BMC Infectious Diseases

RESEARCH

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Comparative analysis of C-Reactive protein levels among Non-comorbid, Comorbid, and Multimorbid Hospitalized COVID-19 patients

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Abstract

Background C-reactive protein (CRP) is one of the most commonly monitored inflammatory markers in patients with COVID-19 to gain insight into the inflammation level in the body and to adopt effective disease management and therapeutic strategies. COVID-19 is now less prevalent, and the study of CRP as a biomarker of inflammation still needs deeper understanding, particularly in understanding its role among patients with comorbidities, which are known to influence inflammatory responses and increase the risk of severe outcomes during acute and chronic infectious diseases. The objective of this study was to evaluate the association of major comorbidities such as ischemic heart diseases, diabetes, chronic kidney disease, hypertension, and lung infections e.g. tuberculosis with serum CRP levels in hospitalized COVID-19 patients.

Methods This study involves a retrospective observational framework to monitor CRP levels among hospitalized COVID-19 patients after getting ethical approval and patient consent. The information on underlying health conditions or comorbidities and age was collected from the patient data files. The requirement of ventilation, ICU admission, mortality & survival, and CRP levels were monitored based on their daily updates in the data file. Furthermore, the association of CRP levels was evaluated with disease severity and mortality.

Results In this study 618 out of 750 hospitalized COVID-19 patients, of which 62.6% were male and 37.4% were female, the levels of serum CRP were significantly influenced by age and comorbidities. No case of hospitalization was observed in children (≤ 14 years) during the study period, while 38.3% of patients belonged to the old age group (≥ 65 years). Comorbidity status varied, with 36.1% of patients without having any comorbidities, 27.8% with one, 23.6% with two, and 12.5% with three or more comorbidities. Descriptive statistics revealed that the CRP levels in the study population averaged 88.92 mg/L (SD = 63.95), ranging from < 1 mg/L to 900 mg/L, with significant variations observed across different comorbidities and age groups. CRP levels, analyzed by the Kruskal-Wallis test, showed significant variations in different age groups of COVID-19 patients ($\chi^2 = 66.741$, df = 3, $p < 0.001$). Moreover,

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pairwise comparisons showed considerable differences between young and middle-aged groups (Z = -2.724, $p < 0.01$) and young and old age groups of COVID-19 patients (Z = -3.970, $p < 0.001$). The most prevalent comorbidities observed in COVID-19 patients in this study were hypertension (42.1%), diabetes (33.8%), ischemic heart disease (16.5%), asthma (11.2%), chronic kidney disease (7.9%) and Tuberculosis (1.9%). The CRP levels fluctuate and also significantly differ among different comorbidities. COVID-19 patients with diabetes were observed to have higher CRP levels than non-diabetics (mean CRP: 126.96 mg/L vs. 88.92 mg/L, Z = -5.724, $p < 0.001$), and those with hypertension also encountered elevated CRP (mean CRP: 355.37 vs. 276.19 mg/L, Z = -5.447, $p < 0.001$). Similar tendencies were detected in COVID-19 patients with ischemic heart disease (mean CRP: 385.43 mg/L, Z = -4.704, $p < 0.001$), chronic kidney disease (mean CRP: 412.37 mg/L, Z = -4.206, $p < 0.001$) as well as with tuberculosis (mean CRP: 458.08 mg/L, Z = -2.914, $p < 0.01$). CRP levels on days 1 and 3 of hospitalization showed a decline (88.92 mg/L to 67.89 mg/L), representative of a response to treatment to reduce the inflammation in the body. Furthermore, high levels of CRP were significantly associated with a high requirement of non-invasive ventilation (mean CRP: 110.80 mg/L vs. 76.82 mg/L, $p < 0.05$), mechanical ventilation (mean CRP: 134.46 mg/L vs. 77.25 mg/L, $p < 0.05$) and ICU admission (mean CRP: 126.96 mg/L vs. 72.79 mg/L, $p < 0.05$). The Cox regression analysis showed that there is a considerable association of CRP level with the expected length of hospitalization, each 1-unit increase in CRP levels was associated with a 0.6% increase in extended stay risk (hazard ratio = 1.006, 95% CI: 1.004–1.008, $p < 0.001$). Furthermore, the logistic regression analysis performed on CRP levels that was monitored on the first day of hospitalization, revealed that there was a 2.7% increase in mortality odds with each unit increase in CRP (odds ratio = 1.027, 95% CI: 1.022–1.033, $p < 0.001$), which suggest CRP as a potential mortality predictor.

Conclusions Elevated CRP levels in COVID-19 patients with comorbidities like diabetes, hypertension, ischemic heart disease, and chronic kidney disease were strongly associated with increased disease severity, including higher ventilation requirements and mortality. Patients with these comorbidities showed significantly higher CRP levels, which correlated with worse outcomes, including ICU admissions and prolonged hospital stays, emphasizing the importance of CRP as a predictor for severe complications in patients with infectious diseases along with one or more comorbidities.

Keywords COVID-19, Comorbidities, Multimorbidity, C-reactive protein (CRP)

Introduction

COVID-19, caused by SARS-CoV-2, is a pulmonary infection that can lead to severe complications through the excessive release of cytokines known as a cytokine storm [1]. This hyper-inflammatory state is associated with severe complications and high mortality rates in COVID-19 patients, particularly those with underlying health conditions or comorbidities such as hypertension, chronic kidney diseases, cardiovascular disease, chronic respiratory disorders, diabetes, etc. Patients with pre-existing comorbidities may experience impaired immune responses, worsening the inflammatory cascade, and producing uncontrolled cytokines [2–5]. The advancement in the development of vaccines against COVID-19 has significantly contributed to disease prevention, however understanding the complex immunological state of cytokine storms, especially in comorbid COVID-19 patients, remains essential to minimize the severe outcomes, mortality rate and adopting effective therapeutic approaches [6–8].

