Journal of Infectious Diseases & Therapy
ISSN: 2332-0877

Research Article Open Access

Clinical and Biochemical Predictors of Mortality of COVID-19 Cases from Pakistan

Aasia Khaliq[1], Fouzia Ashraf[1*], Saamia Tahir[2], Shahzeb Javed[3], Saher Shahid[4] and Huda Abbas[1]

[1]*Department of Pathology and Microbiology, Allama Iqbal Medical College, Lahore, Pakistan*
[2]*Department of Community Medicine, Combined Military Hospital, Lahore, Pakistan*
[3]*Department of Biochemistry, University of Health Sciences, Lahore, Pakistan*
[4]*Department of Biological Sciences, University of the Punjab, Lahore, Pakistan*

Abstract

Coronavirus Disease 2019 (COVID-19) is an infectious respiratory disease with several biochemical alterations reflecting the main pathophysiological characteristics associated with the disease severity and mortality. We have reported clinical and biochemical predictors of mortality among 200 patients: 57 survivors and 143 non-survivors. Data on patient's demographic characteristics, radiological findings, laboratory findings and comorbidities was collected. Categorical variables were expressed as percentages (frequencies) while continuous variables were reported as mean ± SD (Standard Deviation). Mann-Whitney t-test, One-way ANOVA, Pearson's correlation and ROC curves were used for statistical analysis with a p-value of 0.05. Out of 200 patients, 64% were male, and 36% were female. The median age for deceased and recovered cases was 61 (IQR: 24,70) and 36 (IQR: 26,52) years, respectively. Among co-morbid conditions hypertension (p-value 0.079) and cardiac vascular disease (p-value 0.064) was significantly higher in deceased cases. Lymphopenia, GGO, fever, cough are the hallmarks of disease were observed frequently. Increased level of inflammatory biomarkers including CRP (p-value<0.0001), ESR (p-value<0.0001), Ferritin (p-value 0.001), LDH (p-value<0.0001) and PCT (p-value 0.022), coagulation factors such as D-Dimers (p-value 0.0003), Fibrinogen (p-value<0.0001), increased prothrombin time and decreased activated partial thromboplastin time were associated with disease severity. Among serum electrolytes decreased levels of potassium (p-value 0.0004), sodium (p-value<0.0001), chloride (p-value 0.0011) and calcium (p-value 0.0021) but increased level of magnesium (p-value 0.0002) were observed in non-surviving COVID-19 patients. Among hepatocytic biomarkers increased levels of ALT (p-value 0.0001), AST (p-value 0.0011), albumin (p-value 0.0044), alkaline phosphatase (p-value 0.0260) and bilirubin (p-value<0.0001) were observed in non-survivors. The SARS-CoV-2 infection is characterized by several biochemical alterations, which can be recognized by specific biomarkers. Among all biomarkers associated with disease severity and mortality lymphopenia, thrombocytopenia, CRP, ESR, PCT, LDH, AST, ALT, D-dimer, CK, albumin, creatinine phosphatase represents the most predictive parameters of severe COVID-19 infection.

Keywords: COVID-19; Co-morbidities; Biochemical parameters; Hematological profiling; Inflammatory markers

Introduction

COVID-19 is an infectious disease caused by the SARS-CoV-2 virus. The outbreak started from Wuhan China in 2019 and was globally spread [1]. The virus can spread in the form of small droplets through coughing, sneezing, speaking, or breathing from infected individuals. These droplets range from smaller aerosols to larger respiratory droplets and are of >5-10 um in size. People can be infected by breathing in the virus if you are in close contact (within 1 meter) of COVID-19 infected individual, or by touching a contaminated surface and then rubbing the eyes, nose or mouth with hands without sanitization. The virus spreads more easily indoors and in crowded settings [2,3].

The transmission and severity of infection is increasing much rapidly with the emergence of new variants [4]. People infected with COVID-19 experience a wide range of symptoms that range from mild to moderate symptoms and may recover without special treatment. However, some people may not experience any symptoms at all and will remain asymptomatic while some become seriously ill and require urgent medical attention or hospitalization [5]. Symptoms including cough, fever, chills, Shortness of Breath (SOB), fatigue, loss of smell and taste, sore throat, chest congestion, vomiting, nausea, diarrhea, muscle ache etc. Typically, the symptoms may appear after 2-14 days of exposure and can vary from mild to severe symptoms [6]. Many severe or critically ill patients had been hospitalized requiring the intensive [7].The clinical course of COVID-19 ranges from asymptomatic to mild and moderate infection which can lead to critical stages and even death [8].

Diagnosis of the infection is performed usually using PCR reaction which detects viral nucleic acids in the specimens [9]. Despite the supportive help, patients of COVID-19 can face serious respiratory deterioration and ultimately death [10]. Identification of the patients who need supportive help and care can aid in saving the life of the individual. For this, certain signatures need to be identified [11]. Alterations in these signatures can lead a person to its mortality. These biomarkers can aid in confirmation and classification of disease severity, early diagnosis, rationalizing therapies and predicting disease outcomes [12]. Previous studies have demonstrated that there have been high levels of neutrophils, lymphopenia, and C-reactive protein in critical patients of COVID-19 [13,14]. Besides these changes, increase in levels of erythrocyte sedimentation rate and interleukin 6, decreased levels of CD4+ and CD8+ are important marks as both of them are involved in determining disease severity and clinical outcomes [15]. In addition to these inflammation-linked indicators, disease severity can also be linked with co-morbidities. COVID-19 patients could develop a life-threatening situation if they have the co-morbid conditions

***Corresponding author:** Dr. Fouzia Ashraf, Department of Pathology and Microbiology, Allama Iqbal Medical College, Lahore, Pakistan, E-mail: pcrjinnah@yahoo.com

Received: 10-May-2022, Manuscript No. JIDT-22-63466; **Editor assigned:** 12-May-2022, PreQC No. JIDT-22-63466 (PQ); **Reviewed:** 26-May-2022, QC No. JIDT-22-63466; **Revised:** 02-Jun-2022, Manuscript No. JIDT-22-63466 (R); **Published:** 13-Jun-2022, DOI: 10.4172/2332-0877.1000502

Citation: Khaliq A, Ashraf F, Tahir S, Javed S, Shahid S, et al. (2022) Clinical and Biochemical Predictors of Mortality of COVID-19 Cases from Pakistan. J Infect Dis Ther 10: 502.

such as diabetes, Chronic Obstructive Pulmonary Disease (COPD), Cardiovascular Diseases (CVD), hypertension, malignancies, HIV etc. [16,17]. Multiple studies have been conducted to observe various biochemical and hematological parameters from blood samples of COVID infected patients during hospitalization. Gradual decrease of lymphocyte count, thrombocytopenia, elevated CRP, procalcitonin, increased liver enzymes, decreased renal function, and coagulation derangements, were more common in critically ill patient groups and associated with a high incidence of clinical complications [18,19].

In this study, we have performed a comprehensive evaluation of clinical characteristics and biochemical parameter of 200 patients with COVID-19 admitted to Jinnah hospital, Lahore, Pakistan.

Materials and Methods

Study design and patient characterization

This was a retrospective single-center study of 200 patients diagnosed with severe COVID-19 infection admitted in Pulmonology ward of Jinnah hospital, Lahore from March to June 2020. All patients were diagnosed as per interim guidelines of National Institute of Health (NIH) and World Health Organization (WHO). Patients were categorized as "severe" based on the guidelines defined by NIH; a) shortness of breath with >30 times/min Respiratory Rate (RR). b) <93% oxygen saturation at rest, c) Arterial partial oxygen pressure (PaO_2) of <300 mm Hg, d) progression of lesions with >50% in 24-48 hour on Chest X-ray (CXR) or High-Resolution Chest Scan (HRCT), requirement of mechanical ventilation [20].

Data collection

The medical record files of all the admitted patients were reviewed by a team of researchers and physicians. The medical record of each patient was taken from both paper and electronic records using the Medical Record Number (MRN). Extracted data included patients' demographics, clinical and laboratory findings, co-morbidities, treatment outcome was collected by using standardized data collection form.

Demographic data

Demographic data included age and gender of patients. Clinical data included the history and duration of various signs and symptoms of COVID-19 infection such as fever, sore throat, cough, nausea, vomiting, diarrhea, loss of smell, loss of taste, headache, shortness of breath, oxygen saturation level etc. A certified consultant radiologist performed the radiological interpretations, blinded from the clinical presentation of the patients, and made a subjective estimation of severity of the disease from CXR/HRCT or both.

