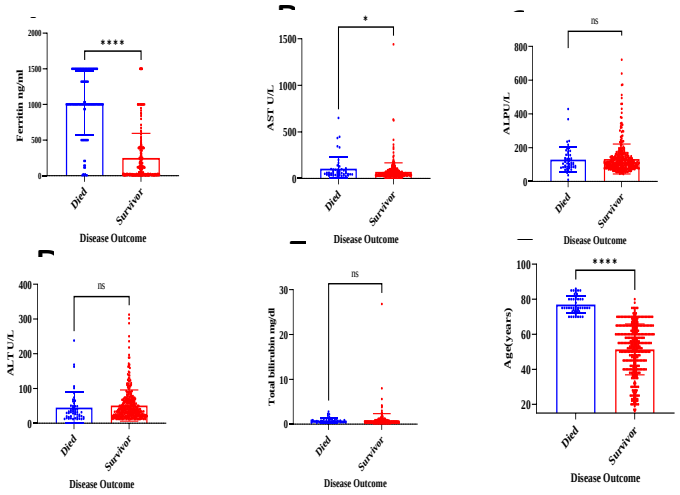

Figure 1. Comparison of different variable between Recovered and Deceased.

A, Ferritin concentrations and disease outcome;
B, AST concentrations and disease outcome;
C, ALP concentrations and disease outcome;
D, ALT concentrations and disease outcome;
E, Total bilirubin concentrations and disease outcome;
F , Ages in year and disease outcome

Binary Logistic regression analysis (Table IV) showed that age has a significant association with mortality followed by ferritin.

According to univariate regression analysis, mortality was found to be significantly associated with AST, age, and ferritin. However, in the multivariate regression analysis, the Odd Ratio of AST was not significant. The ROC curve was carried out for age and ferritin. The cut-off value for ferritin was 490.5 ng/ml with 89.9% sensitivity and 85.7% specificity. Additionally, the cut-off value for age was 73.5 years with a sensitivity of 75.5% and specificity of 98.7%. (Table V)

Discussion

The participants in this study had a mean age of 54.33 ± 16.01 years, with a majority being male (51.9%). This was in accordance with the study by Nafisa et al. (2024)[13]. Another study carried out in the US also reported that 83.8% of COVID-19 patients on ventilators were males[14]. Ranjan et al. (2021) demonstrated that testosterone and dihydrotestosterone resulted in the higher susceptibility of males to COVID-19. They concluded that the Androgen-Androgen receptor can enhance the expression of TMPRSS2 and augment the COVID-19 Severity.[15] The age group 41 to 60 years was the most dominant one and had the maximum number of COVID-19 cases. The majority of fatalities occurred among individuals aged 60 and above in Pakistan.[16] The reason behind this could be pre-existing morbidities, social habits, and physiological characteristics. The increase in COVID-19 severity with age can be attributed to heterologous, adaptive, and innate immunity as well as differences in clotting and endothelial function.[17] The results of this study were compared with the

Table IV: Binary Logistic regression analysis for ALT, AST, LDH, Ferritin, total Bilirubin, age and Gender.

	Univariate regression Analysis		Multivariate regression Analysis	
Variables	Crude odds ratio	p-value	Adjusted AOR(95%CI)	p-value
Age (years)	1.651(1.40, 1.44)	0.0001	1.673(1.372,2.040)	0 .0001
Sex	1.567(0.835, 2.828)	0.167	0.342(0.096,1.222)	0.099
ALT (U/L)	0.462(0.169, 1.265)	0.133	0 .125(0.013,1.157)	0.067
AST (U/L)	3.792(1.56, 9.216)	0.003	2.818(0.603,13.17)	0.188
TB (mg/dl)	1.398(0.446,4.376)	0.565	2.119(0.118,38.210)	0.611
LDH (U/L)	3.674(1.387,9.736)	0.009	1.437(0.223,9.244)	0.703
Ferritin (ng/ml)	27.84(10.01,77.37)	0.0001	2.938 (1.244,6.941)	0.014

Table V: The performance criteria of the inflammatory markers to predict the COVID-19 severity.

Parameters	AUC (95%CI)	Cut-off Value	Sensitivity (%)	Specificity(%)	*p*-value
Age (Years)	0.982	73.5 Years	75.5%	78.7%	0.0001
Ferritin (ng/ml)	0.892	490.5 ng/ml	89.8%	85.7%	0.014

questionnaires filled by different participants and it can be analyzed that there is a strong relationship between increasing age and comorbidities with COVID-19 severity.

This study identified the inflammatory markers in COVID-19 patients. It was observed that except for ALT and total bilirubin, an increased level of inflammatory markers was observed in non-survivors. However, several other studies reported that the level of all liver inflammatory markers, including total bilirubin increases in severe cases of COVID-19.[18-20] The possible reason for liver injury during COVID-19 can be either because of drug-induced liver injury. The course of different antibiotics, corticosteroids, and antivirals during hospitalization can result in liver injury.[21] Further studies are required to check the association between liver injury and drugs during COVID-19.

In the current study, median levels of ferritin were higher in severe and moderate cases as compared to mild cases [121.0(6.2-797.0) & 1000.0(6.30-1500.0) vs 17.2(6.3-416.0) (p=0.0001)].These results were by the study proposed by Kurian et al. in 2023 [545.8 (326.0, 1046.0) vs 97.3 (52.65–155.5) (p = 0.001)].[22] Additionally, they observed higher ferritin levels in patients who experienced complications compared to those without. Another study conducted by Zeng et al.(2020) and Volfovitch et al. (2022) also concluded higher ferritin levels in the severe group[23,24][23,24].The ferritin level increases in different viral infections. The inflammatory cytokines in cytokine storm increase the secretion of ferritin which in turn results in hyperferritinemia. This hyperferritinemia in turn results in multiple organ damage and ultimately death in COVID-19.[25]

The ferritin cut-off value in this study was found to be 490.5 ng/mL, with an 89.9% sensitivity and 85.7% specificity. This is in line with findings of Jabeen et al (2024) who reported a ferritin cut-off value of 506 ng/ml but differ from Cao et al. who reported a lower cut off value of 264.5 ng/ml with 94.2% specificity and 73.9% sensitivity.[26,17] The studies conducted by Nafisa et al.(2024), Jabeen et al. (2024), and Nakanishi et al. (2021) observed the negative out comes of COVID-19 in older subjects.[13,26,27] This indicated that COVID-19 patients having ferritin levels greater than 490.5 ng/ml and age higher than 73.5 years are more likely to have moderate to severe infection. This suggests that hyperferritinemia can also be considered as a risk factor for determining the severity of COVID-19. Therefore, appropriate factors such as higher age, iron level in diet, and use of iron chelators should be considered in COVID-19 patients.

This study had a sample size of 420 patients and included COVID-19 cases from mild to severe which seems to be a major advantage of this study. The retrospective nature of this study is the limitation of this study. Moreover, the role of drugs in liver injury and pre-COVID ferritin levels were also not assessed in this study.

Conclusion

Ferritin and age can be used to predict the severity of COVID-19 in the early stages. Special attention to these inflammatory markers can help in better management and reducing the mortality rate.

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