C-reactive protein (CRP), is an acute-phase protein released primarily by the liver in response to stimulation of pro-inflammatory cytokines, such as interleukin-6 (IL-6). It is a well-established biomarker to monitor the inflammation rate in COVID-19 patients. CRP play an important role in initiating the innate immunological response by binding with damaged cells and to the surface of pathogens, leading to activating the complement system and phagocytosis. This series of activities highlights the importance of monitoring CRP during COVID-19 infection. Elevated levels of CRP act as a key biomarker of high rate of inflammation and disease severity because they represent the intensity of the immune response activated due to infections like COVID-19.

The serum concentration of C-reactive protein (CRP) has been extensively used as an inflammatory biomarker in patients with COVID-19 globally. However, its variation in COVID-19 patients with multimorbidities has received limited attention. Multimorbidity, which is characterized by the coexistence of two or more chronic diseases in a patient, poses unique challenges in COVID-19 or other infectious diseases management, requiring a deep understanding of CPR variation as an inflammatory marker for monitoring infection and overall disease management [8–10] Recent studies demonstrated that COVID-19 patients with diabetes mellitus (DM) and uncontrolled blood glucose level have overstated inflammatory responses resulting in a significantly higher level

of CRP which may exceed 120 mg/L, in comparison to non-diabetic patients [11, 12] Studies also revealed that COVID-19 patients with chronic kidney disease (CKD) as comorbidity also have similar elevated CRP levels trends (≥100 mg/L), which possibly due to systemic inflammation caused by uremia. Patients with chronic heart diseases also face higher levels of systemic inflammation resulting in higher CRP concentrations which increases the risk of adverse outcomes [13].

In pulmonary inflammatory diseases like Tuberculosis (TB), a higher CRP of more than 50 mg/L has been used as an inflammatory biomarker to evaluate the disease severity. TB patients infected with COVID-19 infection further elevate the inflammation in the body due to the concurrent inflammatory processes. These studies suggest monitoring the comorbidities with any infectious disease involving inflammatory response to critically evaluate the body status to defend against the disease. Such as Heart disease, acute or chronic kidney diseases, lung diseases like TB and asthma. These studies have highlighted the role of CRP in evaluating the inflammation in the body keeping in view the fluctuation in CRP level due to the presence of different comorbidities and adopting the specific therapeutic approaches accordingly to manage them [14].

This study aims to evaluate CRP levels across different groups of COVID-19 patients including non-comorbid, comorbid, and multimorbid patients, to investigate how comorbidities affect the CRP levels. This study will help to demonstrate the effect of different prevalent comorbidities such as hypertension, diabetes, chronic kidney diseases and ischemic heart diseases on overall inflammation in COVID-19 patients. The findings of this study are expected to contribute to more personalized treatment approaches and enhance patient care for infectious diseases.

Methodology

This observational retrospective study was conducted on a cohort of 750 hospitalized COVID-19 patients admitted to the "Isolation Hospital & Infectious Treatment Center (IHITC), Islamabad", and the "Pakistan Institute of Medical Sciences (PIMS), Islamabad", from August 2021 to May 2022, adhering to ethical standards and maintaining patient confidentiality. This study design was approved by the Advance Studies and Research Board (ASRB) of Quaid-i-Azam University, Islamabad, and the Ethical Review Committee (ERC) of the National Institute of Health, Islamabad (Pakistan). The study monitored patients throughout their stay after their written consent. The data collected included demographic characteristics such as age and gender, pre-existing comorbidities, CRP level at 1st and 3rd days of hospitalization, and medical interventions including the requirement for ventilation, type of ventilation, and admission to the intensive care unit (ICU). The quantitative monitoring of serum CRP was determined by using iCHROMA™ CRP kit (ICHR070), employing a fluorescence immunoassay technique.

The inclusion criterion for each comorbidity was a minimum of >10 to minimize bias and enhance statistical significance. In addition, the duration of hospitalization, recovery rates, and mortality rates among different groups of patients were recorded. All personally identifiable information was removed during data collection and analysis to ensure compliance with data protection regulations.

Statistical analysis

The data of 618 hospitalized patients with COVID-19 were statistically analyzed, excluding 132 patients due to incomplete information and follow-up. The general characteristics were determined by descriptive statistics and frequency analysis. The data on CRP levels were not normally distributed; therefore, the non-parametric Kruskal-Wallis test was used to determine differences in serum C-reactive protein (CRP) levels across various age groups in the sample population. The non-parametric Mann–Whitney test was performed to explore the association between CRP levels and different comorbidities in patients with COVID-19. To evaluate variations among various multimorbidities, a one-way ANOVA test was performed on CRP levels on the 1st and 3rd days of hospitalization in patients with COVID-19. Two independent sample t-tests were used to determine the correlation between CRP levels and bivariate variables, including the requirement for non-invasive ventilation, mechanical ventilation, ICU admission, and disease outcome (recovery or death). Cox regression analysis was used to evaluate the association between CRP levels and the duration of hospitalization among patients with COVID-19, whereas logistic regression analysis was employed to assess the relationship between CRP levels and the rate of mortality among these patients.

Results

The SRTOBE flow chart (Fig. 1) adopted from our previous study [10] illustrates the inclusion criteria as well as the number of samples for each category for comparative analysis.