Routine laboratory tests

COVID-19 patients were diagnosed with the WHO recommended Real Time Transcription Polymerase Chain Reaction (RT-PCR) assay. Nasopharyngeal/oropharyngeal swabs or both were taken from all the patients at the time of admission and at multiple time points after the admission. Qualitative detection of SARS-CoV-2 was detected as per NIH's guidelines [21]. Routine laboratory tests that were performed on all patients included various hematological, blood gas and biochemical parameters along with coagulation, inflammatory, liver impairment and kidney lymphocyte count, dysfunction markers. Hematological parameters included White Blood Cell Count (WBC), Neutrophil count, Neutrophil to Lymphocyte ratio and platelets count. Blood gas parameters included Hemoglobin (HB), PO_2, PCO_2, pH, bicarbonates (HCO_3) and Sulphates (SO_2). Among inflammatory vitals, CRP (C-Reactive Protein), ESR (Erythrocyte Sedimentation Rate), Ferritin, LDH (Lactate Dehydrogenase) and Pro-Calcitonin were tested. Among co-agulation factors, PT (Prothrombin time), aPTT (activated partial thromboplastin time) and Fibrinogen. Among muscle markers, Creatinine Kinase, Total protein, Glucose and Troponin were measured. Serum electrolytes under observation included Potassium (K^+), Sodium (Na^+), Chloride (Cl^-), Calcium (Ca^{2+}) and Magnesium (Mg^{2+}). To observe the effect of COVID-19 infection on liver impairment, ALT (Alanine Amino Transferase), AST (Aspartate Aminotransferase), Albumin, Total bilirubin and alkaline phosphatase were the markers analyzed. For kidney dysfunction, creatinine, BUN (Blood Urea Nitrogen) and uric acid were tested in blood samples.

Co-morbid conditions and clinical outcome

Among severely ill and hospitalized COVID-19 patients, the comorbidities like hypertension, diabetes (type 2), cardiovascular disease, Chronic Obstructive Pulmonary Disease (COPD), asthma, renal disorders etc. were considered into account. Clinical outcome of patients under analyses was Pneumonia (CAP), ARDS (Acute Respiratory Distress Syndrome) and acute cardiac injury. The final output or reason of death for deceased cases was also noted down as declared on their death documents.

Statistical analysis

To describe patient's demographic characteristics, radiological findings, laboratory findings and comorbidities, the descriptive statistics was used. Categorical variables were expressed as percentages (frequencies) while continuous variables were reported as mean ± SD (Standard Deviation). Chi-square was used to compare of categorical variables with a p-value 0.05 of significance. Mann-Whitney t-test and One-way ANOVA was used for the comparison of the mean value for continuous variables. Pearson correlation coefficient was used to correlate biomarkers and continuous clinical variables. ROC (receiver operator curves) was used to calculate the predictive values of by identifying Youden's index. p-value ≤ 0.05 was considered statistically significant. To identify predictors of clinical characteristics associated with mortality, logistic regression analysis was used, and Odds Ratio (OR) were calculated with 95% Confidence Interval (CI) with a p-value<0.05 SPSS (Statistical Package for the Social Sciences) version 25.0 software (SPSS Inc.) and GraphPad Prism (Version 8, San Diego, CA) were used to perform all statistical analysis.

Results

Demographic characteristics of patients

Demographic and clinical characteristic of COVID-19 patients (n=200) with their final outcomes are summarized in Table 1. Out of 200, 143 (72%) patients were declared dead due to COVID infection and 57 (28%) were discharged once they recovered from infection and were physically stable. In total, 128 patients (64%) were male, and 72 patients (36%) were female. The median age of all patients was 54 years (IQR: 29-65 years). The median age for deceased and recovered cases was 61 (IQR: 24,70) and 36 (IQR: 26,52) years respectively. When stratified with age group, 26% patients (n=53) were of >24-40 years, 29% patients (n=57) were of 40-65 years and 45% patients (n=90) were of >65 years of age. Among deceased cases, maximum deaths (50%) were reported among older peoples of ore that >65 years of age while in recovered cases, maximum no. of deaths (44%) reported were of young age i.e. 24-40 years. Clinical outcome of the patients showed that 55% cases (n=110) had Pneumonia, 35% (n=70) had ARDS and

10% (n=20) had acute-heart injury. The data for co-morbid conditions showed that 35% of patients (n=70) had hypertension, 28% (n-55) had diabetes, 15% (n=30) had cardiac vascular disease. Hypertension and cardiac vascular disease was significantly higher in deceased cases as compared to survived case with a p-value 0.079 and 0.064 respectively. A significant difference for duration of fever was observed between deceased (mean 9.2 days) and recovered cases (mean 6.1 days) with a p-value of 0.02. Deceased cases has a significant longer duration of hospitalization (mean 28 days) as compared to survived cases (mean, 5 days) with a p-value of 0.0019 (Table 1).

Clinical and radiological characteristics of patients

Descriptive statistics and Mann Whitney t-test for clinical symptoms showed that fever (p-value 0.069), cough (p-value 0.032), expectoration (p-value 0.041), weakness (p-value 0.077), diarrhea (p-value 0.085), chills (p-value 0.037), shortness of breath (p-value 0.047), loss of smell and taste (p-value 0.096) were significantly experienced in higher proportion by deceased cases as compared to recovered cases. Radiological features based on High Resolution Chest Scan (HRCT) showed that Ground Glass Opacity (GGO) (p-value 0.040), consolidation (p-value 0.023), infiltration (p-value 0.065), GGO and consolidation in combination (p-value 0.030) and pleural effusion (p value 0.081) were significantly prevalent in deceased cases as compared to recovered cases (Table 1).

Hematological profiling of COVID-19 patients

Different hematological parameters were compared between recovered and deceased group to identify the potential parameter involved in disease severity and ultimate death. All the tests were performed within 48 hours of hospital admission. No significant difference was observed in WBCs count and platelet count among both groups. However, the lymphocyte count and lymphocyte ratio showed a significant difference. The mean lymphocyte count (mean; 0.81, SD 1.06) and lymphocyte ratio (mean; 16%, SD ± 13.1) of deceased cases was significantly lower than the mean lymphocyte count (mean 1.5, SD ± 1.2) and lymphocyte ratio (mean; 24%, SD ± 18.4) of recovered cases with a p-value 0.0003 and 0.005, respectively. The mean value of Hb in deceased group was 10.74 which is significantly lower than that of recovered cases (mean; 13.39) with a p-value of <0.0001. Among blood gas levels, PO2 and PCO2 were significantly lower in deceased cases with a p-value of 0.023 and 0.032 respectively (Table 2).

Characteristics of biochemical parameters

Different inflammatory markers that play a vital role in infection were analyzed that included CRP, ESR, Ferritin, LDH and Pro-Calcitonin. As, compared to recovered cases, the levels of all the inflammatory markers monitored were elevated in deceases cases. CRP level were elevated 7 folds (p-value<0.00001), ferritin levels were elevated 3.4 folds (p-value 0.001), LDH levels were increased by 1.4 folds (p-value<0.00001) and pro-calcitonin levels were increased by 5.6 folds (p-value 0.0223) in deceased patients. To observe the serum electrolyte imbalance, Potassium (K+), Sodium (Na+), Chloride (Cl-), Calcium (Ca+2) levels were checked. Potassium levels were significantly lower in deceased patients (mean; 3.45, SD 0.52) as compared to recovered cases (mean; 3.70, SD ± 0.58) with a p-value of 0.0004. Sodium levels were significantly low in deceased cases (mean; 133, SD ± 4.42) as compared to recovered cases (mean; 137 ± 1.87) with a p-value of <0.00001). Similarly, low levels of Chloride and Calcium were observed in deceased patients as compared to recovered cases with a p-value of 0.0011 and 0.0021, respectively (Table 2 and Figure 1).

Variables	Total cases N (%)	Survivors N (%)	Non-survivors N (%)	p-value
Total Patients	200 (100)	57 (28)	143 (72)	0.04
Age (median, Y)	47	38	62	0.0001
>24-40 Y	42 (21)	14 (24)	28 (20)	0.005
>40-65 Y	68 (34)	23 (40)	43 (30)	0.0003
> 65 Y	90 (45)	20 (35)	72 (50)	0.105
Gender				
Male	128 (64)	25 (20)	103 (80)	0.201
Female	72 (36)	18 (44)	54 (56)	0.091
Clinical outcome				
Pneumonia	110 (55)	37 (42)	73 (58)	0.004
ARDS	70 (35)	8 (11)	62 (89)	0.398
Acute Heart Injury	20 (10)	2 (2)	18 (98)	0.422
Co-morbidity				
Hypertension	70 (35)	18 (40)	52 (60)	0.079
Diabetes	55 (28)	11 (33)	44 (67)	0.191
Chronic Lung disease	45 (22)	3 (6)	42 (94)	0.497
Cardiac Vascular disease	30 (15)	8 (26)	22 (74)	0.064
Duration of Fever, days	7.9 + 4.8	6.1 + 4.4	9.2 + 4.7	0.021
Hospital admission, days	20 (9, 28)	15 (9, 20)	28 (18, 31)	0.0019
Noninvasive mechanical ventilation	65 (56)	7 (11)	58 (89)	0.405
Invasive mechanical ventilation	98 (49)	3 (3)	95 (97)	0.573
Clinical characteristics				
Fever	190	50 (88)	140 (98)	0.069
Cough	154	45 (79)	109 (76)	0.032
Expectoration	134	38 (67)	96 (67)	0.041
Dyspnea	146	21 (37)	125 (87)	0.32
Headache	170	37 (65)	133 (93)	0.153
Weakness	190	49 (86)	141 (99)	0.077
Diarrhea	158	40 (70)	118 (83)	0.085

Chills	185	46 (81)	139 (97)	0.037
Shortness of Breadth	182	53 (93)	137 (96)	0.047
Alternate taste/smell	158	39 (68)	119 (83)	0.096
Radiological characteristics				
GGO	200	57 (100)	143 (100)	0.04
Consolidation	136	41 (72)	95 (66)	0.023
Infiltration	139	37 (65)	102 (71)	0.065
GGO + Consolidation	189	45 (79)	108 (76)	0.03
Pl. Effusion	120	23 (40)	67 (47)	0.081

Table 1: Demographic and Clinical outcome of COVID-19 patients.

Characteristics	Normal range	Total cases N=200	Survivors N=57	Non-survivors N=143	p-value
Hematological parameters (Mean + SD)					
WBCs *10^9/L	04-10	4.9 ± 3.6	4.5 ± 3.3	5.4 ± 4.2	0.217
Lymphocyte Count *10^9/L	0.8-4	1.6 ± 1.39	1.5 ± 1.2	0.81 ± 1.06	0.0003
Neutrophil to Lymphocyte Ratio %	20-40%	25 % ±12.8	24% ± 18.4	16% ± 13.1	0.005
Platelets *10^9/L	100-300	195.3 ± 60.5	187.8 ± 92.3	206.1 ± 81.9	0.381
Blood gas (Mean + SD)					
Hemoglobin g/dL	11-16	12.49 ± 1.80	13.39 ± 1.65	10.74 ± 2.68	<0.0001
PO_2 (mmHg)	80- 100	76.91 ± 42.9	86.70 ± 54.9	68.37 ± 38.0	0.023
PCO_2 (mmHg)	35-405	38.8 ± 18.5	41.53 ± 19.5	35.38 ± 14.09	0.032
pH	7.35-7.45	7.43 ± 1.2	7.46 ± 0.44	7.39 ± 1.29	0.568
HCO_3 (mmol/L)	22-28	26.62 ± 10.5	27.8 ± 9.52	24.0 ± 9.75	0.012
SO_2 (mmHg)	75-100	87.7 ± 41.1	82.4 ± 40.2	80.84 ± 43.0	0.803
Inflammatory markers (Mean + SD)					
CRP mg/L	< 10	62 ± 85.5	15.6 ± 21.04	76.59 ± 90.06	<0.0001
ESR mm/h	0-15	29.8 ± 13.9	23.4 ± 12.3	43.6 ± 15.5	<0.0001
Ferritin mg/L	10-322	205 ± 1762	86 ± 244	300 ± 2058	0.001
LDH U/L	109-245	272 ± 174	214 ± 68	303 ± 205	<0.0001
Pro-Calcitonin ng/ml	< 0.5	0.22 ± 0.64	0.06 ± 0.26	0.28 ± 0.71	0.0223
Coagulation factors (Mean + SD)					
PT seconds	60-70	65 ± 2.4	64.91 ± 7.50	69.06 ± 8.45	<0.0001
aPTT seconds	30-40	36 ± 1.8	35.47 ± 5.89	34.06 ± 15.48	0.005
D-Dimers ng/ml	<250	1028 ± 1519	534.3 ± 531.6	1226 ± 1698	0.0003
Fibrinogen mg/dL	200-400	595.5 ± 225	478.7 ± 156.7	713.9 ± 292.5	<0.0001
Muscle markers (Mean + SD)					
Creatinine Kinase U/L	30-170	195 ± 366.5	123.8 ± 147.9	272.2 ± 578.9	0.0488
Total protein g/L	60-83	71.54 ± 6.85	68.75 ± 7.30	74.47 ± 8.44	<0.0001
Glucose mg/dL	<140	88 ± 8.54	76.75 ± 8.31	108.6 ± 11.77	<0.0001
Troponin ng/ml	0-0.15	0.03 ± 0.24	0.10 ± 0.01	0.18 ± 0.59	<0.0001
Serum electrolytes (Mean + SD)					
Potassium mmol/L	3.40-4.50	3.98 ± 0.63	3.70 ± 0.58	3.45 ± 0.52	0.0004
Sodium mmol/L	136-145	138 ± 3.2	137.8 ± 1.88	133 ± 4.42	<0.0001
Chloride mmol/L	95-105	98 ± 7.82	101.7 ± 8.68	98.5 ± 9.08	0.0011
Calcium mg/L	8.5-10.2	8.21 ± 5.41	9.51 ± 4.48	7.99 ± 2.72	0.0021
Magnesium mg/dL	1.7-2.2	2.0 ± 0.94	1.91 ± 0.20	2.11 ± 0.34	0.0002
Hepatic markers					
ALT U/L	7-40	39.2 ± 80.9	21.9 ± 15.6	45.54 ± 93.75	0.0001
AST U/L	13-40	43 ± 74.8	23.2 ± 8.9	50.19 ± 86.33	0.0011
Albumin g/L	35-50	39.3 ± 5.2	25.52 ± 7.21	29.29 ± 7.53	0.0044
Total bilirubin umol/L	3.4-17.1	11.5 ± 4.6	10.90 ± 1.88	14.66 ± 2.81	<0.0001
Alkaline Phosphatase IU/L	440-147	91 ± 41.58	90.33 ± 35.96	87.73 ± 81.02	0.026
Renal markers					
Creatinine mg/L	05-08	10.3 ± 8.2	8.30 ± 4.28	10.76 ± 8.79	0.0001
BUN nmol/L	2-7	3.8 ± 2.3	3.92 ± 0.78	5.66 ± 2.81	0.0005
Uric acid mg/dL	2.7-8.5	3.9 ± 0.72	4.68 ± 0.82	3.31 ± 0.76	<0.0001

Table 2: Characteristics of Biochemical parameters of COVID-19 patients.