Descriptive statistics and baseline characteristics

The sample consisted of 618 patients, of whom 62.6% were male and 37.4% were female. Interestingly, no children (≤14 years) were observed among hospitalized

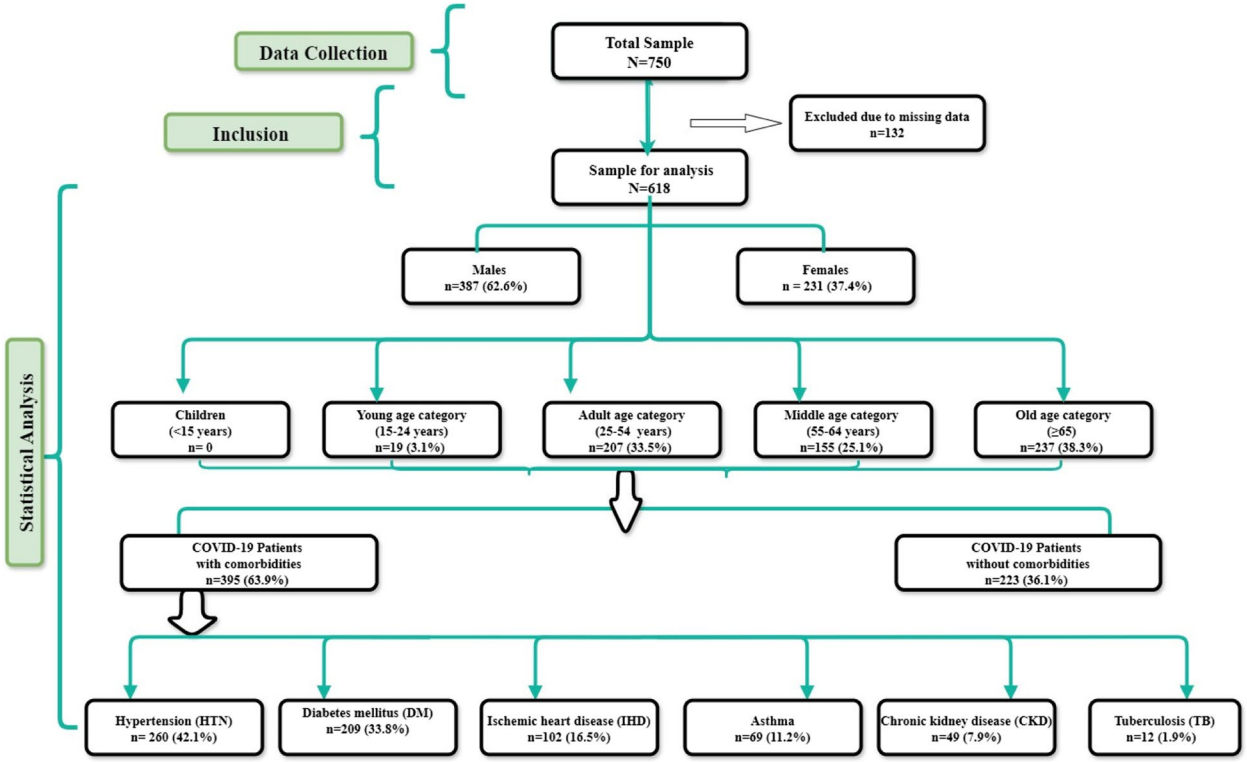

Fig. 1 The SRTOBE Flow Chart depicting the inclusion criteria and sample distribution

patients, whereas the majority of them belonged to the old age group (≥65 years) at 38.3% followed by other age groups. Regarding comorbidities, 36.1% of patients had no comorbidities, while 27.8% had one, 23.6% had two, and 12.5% had three. Disease outcomes showed that 73.9% of patients recovered and 26.1% expired (Table 1).

C-reactive protein (CRP) as an inflammatory marker

The descriptive statistics for CRP levels revealed a mean value of 88.92 mg/L, with a standard deviation of 63.949 mg/L for the overall study population. The minimum CRP level observed was <1 mg/L, whereas the maximum CRP level was 900 mg/L. The statistical analysis revealed significant variations in CRP levels among COVID-19 patients with different comorbidities and age groups.

Table 1 Baseline characteristics of COVID-19 patients

Characteristics	Category	No. of patients	Frequency %
Gender	Male	387	62.6
	Female	231	37.4
Age groups	Children (≤ 14 years)	0.0	0.0
	Young (15–24 years)	19	3.1
	Adult (25–54 years)	207	33.5
	Middle age (55–64 years)	155	25.1
	Old age (≥ 65 years)	237	38.3
No. of comorbidities	No Comorbidity	223	36.1
	1 Comorbidity	172	27.8
	2 Comorbidities	146	23.6
	3 Comorbidities	77	12.5
Disease outcome	Discharged after recovery	457	73.9
	Death	161	26.1
Total		618	100.0

Fluctuations in the concentration of serum C-reactive protein (CRP) in patients with COVID-19

Different age groups

The Kruskal-Wallis test was applied to determine the association between C-reactive protein (CRP) levels and various age groups, including children (≤14 years), young (15–24 years), adults (25–54 years), middle age (55–64 years) and old age (˃65 years) COVID-19 patients. The results revealed a significant overall difference in CRP levels among the age groups ($\chi^2 = 66.741$, df=3, $p<0.001$) (Table 2). Furthermore, to determine specific intergroup differences, post hoc Mann–Whitney tests were employed. In the young and adult group pair ($N=19$ and $N=207$, respectively), no significant difference in CRP levels was observed ($Z = -1.493$, $p=0.136$). In the young and middle-aged groups ($N=19$ and $N=155$, respectively), a statistically significant difference in CRP levels ($Z = -2.724$, $p=0.006$) was observed. Moreover, in the young and old age groups ($N=19$ and $N=237$, respectively), a highly significant difference in CRP levels was observed ($Z = -3.970$, $p<0.001$). The observed lower CRP levels in the younger group and the highest values in the older group demonstrate potential age-related variations in CRP levels within the studied population.