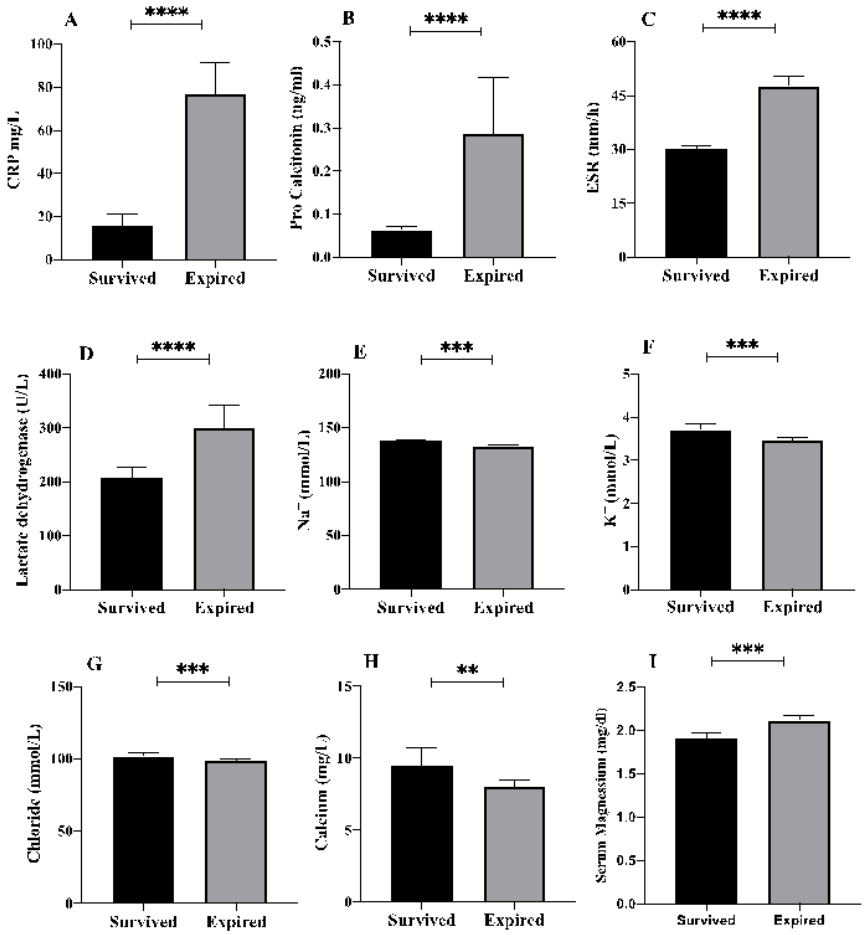

Figure 1: Comparison of inflammatory markers and serum electrolytes among survived and expired COVID-19 cases using non-parameter Mann-Whitney t-test. Bar shows the median values for survived (black) and Expired (grey) and error bars show 95% Confidence Interval. P-value of 0.005 was considered as significant.

Characteristics of coagulation and muscle markers

Among coagulation factors, four factors were observed, PT, aPTT, D-Dimers and Fibrinogen. The delay in the coagulation for PT was increased 1.06 folds (p-value<0.0001) and that of aPTT was increased by 1.18 folds (p-value 0.0050) in deceased cases. D-Dimers were increased 2.3 folds high in deceased cases (p-value<0.0003) whereas Fibrinogen was 1.5 folds high in deceased cases (p-value<0.0001) in comparison to the recovered cases. Among muscle markers, creatinine kinase was 2.21 folds high (p-value 0.048), total proteins were 1 fold high (p-value<0.0001), Troponin was 1.8 folds high (p-value<0.0001) and blood glucose was 1.4 folds high (p-value<0.0001) in deceased cases as compared to record cases (Table 2 and Figure 2).

Markers for liver impairment and renal dysfunction

To administer the effect of COVID infection on liver impairment, the alteration of hepatocytes damage biomarkers, such as Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), albumin, bilirubin and alkaline phosphatase were observed. The data showed that both alanine aminotransferase and aspartate aminotransferase were significantly increased in deceased cases (mean ALT; 45.54, mean AST; 50.19) as compared to recovered cases (mean ALT; 21.9, mean AST; 23.2) with a p-value of 0.001 and 0.0011 respectively. The amount of albumin in deceased patients was significantly lower (mean; 29.29, SD ± 7.53) as compared to recovered cases (mean; 25.52, SD ± 7.21) with a p-value of 0.0044. The amount of total bilirubin was significantly increased in deceased cases (mean; 14.66, SD ± 2.81) as compared to recovered cases (mean; 10.90, SD ± 1.88) with a p-value of <0.0001. On the other hand, alkaline phosphatase was observed to be in low concentration in deceased patients (mean; 87.73, SD + 81.02) as compared to recovered cases (mean; 90.33, SD ± 35.96) with a p-value of 0.026. For renal dysfunction, creatinine, BUN and uric acid were observed. The data showed that amount creatinine was 1.3 folds high (p-value 0.0001) and BUN was 1.5 folds high (0.0005) in deceased cases as compared to recovered cases. However, uric acid was 1.4 folds low in deceased cases as compared to recovered cases (Table 2 and Figure 3).

Pearson's correlation coefficient showed a strong correlation for gender (r= 0.85) while moderate correlation was found for serum sodium (r=0.545), Ferritin (r=0.445), D-dimers (r=0.469), LDH (r=0.584), pro-calcitonin (r=0.435), bilirubin (r=0.434) and co-morbidities (r=0.461) with a p-value of <0.001. A negative correlation of moderate level was found for albumin (r=-0.599) with a p-value of >0.0001 (Table 3).

Parameters	R*	p-value	Parameters	R*	p-value
Age	0.856	<0.0001	LDH	0.584	<0.0001
Gender	0.129	<0.0001	Pro-calcitonin	0.435	<0.0001
WBCs	0.241	0.0005	Fibrinogen	0.212	<0.0001
Platelets	0.23	0.0001	Pt	0.263	<0.0001
Lymphocyte Count	-0.285	0.0004	aPTT	0.29	0.0003
ESR	0.212	0.002	ALT	0.246	0.0004
Serum Na	0.545	<0.001	AST	0.324	<0.0001
Serum K	-0.249	0.003	Albumin	-0.599	<0.0001
Serum Ca	0.215	0.002	Bilirubin	0.43	<0.0001
Serum Chloride	0.27	0.001	Creatinine	0.261	<0.0001
Serum Magnesium	0.31	<0.0001	Urea	0.248	0.0003
CRP	0.347	<0.0001	BUN	0.312	0.002
Ferritin	0.445	<0.0001	Co-morbidities	0.461	<0.0001
D-Dimers	0.469	<0.0001	Plasma Glucose	0.363	<0.0001
Note: *Pearson's correlation coefficient					

Table 3: Correlation of demographic factor and disease parameters with COVID-19 infection.

Characteristics	AUC	95% Confidence interval	Cut-off	Cut-off	Sensitivity	Specificity	p-value
		Lower limit	Upper limit				
Inflammatory markers							
CRP mg/L	0.746	0.678	0.8155	10.17	75.71	66.07	<0.0001
ESR mm/h	0.811	0.747	0.8753	38	81.12	100	<0.0001
Ferritin mg/L	0.746	0.662	0.8311	127.3	72.94	60.87	<0.0001
LDH U/L	0.716	0.628	0.8045	215	70.93	66	<0.0001
Pro-calcitonin ng/ml	0.687	0.6756	0.725	?	72.79	65.45	0.0002
Coagulation factors							
PT seconds	0.716	0.6417	0.792	67.5	68.53	64.91	<0.0001
aPTT seconds	0.626	0.5498	0.703	36.5	66.43	64.91	0.0053
D-Dimers ng/ml	0.7	0.6033	0.7977	463.5	70	70	0.0004
Fibrinogen mg/dL	0.793	0.723	0.8639	515	78.17	66.67	<0.0001
Muscle markers							
Creatinine Kinase	0.513	0.417	0.6105	145	56.01	52.11	0.8067
Total protein	0.707	0.6175	0.7969	68.5	78.4	68.23	<0.0001
Glucose	1	1	1	126	100	100	<0.0001
Troponin	0.89	0.8382	0.9428	0.05	89.05	100	<0.0001
Serum electrolytes							
Potassium mmol/L	0.658	0.5683	0.7478	3.7	75.18	71.43	0.0005
Sodium mmol/L	0.812	0.7529	0.8721	136.5	76.06	89.47	<0.0001
Chloride mmol/L	0.651	0.5723	0.7315	101.5	60.56	56.14	0.0012
Calcium mg/L	0.639	0.5611	0.7181	8.7	60.71	62.4	0.0021
Hepatic markers							
ALT U/L	0.696	0.6035	0.7894	19.5	72.41	64.29	0.0002
AST U/L	0.67	0.5829	0.7577	20.56	72.17	65.85	0.0012
Albumin g/L	0.651	0.5522	0.7515	27.1	65.77	63.89	0.0047
Bilirubin umol/L	0.874	0.8174	0.9306	11.8	99.3	70.69	<0.0001
Alkaline Phosphatase IU/L	0.608	0.5086	0.7086	80.5	70.3	64.44	0.026
Renal markers							
Creatinine mg/L	0.674	0.5915	0.7581	7.9	72.79	65.45	0.0002
BUN nmol/L	0.652	0.5787	0.7265	3.92	73.43	72.41	0.0007
Uric acid mg/dL	0.891	0.849	0.9338	7.8	78.91	72.45	<0.0001

Table 4: Bivariate analysis of biochemical parameters associated with disease severity and mortality in COVID-19 patients.