Different comorbidities

The descriptive statistics for CRP levels revealed a mean value of 88.92 mg/L, with a standard deviation of 63.949 mg/L for the overall study population. The minimum CRP level observed was <1 mg/L, whereas the maximum CRP level was 900 mg/L. The statistical analysis revealed significant variations in CRP levels among COVID-19 patients with different comorbidities (Table 3).

No comorbidity The Mann–Whitney test results indicate a substantial difference in CRP levels between COVID-19 patients with comorbidities ($N=395$) and those without comorbidities ($N=223$). Notably, patients with comorbidities exhibited significantly different ($Z=-9.187$, $p=0.000$) CRP levels (359.04 mg/L) compared with those without comorbidities (223 mg/L).

Table 2 Mann-Whitney tests analysis to predict the association of CRP with different age groups

Age group comparison	Age Group	*N*	Mean Rank	Z	*p* (Asymp. Sig. (2-tailed)
Young vs. Adult	Young	19	92.08	−1.493	0.136
	Adult	207	115.47		
Young vs. Middle age	Young	19	57.82	−2.724	0.006
	Middle age	155	91.14		
Young vs. Old age	Young	19	63.68	−3.97	0.00
	Old age	237	133.7		

Table 3 Association between CRP level and comorbidities analyzed by Mann-Whitney U test

Comorbidity	No/Yes	No of patients	%	Mean Rank CRP	Z	*p* (2-tailed)
No Comorbidity	No	223	36.1	221.75	−9.187	0.000
Diabetes Mellitus (DM)	No	409	66.2	280.14	5.724	0.000
	Yes	209	37.4	366.96		
Hypertension (HTN)	No	358	57.9	276.19	5.447	0.000
	Yes	260	42.1	355.37		
Asthma	No	549	11.2	302.31	2.827	0.005
	Yes	69	88.8	366.72		
Ischemic heart disease (IHD)	No	516	83.5	294.49	4.704	0.000
	Yes	102	16.5	385.43		
Chronic kidney disease (CKD)	No	569	92.1	300.64	4.206	0.000
	Yes	49	7.9	412.37		
Tuberculosis (TB)	No	606	98.1	306.56	2.914	0.004
	Yes	12	1.9	458.08		

Diabetes mellitus A statistical analysis of COVID-19 patients ($N=618$), including 33.8% diabetic ($N=209$) and 66.2% non-diabetic ($N=409$) study population, with their C-reactive protein (CRP) values, was conducted. Descriptive statistics for the CRP variable indicated that the mean CRP values for the non-diabetic and diabetic patients were 88.92 mg/L (SD = 63.949) and 126.96 mg/L (SD = 72.947), respectively. Patients with diabetes exhibited higher CRP levels than those without diabetes. The Mann–Whitney U test was conducted to compare the CRP values between these two groups, considering the non-normality of the data. The test revealed a statistically significant difference ($Z = -5.724$, $p < 0.05$) in CRP levels between patients with and without diabetes comorbidity (Table 3).

Hypertension Among the total study population ($N=618$), 260 (42.1%) had hypertension. The Mann–Whitney test results revealed a significant difference ($p < 0.001$) in CRP levels between the two groups of patients with and without hypertension (HTN). The mean CRP levels in patients with and without hypertension were 355.37 and 276.19 mg/L, respectively, indicating a significant difference. Patients with hypertension exhibited higher CRP levels, indicating a stronger systemic inflammatory response. The study demonstrated a significant association between hypertension and elevated CRP levels in COVID-19 patients ($Z=-5.447$, $p=0.000$) (Table 3).

Asthma The results of the Mann-Whitney U test on the total sample population, including 549 (88.8%) patients with no asthma and 69 (11.2%) patients with asthma as a comorbidity, showed a statistically significant association ($Z= -2.827$, $p=0.005$), suggesting a strong relationship between CRP levels and asthma comorbidity in COVID-19 patients. The mean CRP levels were 302.31 mg/L in patients without asthma and 366.72 mg/L in patients with asthma. The higher mean rank in the asthma group implies that patients with asthma tend to have higher CRP levels than those without asthma. Based on these results, it can be inferred that CRP levels significantly vary with asthma comorbidity in patients with COVID-19. The observed association highlights the potential clinical relevance of monitoring CRP levels in patients with asthma because elevated CRP levels may indicate a more severe inflammatory response in these individuals (Table 3).

Ischemic heart disease Among the 618 COVID-19 patients, 102 (16.5%) had IHD as a comorbidity. Results of the Mann–Whitney test indicated a significant difference in CRP levels between participants with and without IHD ($p < 0.001$). The mean CRP level in the IHD group was 385.43 mg/L, which was higher than that in the group without IHD (294.49 mg/L). These findings demonstrate a statistically significant association between CRP levels and IHD as a comorbidity in COVID-19 patients with a high risk of severity due to inflammation ($Z= -4.704$, $p=0.000$) (Table 3).

Chronic kidney disease (CKD) The analysis by the Mann-Whitney U test of the total sample population including 569 (92.1%) patients with no chronic kidney disease and 49 (7.9%) patients with chronic kidney disease resulted in a statistically significant finding ($Z=-4.206$, $p < 0.001$), suggesting a significant association between CRP levels and chronic kidney disease comorbidity in COVID-19 patients. The mean CRP levels were 300.64 mg/L in patients without CKD and 412.37 mg/L in patients with CKD. The higher mean rank in the CKD group implies that patients with this comorbidity tend to have higher CRP levels than those without CKD. Based on these results, it can be inferred that CRP, as a biomarker, significantly differs with CKD comorbidity in COVID-19 patients. The observed association highlights the potential clinical relevance of monitoring CRP levels in patients with chronic kidney disease, as elevated CRP levels may indicate a more severe inflammatory response in these individuals (Table 3).