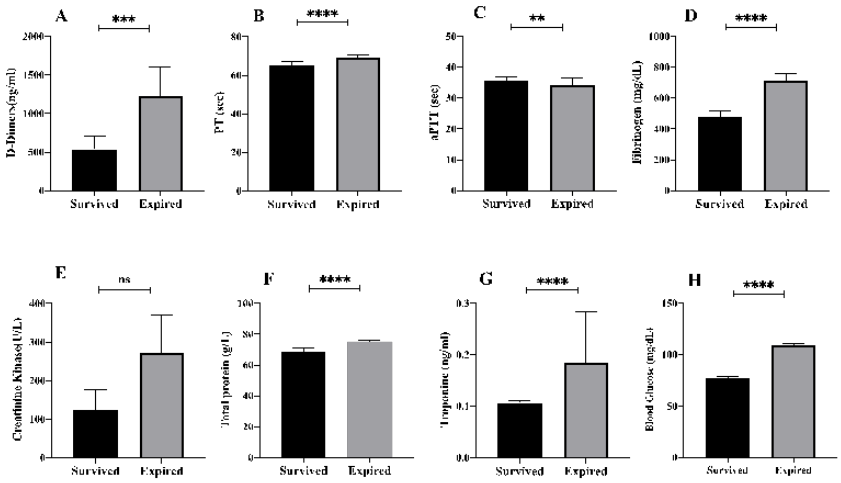

Figure 2: Comparison of coagulation and muscle markers acquired within 48 hours of admission of COVID-19 cases using non-parameter Mann-Whitney t-test. Bar shows the median values for survived (black) and Expired (grey) and error bars show 95% Confidence Interval. P-value of 0.005 was considered as significant.

Association of inflammatory markers and coagulation factors with disease severity and mortality in COVID-19 patients

ROC analysis inflammatory markers and coagulation factors showed a significant association with disease severity and mortality between survived and expired patients infected with COVID-19 infection (Table 4). All the variables were dichotomized, and logistic regression showed a significant association under ROC defined cut-off among survived and expired patients. The ROC curve for CRP (AUC=0.746, CI 0.768-0.815, p-value <0.0001) suggested the best cut off point was 10.17 mg/L with a sensitivity of 75.71% and specificity of 66.07% (Figure 4A). The ROC curve for ESR (AUC=0.811, CI 0.747-0.875, p-value<0.0001) suggested the best cut-off point was 38 mm/h with a sensitivity of 81.12% and specificity of 100% (Figure 4B). The ROC curve for ferritin (AUC=0.746, CI 0.662-0.831, p-value<0.0001) suggested the best cut off point was 127.3 μg/L with a sensitivity of 72.94% and specificity of 60.87% (Figure 4C). The ROC curve for LDH (AUC=0.716, CI 0.628-0.804, p-value<0.0001) suggested the best cut off point was 215 U/L with a sensitivity of 70.93% and specificity of 66% (Figure 4D). The ROC curve for pro-calcitonin (AUC=0.687, CI 0.675-0.725, p-value 0.0002) suggested the best cut off point was 0.45 ng/ml with a sensitivity of 72.79% and specificity of 65.45% (Figure 4E). The ROC curve for coagulation factors such as PT indicated the best cut-off point was 67.5 seconds (AUC= 0.716, CI 0.641-0.792, p-value<0.0001) with a sensitivity and specificity of 68.53% and 64.91% respectively (Figure 4F). The ROC curve for aPTT indicated the best cut-off point was 36.5 seconds (AUC=0.626, CI 0.549-0.703, p-value 0.005) with a sensitivity and specificity of 66.43% and 64.91% respectively (Figure 4G). The ROC curve for D-Dimers indicated the best cut-off point was 463.5 ng/ml (AUC=0.702, CI 0.603-0.797, p-value 0.0004) with a sensitivity and specificity of 70% and 70%, respectively (Figure 4H). The ROC curve for fibrinogen indicated the best cut-off point was 515 mg/dL (AUC=0.793, CI 0.723-0.863, p-value<0.0001) with a sensitivity and specificity of 66.43% and 64.91%, respectively (Figure 4I).

Association of serum electrolytes with disease severity and mortality in COVID-19 patients

Serum electrolytes were also found to be significantly associated with disease severity and mortality in COVID-19 patients (Table 4). The ROC curve for Na^+ showed the best cut-off value of 136.5 mmol/L (AUC=0.812, CI 0.752-0.872, p-value<0.0001) with a sensitivity of 76.06% and specificity of 89.47% (Figure 5A). The ROC curve for K^+ showed the best cut-off value of 3.7 mmol/L (AUC=0.658, CI 0.568-0.747, p-value 0.0005) with a sensitivity of 75.18% and specificity of 71.43% (Figure 5B). The ROC curve for Cl- showed the best cut-off value of 101.5 mmol/L (AUC=0.651, CI 0.572-0.731, p-value 0.001) with a sensitivity of 60.56% and specificity of 56.14% (Figure 5C). The ROC curve for Ca^{2+} showed the best cut-off value of 8.7 mg/L (AUC=0.639, CI 0.561-0.718, p-value 0.002) with a sensitivity of 60.71% and specificity of 62.40% (Figure 5D).

Association of marker of liver impairment and kidney dysfunction with disease severity and mortality in COVID-19 patients

Among markers of liver impairment (Table 4), the ROC curve of ALT depicted the best cut-off value of 19.50 U/L (AUC=0.696, CI 0.603-0.789, p-value 0.0002) with a sensitivity of 72.41% and specificity of 64.29% (Figure 6A). The ROC curve of AST depicted the best cut-off value of 20.56 U/L (AUC=0.670, CI 0.582-0.757, p-value 0.0012) with a sensitivity of 72.17% and specificity of 65.85% (Figure 6B). The ROC curve of albumin depicted the best cut-off value of 27.10 g/L (AUC=0.651, CI 0.552-0.751, p-value 0.0047) with a sensitivity of 65.77% and specificity of 63.89% (Figure 6C). The ROC curve of bilirubin depicted the best cut-off value of 11.80 umol/L (AUC=0.874, CI 0.817-0.930, p-value<0.0001) with a sensitivity of 99.30% and specificity of 70.69% (Figure 6D). The ROC curve of alkaline phosphatase depicted the best cut-off value of 80.50 g/L (AUC=0.608, CI 0.508-0.708, p-value 0.0047) with a sensitivity of 70.30% and specificity of 64.44% (Figure 6E). The muscle marker under observation was creatinine kinase showed no significant association. The ROC curve suggested the cut-off value of 145 U/L (AUC=0.513, CI 0.417-0.61, p-value 0.806) with the sensitivity of 56.01% and specificity of 52.11% (Figure 6F). In ROC analysis of renal dysfunction markers, the best cut-off value for Creatinine was 7.9 mg/L (AUC=0.674, CI 0.591-0.758, p-value 0.0002) with the sensitivity and specificity of 72.93% and 65.45%, respectively (Figure 6G). The best cut-off value for BUN was 3.92 nmol/L (AUC=0.652, CI 0.578-0.726, p-value 0.0007) with the sensitivity and specificity of 73.43% and 72.41% respectively (Figure 6H). The best cut-off value for uric acid was 7.8 mg/L (AUC=0.891, CI 0.849-0.933, p-value<0.0001) with the sensitivity and specificity of 78.91% and 72.45%, respectively (Figure 6I).

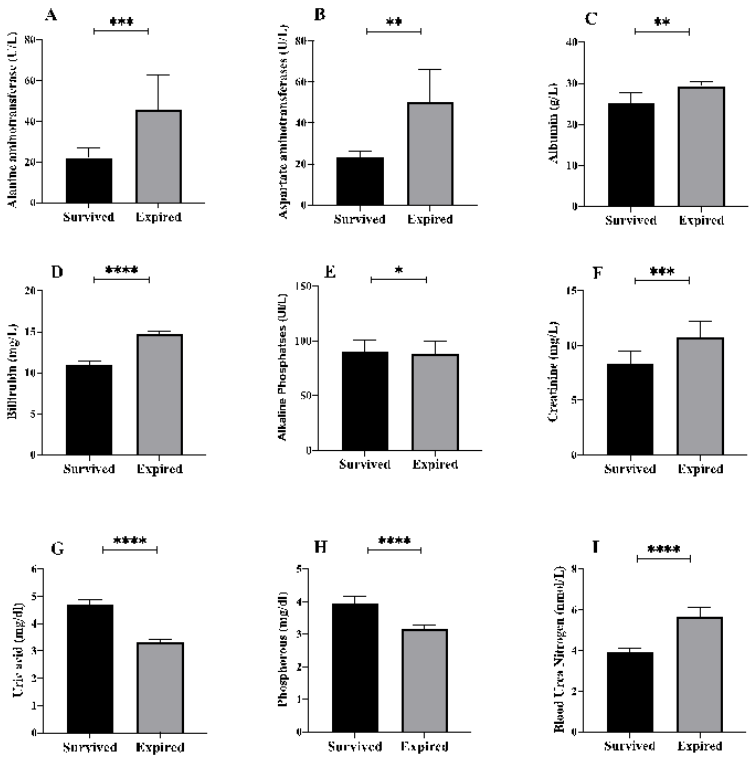

Figure 3: Comparison of markers for Liver impairment and renal dysfunction of COVID-19 cases using non-parameter Mann-Whitney t-test. Bar shows the median values for survived (black) and Expired (grey) and error bars show 95% Confidence Interval. P-value of 0.005 was considered as significant.