Tuberculosis (TB) TB was a less prevalent (1.9%) comorbidity in patients with COVID-19, but it significantly affected the outcomes of COVID-19.

The results of The Mann–Whitney U test showed a significant association between serum CRP levels and TB comorbidity in COVID-19 patients ($Z=-2.914$, $p=0.004$). Patients with TB as a comorbidity exhibited higher mean CRP levels (458.08 mg/L) than those without TB (306.56 mg/L). Monitoring CRP levels in patients with TB may be clinically relevant because elevated CRP levels could indicate a more severe inflammatory response in these patients. However, due to the limited number of patients with TB in the sample, further research with a larger cohort is required to validate these results and gain a deeper understanding of the implications of this association in the context of COVID-19 patient management (Table 3).

The Kaplan-Meier survival analysis

The Kaplan-Meier survival analysis was used to compare the trend of survival/hazard distributions among patients with underlying health conditions or comorbidities. The significant Log Rank (Mantel-Cox) test result ($\chi^2 = 89.340$, $df=16$, $p < 0.001$) revealed that the length of

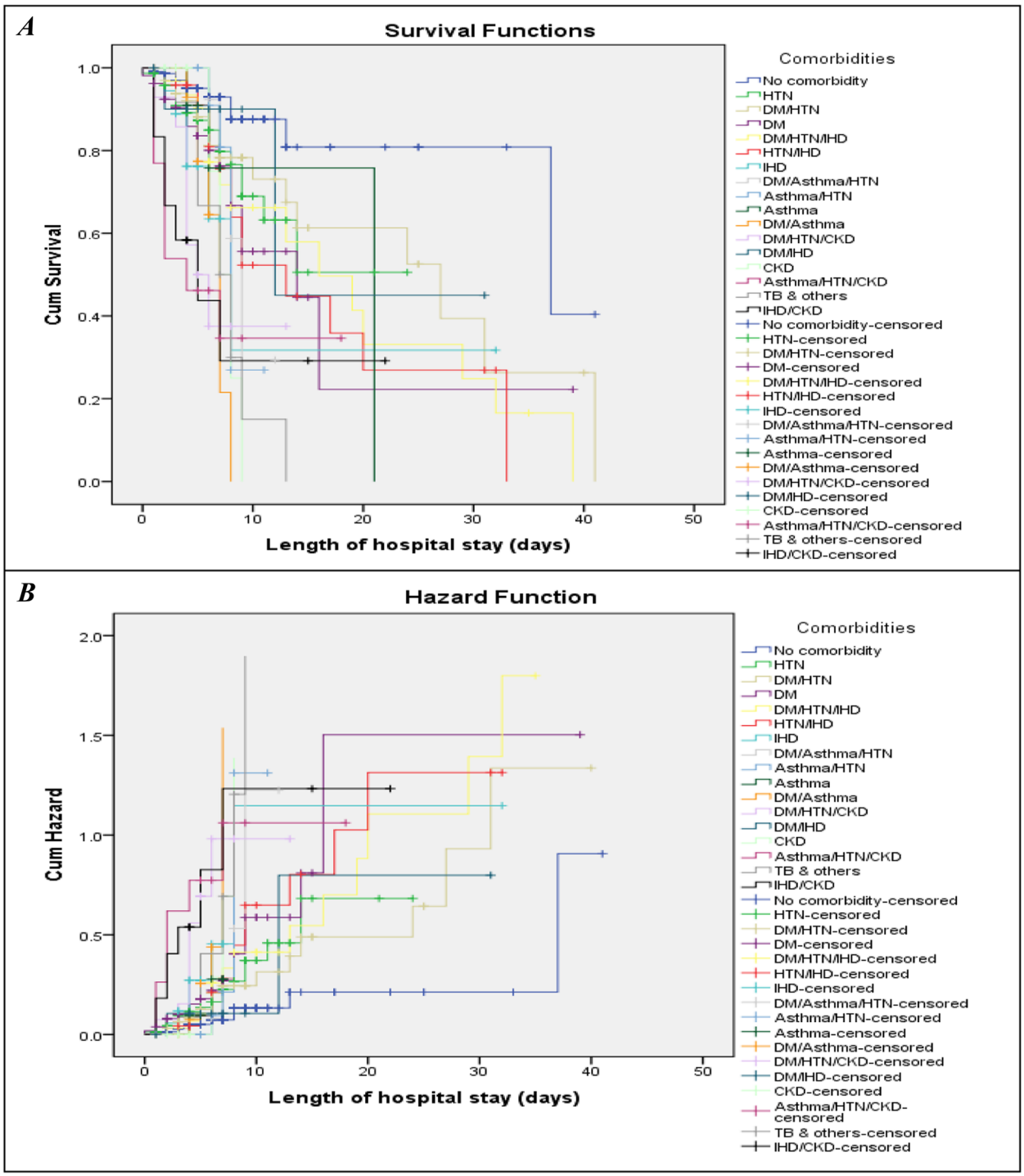

Fig. 2 The Kaplan-Meier survival analysis to predict the survival (**A**) and risk/hazard (**B**) in COVID-19 patients with different comorbidities

hospitalization differ significantly due to different comorbidities. COVID-19 patients with specific comorbidities including CKD, diabetes, or cardiovascular diseases were associated with longer hospital stays or higher mortality risk. These differences demonstrate that comorbidities affect the probabilities of survival and hazard rates during hospitalization (Fig. 2A and B).