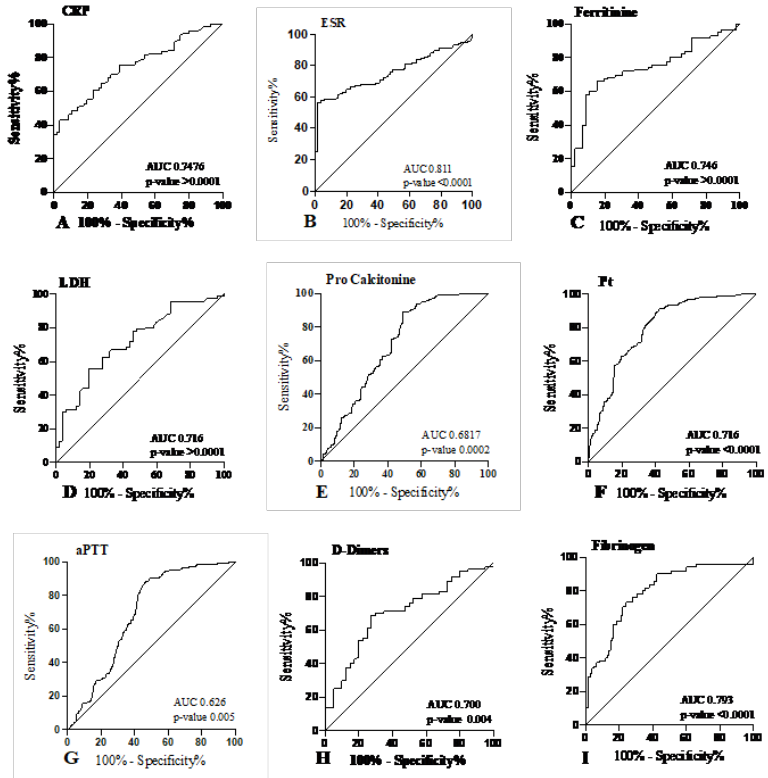

Figure 4: ROC curve of inflammatory markers and coagulation factors between survived and expired COVI-19 patients. A) CRP; B) ESR; C) Ferritin; D) LDH; E) Pro-Calcitonin; F) PT; G) aPTT; H) D-Dimers; I) Fibrinogen.

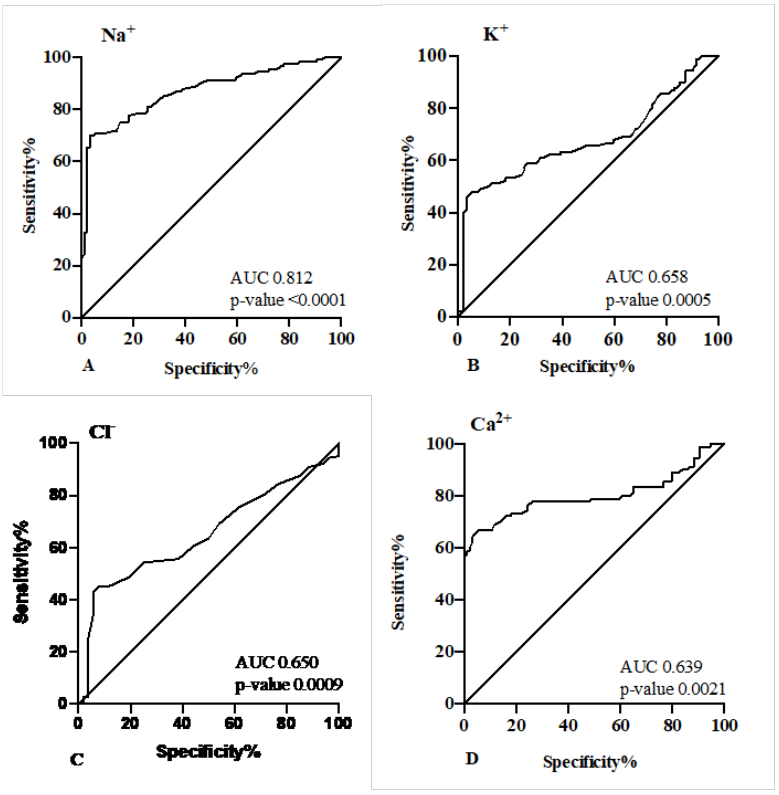

Figure 5: ROC curves for association of serum electrolytes between survived and expired COVID-19 patients. A) Na+; B) K+; C) Cl-; D) Ca2+.

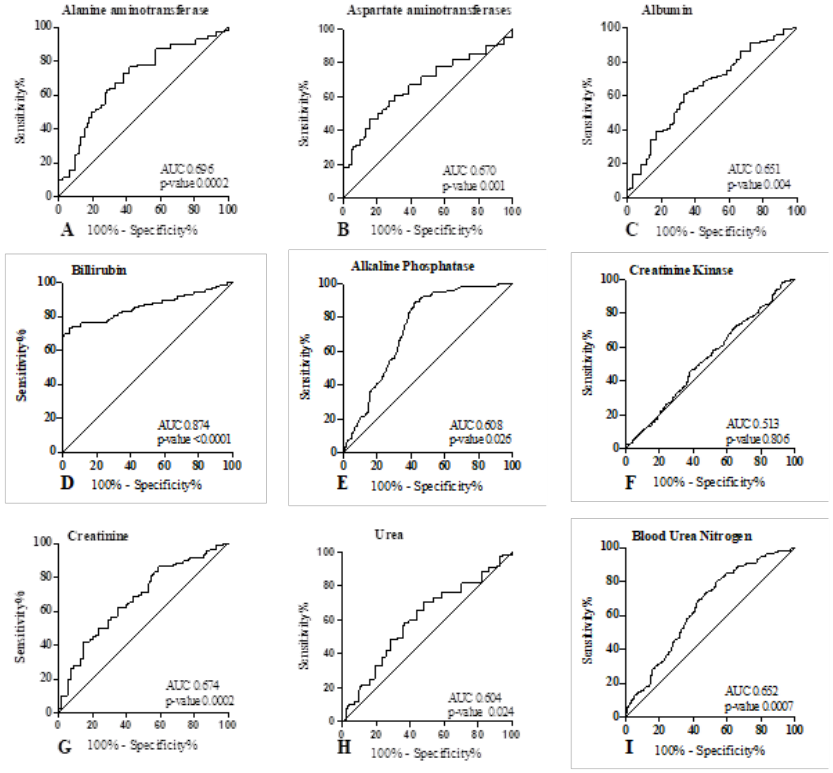

Figure 6: ROC curves for the association of marker of liver impairment and kidney dysfunction between survived and expired COVI-19 patients. A) ALT; B) AST; C) Albumin; D) Bilirubin; E) Alkaline Phosphatase; F) Creatinine Kinase; G) Creatinine; H) Urea; I) Blood Urea Nitrogen.

Discussion

The ongoing global pandemic of COVID-19 is due to emergence of a novel infection caused by Sever Acute Respiratory Syndrome (SARS Cov-2) started from Wuhan China in 2019. Almost every country and territory in the world has been impacted by this disease. World Health Organization (WHO) has reported more that 290 million cases of COVID-19 by the end of December 2021 and 5.4 million deaths [22]. Till date, the world has faced four waves of the infection and three variants of concern known as Alpha, Delta and Omicron. The fifth wave with the emergence of Omicron has started at the end of 2021 [23]. In the first 2 weeks of January 2022 a record number of cases i.e. 15 million cases of COVID 19 cases have been reported to WHO and most of them are infected with omicron [24]. By December 2021, Pakistan has reported 1.3 million cases of COVID 19 with 28,000 deaths which accounts for the 0.5% of the global burden of the infection [25]. Various studies have reported that overall death rate due to COVID-19 varies from 2%-12% in different countries [26,27]. The major cause of death among COVID-19 patients reported in this study and many previous studies is heart attack and multiple organ dysfunction and failure [28]. Furthermore, accurate, rigorous and timely recording and reporting of different aspects of the disease is crucial for determining the disease severity and risk of mortality. Thus, a clear characterization of clinical, radiological, immunological and laboratory parameters is of paramount importance for effective treatment strategies and to reduce the disease severity and risk of mortality.

In this study, we have comprehensively analyzed the correlation of clinical, hematological and biochemical parameters with disease severity and mortality. We have observed that among hospitalized cases, 72% cases (n=143) died due to complication of COVID-19. Among died cases, the proportion of male patients (82%) was significantly higher than female patients and maximum deaths were reported in older people (62%) of age >65 years. The clinical features of COVID-19 patients of the present study were comparable with those of previous studies. Co-morbid conditions such as hypertension, diabetes, chronic lung diseases and cardiovascular diseases were predominately linked with the high risk of disease severity in COVID-19 patients, which was in accordance with the observations of previous published studies [29]. A significant proportion of COVID-19 patients were presented with fever and chest imaging alterations. Clinical outcome of the admitted patients showed that 55% cases were presented with pneumonia, 35% with ARDS and 20% with acute heart injury. The proportion of these outcomes was higher in non-survivors as compared to survivors. The data illustrated that higher body temperature before admission might be a risk factor for disease deterioration in COVID-19 patients. The duration of fever and stay in hospital was longer from onset to hospitalization in non-survivors as compared to survivors (p-value 0.021 and 0.001) respectively which was consistent with a previous study [30]. The survived patients had normal body temperatures within 7 days after admission. Consistent fever during hospitalization might be an indicator of the mortality in COVID-19 patients.

The clinical and radiological parameters of the study cases revealed that fever (p-value 0.0006), cough (p-value 0.032), chills (p-value 0.037), shortness of breath (p-value 0.047) were predominant in non-survivors as compared to survivors. The predominant radiological characteristics of non-survivors were GGO (p-value 0.040), consolidation (p-value 0.023), GGO and consolidation in combination (p-value 0.030). These findings have been reported by many researchers [31-33].

Lymphocytes play a critical role in the viral clearance and preservation of immune system function during an infection [34,35]. Researchers have showed that reduced lymphocyte counts were observed in most severe patients with COVID-19 but remained within the normal range in non-severe patients [36,37]. In the CBC of COVID-19 patients, we found that lymphopenia was the most common in non-survivors than in survivors (0.81×10^9/L vs. 1.5×10^9/L, p-value 0.0003) Neutrophil to lymphocyte ratio was also reduced in non-survivors than survivors (16% vs. 24%, p-value 0.005). It is suggested that lymphocytes are directly infected by virus, principally T cell resulting in reduction of CD^{4+}, and CD^{8+} cells, thus suppressing the immune response [38,39].

Hyperinflammation due to cytokine storm is the hallmark of COVID-19 infection. Among the inflammatory markers, increased levels of CRP (p-value <0.0001), ESR (p-value<0.0001), Ferritin (p-value 0.001), LDH (p-value <0.0001) and PCT (p-value 0.022) were observed in non-survivors were positively correlated with disease severity. Overall, literature evidence suggests that increased levels of various inflammatory markers are associated with disease severity and mortality in the early stage of COVID-19, [40,41]. Another common complication of COVID-19 is a hypercoagulable state, which might promote thrombotic coagulopathies. The underlying pathological mechanisms include infection-related dysfunction of endothelial cells, which causes an increased production of thrombin and inhibition of the fibrinolysis; cardiovascular injury; hyperinflammation state [42,43]. In our patients, increased levels of D-Dimers (p-value 0.0003), Fibrinogen (p-value<0.0001) in COVID-19 patients reflect the coagulation alterations which can increase the risk of ARDS and mortality as reported previously [44,45]. We also observed that increased Prothrombin Time (PT) and decreased activated Partial Thromboplastin Time (aPTT) which has been reported to be associated with disease severity during the early phase of COVID-19 [46].

Electrolyte imbalance in COVID-19 patients has also been reported by many studies to be associated with disease severity particularly hyponatremia, hypokalemia, and hypocalcemia [39,47]. Our findings were also in agreement with previous studies as we also observed decreased levels of potassium (p-value 0.0004), sodium (p-value<0.0001), chloride (p-value 0.0011) and calcium (p-value 0.0021) but increased level of magnesium (p-value 0.0002) in serum of COVID-19 patients who did not survive. Our data suggests that increased levels of CK (p-value 0.048) and troponin (p-value<0.0001) in non-survivor cases could be a direct effect of the SARS-CoV-2, which can also infect cells of the muscle tissue due to the expression of the ACE2 receptor. Typically, increased levels of biomarkers of muscle injury such as creatinine kinase and troponin are related to kidney dysfunction and cardiac injury [48].

Another common laboratory finding is the alteration of hepatocytic biomarkers such as ALT, AST, albumin, alkaline phosphatase and bilirubin. We found the increased levels of all these biomarkers in non-survivor COVID-19 patients. Although the underlying mechanism of this alteration is not well depicted but higher levels of biomarkers related to liver dysfunction have been observed to be linked associated with severity and mortality due to COVID-19 [48]. Multiple studies have demonstrated that alterations in renal biomarkers, as depicted by raised levels of serum creatinine, in COVID-19 patients. The virus can could directly infect kidney tubular cells expressing the ACE2 receptor on their cellular surface. According to an Italian report, about 25%-30% of deaths due to COVID-19 are due to Acute Kidney Injury (AKI). Our findings of the study are also in pertinent with the observation reported

by other researchers globally.

Conclusion

For the pertinent diagnosis and treatment management of COVID-19, the laboratory medicine plays a key role. The SARS-CoV-2 infection is characterized by several biochemical alterations, which can be recognized by specific biomarkers helping clinicians to clinch requisite clinical monitoring for an ameliorate clinical outcome. Among all biomarkers associated with disease severity and mortality lymphopenia, thrombocytopenia, CRP, ESR, PCT, LDH, AST, ALT, D-dimer, CK, albumin, creatinine phosphatase represents the most predictive parameters of severe COVID-19 infection.

Limitation of the Study

The major limitation of the present study is that the data presented is of a single hospital with relatively small number of non-survivor cases. Due to this limitation, the proportion of some clinical manifestations of the patients might be different from the reports from other cohort studies with large sample size. Therefore, a cohort study with large numbers of patients is needed to verify our conclusions.

Ethical Approval

This study was approved by Institutional Ethical Review Board (IERB) of Allama Iqbal Medical College/Jinnah hospital, Lahore (Ref No. 8/15/12/2020/S2/ERB) in 79th meeting of the board. Participant confidentiality was maintained throughout the investigation. All subjects who participate in the investigation were assigned a study identification number by the investigation team for the labelling of questionnaires and specimens. As this was a retrospective study using patient data form hospital record, so patient's consent was not required. The study was only approved by IERB.

Consent for Publication

All authors have consented for this publication.

Available Data and Materials

The data can be obtained from corresponding author on reasonable request and through the permission or approval of ethical review board.

Competing Interest

None

Funding

This study did not receive any funding in partial or whole.

Author Contribution

Conceptualization: AK, FA ; Data Curation: AK, FA, HA, ST, SJ, SS ; Formal Analysis: AK, HA, SS ; Investigation: AK, FA, HA, ST, SJ, SS ; Methodology: AK, FA, HA, ST, SS ; Project Administration: AK, FA, HA, ST, SJ ; Resources: AK, FA ; Supervision: AK, FA ; Validation: AK, FA, SS ; Visualization: AK, FA, SS ; Writing-original draft: AK, SS ; Writing review and editing: AK, FA, SS

Acknowledgment

The authors would like to acknowledge Muhammad Tajjamul, Muhammad Asim and Saima Anwer or helping in data acquisition.