Comparison of C-reactive protein (CRP) levels in COVID-19 patients with comorbidities/multimorbidities

The findings of CRP in patients with COVID-19 from the perspective of one particular comorbidity do not present the true variation in serum CRP concentration because it was observed that most patients had more than one comorbidity. Therefore, the prediction of serum CRP levels in patients with multimorbidity is necessary to evaluate the associations and variations of CRP values within that population. To evaluate differences due to various multimorbidities, one-way ANOVA was performed on the CRP levels on the 1st and 3rd day of hospitalization in COVID-19 patients.

The descriptive statistics indicate that the overall mean CRP level on the 1st day of hospitalization was 88.92 mg/L, whereas on the 3rd day; it was slightly lower at 67.89 mg/L. This demonstrates that CRP levels tend to decrease throughout hospitalization, which may indicate the body's response to treatment and recovery from the acute phase of infection.

However, it is important to note that CRP levels exhibit significant variations across different comorbidity and multimorbidity categories. Each category depicted a unique range of CRP values correlating it with a specific level of inflammation and internal immune state due to underlying conditions (Fig. 3).

For instance, patients with diabetes/hypertension/ischemic heart disease (DM/HTN/IHD) showed the highest mean CRP levels on both the 1st and 3rd days of hospitalization (132.34 mg/L and 82.84 mg/L, respectively). This finding indicates that individuals with multiple comorbidities may experience more severe inflammation, leading to a higher production of CRP as an inflammatory biomarker. On the other hand, patients with asthma comorbidity had the lowest mean CRP levels on both the 1st and 3rd days (73.45 mg/L and 53.00 mg/L, respectively). This shows that asthma patients might have a milder inflammatory response to COVID-19 than other comorbidities. These findings highlight the complex relationship between different comorbidities/multimorbidities and inflammation, as indicated by CRP levels, in COVID-19 patients.

Ventilation requirements with fluctuations in C-reactive protein levels

The effect of C-reactive protein (CRP) levels in patients with COVID-19 based on their requirement for ventilation (non-invasive and mechanical ventilation) was measured using two independent sample t-tests that compared the means of CRP levels in ventilated and non-ventilated patients (Table 4).

Non-invasive ventilation There was a significant difference ($p < 0.05$) in CRP levels between the two groups of patients who received non-invasive ventilation and those who did not. Notably, patients who underwent non-invasive ventilation had a higher mean CRP level (M = 110.80 mg/L, SD = 47.199) than those who did not receive non-invasive ventilation (M = 76.82 mg/L, SD = 68.663). This suggests that patients with higher CRP levels tend to have more chances of requiring non-invasive ventilation, which could be indicative of a more severe clinical condition and inflammation (Table 4).

Mechanical ventilation A significant association was observed between serum CRP levels and the requirement for mechanical ventilation among hospitalized patients with COVID-19 ($p < 0.05$).

Patients who required mechanical ventilation had a considerably higher mean CRP level (M = 134.46 mg/L,

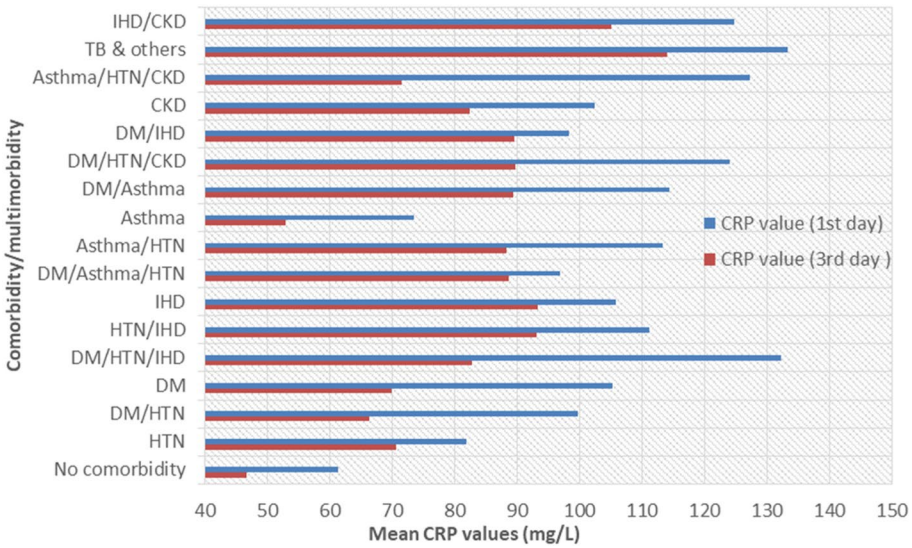

Fig. 3 CRP variations in COVID-19 patients with different comorbidities and multimorbidity

Table 4 Independent samples t-tests to predict the association between CRP level and medical interventions in COVID-19 patients

Disease Variables		*N*	Mean	SD	SE	t	df	*p*
Non-invasive ventilation	No	398	76.82	68.663	3.442	−7.25	587.542	0.00
	Yes	220	110.80	47.199	3.182			
Mechanical ventilation	No	492	77.25	64.773	2.920	13.837	397.475	0.00
	Yes	126	134.46	32.853	2.927			
ICU admission	No	434	72.79	51.986	2.495	−9.138	265.097	0.00
	Yes	184	126.96	72.947	5.378			
Disease outcome	Discharged	457	71.47	50.337	2.355	10.877	217.275	0.00
	Death	161	138.44	72.188	5.689			

SD = 32.853) than those who did not require mechanical ventilation (M = 77.25 mg/L, SD = 64.773). These findings showed that patients with elevated CRP levels have more pronounced inflammation and disease severity, thereby leading to a higher likelihood of mechanical ventilation (Table 4).

Association between C-reactive protein levels and admission in intensive care unit (ICU)

The COVID-19 patients who were admitted to the intensive care unit (ICU) had a considerably higher mean CRP level (M = 126.96 mg/L, SD = 72.947) than those who did not require ICU admission (M = 72.79 mg/L, SD = 51.986), revealing a significant association ($p < 0.05$). This finding indicates that patients with higher CRP levels have a higher probability of disease severity ultimately requiring more ICU admissions than those with lower CRP levels (Table 4).

Cox regression analysis: CRP levels as a predictor of length of hospitalization among patients with COVID-19

Cox regression analysis was conducted to investigate the relationship between C-reactive protein (CRP) levels on the first day of hospitalization and the duration of hospital stay among patients with COVID-19. Disease outcomes were categorized as "Event" for patients who experienced an event of interest, such as death or discharge, and "Censored" for patients still hospitalized at the end of the study or lost to follow-up. The forward stepwise (conditional LR) method was used for variable selection, and the criteria for entry and removal were set at $p < 0.05$ and $p > 0.10$, respectively.

The results revealed that CRP level significantly predicted the length of hospital stay ($p < 0.001$). For every 1-unit increase in CRP level on the first day of hospitalization, the risk of an extended hospital stay increased by approximately 0.6%. The hazard ratio for CRP level was 1.006 (95% CI: 1.004–1.008), indicating its importance as a predictor. When the CRP value of the 1st day of hospitalization was removed from the model, the chi-squared loss was 52.522 (df = 1, $p < 0.001$), highlighting the significance of CRP in explaining variations in the duration of hospitalization. The mean CRP level in the sample was 88.917. These findings suggest that monitoring CRP levels on admission can help identify patients with COVID-19 at a higher risk of prolonged hospitalization, enabling early intervention and targeted care management strategies (based on calculations in brackets) [Cox regression analysis, $p < 0.001$, hazard ratio: 1.006 (95% CI: 1.004–1.008), chi-square test: $p < 0.001$].

Logistic regression analysis: CRP as a predictor of mortality in patients with COVID-19

Logistic regression analysis was performed to examine the relationship between the binary outcome (recovery or mortality) and the predictor variable "CRP level on the 1st day of hospitalization." The model correctly predicted 73.9% of cases, with a significant association between CRP levels and the likelihood of mortality ($p < 0.001$). For each unit increase in CRP level on the first day of hospitalization, the odds of mortality increased by approximately 2.7% (odds ratio = 1.027, 95% CI: 1.022–1.033). These results indicate a positive association between higher CRP levels and a high mortality rate among patients with COVID-19. These insights can help identify high-risk patients who require intensive monitoring and targeted treatment.

Discussion

The findings of this study offer important insights into the complicated association of C-reactive protein (CRP) in patients with comorbid and multimorbid COVID-19. Infectious diseases like COVID-19 taught the world a lesson to prepare for unexpected pandemics. It has become evident that underlying health conditions increase the risk of severe outcomes. This study comprehensively explored the role of CRP as an inflammatory marker in

patients with COVID-19 with different comorbidities and multimorbidities.

Age-related variations in CRP levels were observed in this study, revealing that the older age group had the highest possible risk of inflammation and number of cases while the opposite was observed in the case of children. No hospitalized case of COVID-19 patients among children (≤14 years) was observed during this study. The possible reason for the absence of hospitalized COVID-19 cases could be attributed to several factors such as lower susceptibility to disease severity as the innate immune response in this age is more active. Furthermore, the chances of comorbidities in children are less as compared to other age group. The exposure risk was also limited due to indirect protection through public health measures including closures of schools and fewer social interactions. These findings highlight the importance of age as an important factor in COVID-19 disease severity and hospitalization trends [15, 16]. This finding is consistent with previous studies showing that age is a significant risk factor for severe COVID-19 outcomes. Higher CRP levels in older patients can be attributed to age-related changes in the immune system [17] and chronic low-grade inflammation, known as "inflammaging" [18]. As people age, there is a gradual increase in pro-inflammatory cytokine production, activation of the chronic inflammasome, and a decline in anti-inflammatory responses, leading to a state of chronic low-grade inflammation in the body [19]. e.g. the NLRP3 inflammasome responds to DAMPs and plays a crucial role in age-related inflammation and disease, affecting immune responses during infections [20]. Certain lifestyle factors, such as poor diet, smoking, and sedentary lifestyle, can further increase inflammation with the passage of age. Monitoring CRP levels in older adults can help assess their overall health status and identify potential health risks associated with inflammation.

The study also demonstrated an association between CRP levels and different comorbidities. Patients with Diabetes mellitus (DM), hypertension (HTN), ischemic heart disease (IHD), chronic kidney disease (CKD), and tuberculosis (TB) exhibited significantly higher CRP levels than those without these comorbidities. Multimorbidity of DM/HTN/IHD in combination affected patients with COVID-19 most severely in this study population. These findings indicate that the presence of these underlying conditions may contribute to an enhanced inflammatory response to COVID-19, potentially leading to more severe disease outcomes [21–23]. Patients with these comorbidities experience elevated CRP levels because of underlying chronic inflammation, immune responses to infections or tissue damage, and related metabolic abnormalities [24, 25]. In diabetes, chronic hyperglycemia and insulin resistance trigger systemic inflammation, leading to increased CRP production [26, 27] HTN causes vascular damage and oxidative stress, prompting the liver to produce CRP [28–30]. IHD leads to inflammation and atherosclerosis, further elevating CRP levels [31, 32]. Patients with CKD have a chronic inflammatory state due to impaired kidney function [33, 34], and TB induces a strong immune response [35], resulting in acute-phase CRP elevation. Monitoring CRP levels under these conditions provides valuable insights for disease management and risk assessment.