References

1. Chen N, Zhou M, Dong X, Qu J, Gong F, et al. (2020) Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: A descriptive study. Lancet 395:507-513.
2. Ge H, Wang X, Yuan X, Xiao G, Wang C, et al. (2020) The epidemiology and clinical information about COVID-19. EurJ Clin Microbiol Infect Dis 39:1011-1019.
3. Adhikari SP, Meng S, Wu Y, Mao Y, Ye R, et al. (2020) Epidemiology, causes, clinical manifestation and diagnosis, prevention and control of coronavirus disease (COVID-19) during the early outbreak period: a scoping review. Infect Dis Poverty 9:1-12.
4. CDC (2021) National Center for Immunization and Respiratory Diseases (NCIRD), Division of Viral Diseases.
5. Zheng Y, Sun L, Xu M, Pan J, Zhang Y, et al. (2020) Clinical characteristics of 34 COVID-19 patients admitted to intensive care unit in Hangzhou, China. J Zhejiang Univ Sci B 21:378-387.
6. Wang F, Liu J, Zjang P, Jiang W, Zhang L, et al. (2020) Expert consensus on prevention and control of COVID-19 in the neurological intensive care unit (first edition). Stroke Vasc Neurol 5:242-249.
7. Zeng F, Huang Y, Guo Y, Yin M, Chen X, et al. (2020) Association of inflammatory markers with the severity of COVID-19: A meta-analysis. Int J Infect Dis 96:467-474
8. Huang C, Wang Y, Li X, Ren L, Zhao J, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 395:497-506.
9. Chang HL, Chen K, Lai S, Kuo H, Su I, et al. (2006) Hematological and biochemical factors predicting SARS fatality in Taiwan. J Formos Med Assoc 105:439-450.
10. Xu L, Mao Y, Chen G (2020) Risk factors for 2019 novel coronavirus disease (COVID-19) patients progressing to critical illness: a systematic review and meta-analysis. Aging (Albany NY) 12:12410.
11. Qian GQ, Yang N, Ding F, Ma AHY, Wang Z, et al. (2020) Epidemiologic and clinical characteristics of 91 hospitalized patients with COVID-19 in Zhejiang, China: a retrospective, multi-centre case series. QJM Int J Med 113:474-481.
12. Samprathi M, Jayashree M (2021) Biomarkers in COVID-19: An Up-To-Date Review. Front Pediatr 8:607-647.
13. Wang JT, Sheng W, Fang C, Chen Y, Wang J, et al. (2004) Clinical manifestations, laboratory findings, and treatment outcomes of SARS patients. Emerg Infect Dis 10:818-824.
14. Tjendra Y, Mana AF, Espejo AP, Akgun Y, Millan NC, et al. (2020) Predicting Disease Severity and Outcome in COVID-19 Patients: A Review of Multiple Biomarkers. Arch Pathol Lab Med 144:1465-1474.
15. Wen Xs, Jiang D, Gao L, Zhou J, Xiao J, et al. (2021) Clinical characteristics and predictive value of lower CD4+T cell level in patients with moderate and severe COVID-19: A multicenter retrospective study. BMC Infect Dis 21:57.
16. Ejaz H, Alsrhani A, Zafar A, Javed H, Junaid K, et al. (2020) COVID-19 and comorbidities: Deleterious impact on infected patients. J Infect Public Health 13:1833-1839.
17. Singh AK, Gillies CL, Singh R, Singh A, Chudasama Y, et al. (2020) Prevalence of co-morbidities and their association with mortality in patients with COVID-19: A systematic review and meta-analysis. Diabetes Obes Metab 22:1915-1924.
18. Alamin AA, Yahia AIO (2021) Hematological parameters predict disease severity and progression in patients with COVID-19: A Review Article. Clin Lab 67.
19. China (2020) Notice on the novel coronavirus infection diagnosis and treatment plan (trial version sixth). In: National Health Commission of the People's Republic of China, editor.
20. RT-PCR- NG (2020) National testing guidelines for RT-PCR.
21. WHO (2022) COVID-19 dashboard.
22. CDC (2021) Centre for Disease Control and Prevention.
23. NIH (2022) National Institute of Health.
24. Mahase E (2020) Coronavirus: COVID-19 has killed more people than SARS and MERS combined, despite lower case fatality rate. BMJ 368:m641
25. Yang X, Yu Y, Cu J, Shu H, Xia J, et al. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. 8:475-481.

26. Carlos WG, Cruz CSD, Cao B, Pasnick S, Jamil S (2020) Novel Wuhan (2019-nCoV) Coronavirus. Am J Rspir Crit Care Med P7-P8.

27. Tian W, Jiang W, Yao J, Nicholson CJ, Li RH, et al. (2020) Predictors of mortality in hospitalized COVID-19 patients: a systematic review and meta-analysis. 92:1875-1883.

28. Li X, Xu S, Yu M, Wang K, Tao K, et al. (2020) Risk factors for severity and mortality in adult COVID-19 inpatients in Wuhan. 146:110-118.

29. Durrani M, Haq I, Kalsoom U, Yousaf A (2020) Chest X-rays findings in COVID 19 patients at a University Teaching Hospital - A descriptive study. Pak J Med Sci 36: S22-S26.

30. Wong HYF, Lam HYS, Fong AH, Leung ST, Chin TW, et al. (2020) Frequency and distribution of chest radiographic finding and distribution in COVID-19 positive patients. Radiology 296:E72-E78.

31. Bai Y, Yao L, Wei T, Tian F, Jin D, et al. (2020) Presumed asymptomatic carrier transmission of COVID-19. 32:1406-1407.

32. Yap K, Ada G, McKenzie IJN (1978) Transfer of specific cytotoxic T- lymphocytes protects mice inoculated with influenza virus 273:238-239.

33. Sitati EM, Diamond MS (2006) CD4+ T-cell responses are required for clearance of West Nile virus from the central nervous system. J Virol 80:12060-12069.

34. Wang F, Hou H, Luo Y, Tang G, Wu S, et al. (2020) The laboratory tests and host immunity of COVID-19 patients with different severity of illness. JCI Insight 5:e137799.

35. Chang D, Lin M, Wei L, Xie L, Zhu G, et al. (2020) Epidemiologic and clinical characteristics of novel coronavirus infections involving 13 patients outside Wuhan, China. JAMA 323:1092-1093.

36. Zhang G, Zhang J, Wang B, Zhu X, Wang Q, et al. (2020) Analysis of clinical characteristics and laboratory findings of 95 cases of 2019 novel coronavirus pneumonia in Wuhan, China: A retrospective analysis. Respir Res 21:74.

37. Fu L, Wang B, Yuan T, Chen X, Ao Y, et al. (2020) Clinical characteristics of coronavirus disease 2019 in China. J Infect 80:656-665.

38. Guan W, Ni Z, Hu Y, Liang W, Ou C, et al. (2020) Clinical characteristics of 463 patients with common and severe type coronavirus disease. N Engl J Med 2002032.

39. Qin C, Zhou L, Hu Z, Zhang S, Yang S, et al. (2020) Dysregulation of immune response in patients with coronavirus 2019 (COVID-19) in Wuhan, China. 71:762-768.

40. Bikdeli B, Madhavan MV, Jimenez D, Chuich T, Dreyfus I, et al. COVID-19 and thrombotic or thromboembolic disease: implications for prevention, antithrombotic therapy, and follow-up: JACC state-of-the-art review. J Am Coll Cardiol. 75:2950-2973.

41. Levi M, Poll TV (2017) Coagulation and sepsis. Thrmob Res 149:38-44.

42. Wu C, Chen X, Cai Y, Xia J, Zhou X, et al. (2020) Risk factors associated with acute respiratory distress syndrome and death in patients with coronavirus disease 2019 pneumonia in Wuhan, China. JAMA Intern Med 180:934-943.

43. Zhou F, Fan G, Liu Z, Cao B (2020) SARS-CoV-2 shedding and infectivity–Authors' reply. Lancet 395:10233.

44. Tang N, Bai H, Chen X, Gong J, Li D, et al. Anticoagulant treatment is associated with decreased mortality in severe coronavirus disease 2019 patients with coagulopathy. J Thromb Haemost 18:1094-1099.

45. Lippi G, South AM, Henry BM (2020) Electrolyte imbalances in patients with severe coronavirus disease 2019 (COVID-19). Ann Clin Biochem 57:262-265.

46. Lippi G, Plebani M (2020) The critical role of laboratory medicine during coronavirus disease 2019 (COVID-19) and other viral outbreaks. Clin Chem Lab Med 58:1063-1069.

47. Xu Z, Shi L, Wang Y, Zhang J, Huang L, et al. (2020) Pathological findings of COVID-19 associated with acute respiratory distress syndrome. 8:420-422.

48. News (2020) The report to parliament.