It was observed that the CRP levels of asthma were comparatively lower than those of other comorbidities. As evident from previous studies, asthma is characterized by chronic airway inflammation driven primarily by eosinophils and Th2 lymphocytes, which produce cytokines like IL-4, IL-5, and IL-13, leading to IgE production and eosinophil recruitment [36–39]. While CRP is an acute-phase protein biomarker produced in response to different types of inflammation, such as infections and tissue damage, it is less likely to be elevated in asthma, where inflammation is eosinophilic. While CRP levels may be lower, other markers related to eosinophilic inflammation, like eosinophil counts and certain cytokine levels, may be elevated in asthma patients, emphasizing the distinct immune profile of each inflammatory condition [40, 41].

Furthermore, the results demonstrated a significant association between CRP levels and the need for noninvasive ventilation (NIV), mechanical ventilation (MV), intensive care unit (ICU) admission, and mortality. Patients requiring respiratory support and ICU admission had higher CRP levels, indicating a more severe inflammatory response and disease progression. Similarly, patients who did not survive COVID-19 had higher CRP levels than survivors, demonstrating that elevated CRP levels may be a predictor of poor prognosis. Elevated CRP levels in patients with COVID-19 indicate more intense and prolonged immune system activation, leading to a cytokine storm [42, 43] This excessive release of pro-inflammatory cytokines can result in severe lung inflammation, acute respiratory distress syndrome (ARDS), and multi-organ dysfunction. As a result, patients with high CRP levels are more likely to experience respiratory failure, requiring respiratory support such as noninvasive or mechanical ventilation, and may need admission to the intensive care unit (ICU) for critical care. Unfortunately, persistently high CRP levels can also be associated with a higher risk of mortality as the immune system's hyperactivity can cause significant damage to vital organs and impair their proper functioning [44]. Overall, the results indicate that CRP is a valuable biomarker for assessing inflammation and disease

severity in patients with multimorbid COVID-19. Monitoring CRP levels in patients can provide crucial information for risk stratification and clinical decision-making, ultimately improving patient outcomes.

As this study was limited to data collection from only two hospitals in Pakistan and was dependent on the availability of medical records and patient follow-up, we acknowledge that these constraints may have affected data accuracy and its applicability to a broader population.

Conclusion

Elevated C-reactive protein (CRP) levels in patients with multimorbid COVID-19 are associated with severe disease outcomes, including the need for ventilation, ICU admission, and mortality. Age-related variations in CRP levels and their significant variations among different comorbidities emphasize the importance of CRP levels in risk assessment. Monitoring CRP levels can predict hospitalization duration and identify high-risk patients.

Future perspective

Future studies should consider conducting gender-based studies, pathway analysis and genetic-based studies to explore the underlying mechanisms and pathogenesis related to C-reactive protein (CRP) in patients with COVID-19 and other infectious diseases. Additionally, functional studies using cellular and animal models can validate genetic findings and provide mechanistic insights. Furthermore, investigating therapeutic interventions that modulate CRP levels will help determine their efficacy in improving clinical outcomes. Integrating these aspects into research can enhance our understanding of CRP's role in COVID-19 pathogenesis and guide personalized treatment approaches to improve patient care and outcomes.

Abbreviations

CRP	C-reactive protein
COVID-19	Coronavirus disease 2019
IHD	Ischemic heart disease
CKD	Chronic kidney disease
TB	Tuberculosis
HTN	Hypertension
NIV	Non-invasive ventilation
MV	Mechanical ventilation
ICU	Intensive care unit
NLRP3	NOD-like receptor protein 3
DAMPs	Damage associated molecular patterns

Acknowledgements

The authors would like to thank Professor Dr Bushra Jamil and Dr Nasim Akhtar for allowing to collect data from Isolation Hospital & Infectious Treatment Center (IHITC), Islamabad and Pakistan Institute of Medical Sciences (PIMS), Hospital, Islamabad, respectively. The authors extend their appreciation to the researcher supporting project number (RSP2025R218), King Saud University, Riyadh, Saudi Arabia.

Clinical trial number

Not applicable.

Authors' contributions

MS and HK conceptualized the study, analyzed the data, and wrote the manuscript. AR, SR, SA, WZ, WM, and MOB collected samples, completed data curation, and assisted with data analysis and interpretation. WM, NA, WZ and MU provided critical revisions and resources for data collection. MN and MB reviewed and edited the manuscript, contributing to visualization, resource management, and supervision. MN and AFA provided resources, acquired funding, and contributed to reviewing and editing the manuscript.

Funding

The authors extend their appreciation to the researcher supporting project number (RSP2025R218), King Saud University, Riyadh, Saudi Arabia.

Data availability

The accessibility of raw data is limited due to confidentiality and ethical reasons. However, it will be made available from the corresponding author on an appropriate request.

Declarations

Ethics approval and consent to participate

This study was approved by the Advance Studies and Research Board (ASRB) of Quaid-i-Azam University, Islamabad, and the Ethical Review Committee (ERC) of the National Institute of Health, Islamabad (Ethical Approval Reference No: F.1–5/RAPiD/2020–21/ERC) dated April 2021. Informed consent was obtained from all subjects involved in the study. The study was conducted in compliance with the Council for International Organizations of Medical Sciences (CIOMS) guidelines.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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Received: 17 August 2024 Accepted: 5 December 2024
Published online: 14 January 2025

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