CORRELATION OF COVID-19 SEVERITY with HEMATOLOGICAL, BIOCHEMICAL, COAGULATION AND INFLAMMATORY MARKERS- A CROSS-SECTIONAL STUDY

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ABSTRACT

Coronaviruses cause exceptionally contagious infections and at present they pose a major concern of public health worldwide. This study was conducted to evaluate the severity of Corona Virus (COVID)-19 through hematological, biochemical, coagulation and inflammatory markers. This was a cross-sectional study including 200 COVID-19 patients (97 with non-severe and 103 with severe diseases) admitted to Hayatabad Medical Complex, Peshawar, Pakistan from December 2020 to June 2021. The patients were initially screened through Real-time PCR and positive confirmed patients' were further evaluated for serum ferritin, C-reactive protein (CRP), lactate dehydrogenase (LDH), D-dimer, and complete blood count (CBC) by using the standard protocols. Among the 200 COVID-19 positive patients, male positive patients were predominant (n= 138, 69%), and the most prevalent age group was 41 to 60 years (i.e. 49%). CRP was found to be most frequently deranged (95%) followed by D-dimer and LDH levels in 92% of patients. The abnormal levels of neutrophils, lymphocytes, ferritin, and hemoglobin were recorded as 83%, 82%, 79%, and 20%, respectively. All studied inflammatory markers significantly ($p < 0.005$) correlated with the severity of COVID-19 patients.

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INTRODUCTION

Novel Corona virus-2019 (COVID-19) was classified as the sixth public health emergency of international concern (PHEIC) by the World Health Organization (WHO) on January 30, 2020, and later it was declared as a pandemic by WHO on March 11, 2020. Up to April 9, 2020, there were approximately 1436198 positive patients of COVID-19 reported with an overall case fatality rate (CFR) of 5.95%(1,2). Cough, sneezing, talking, and mucus in respiratory droplets, are the most common means of transmission. The risk of transmission is greatest in the early stages when symptoms first occur since viral RNA levels are at their highest. It may, however, transmit throughout the initial stage. The incubation period is usually two weeks after exposure, with the majority of symptoms developing within 4 to5 days(3). Polymorphonuclear (PMN) cell activation in the immunological response caused by the virus was recently reported. Additionally, neutrophilia has been linked to significant respiratory symptoms and a poor prognosis in COVID-19 patients(4). Lymphopenia, or increased inflammatory cascade activation, is a key feature of COVID-19 disease and has a strong prognostic significance. The fundamental processes, however, are still poorly understood. According to the clinical work, coronaviruses can also directly infect stem cell precursors, leading to defective hemopoiesis, or triggering an auto-immune reaction to blood cells(5). C-reactive protein (CRP) up-regulation was observed during the 2002 SARS outbreak and was linked to respiratory dysfunction and mortality. Various investigations in COVID-19 patients were done in this regard considering CRP as one of the potential biomolecules connected to the mortality of infected individuals. However, the results of the studies remained conflicting. The CRP was shown to be considerably elevated in the early stages of infection in severe COVID-19 individuals, even before CT results indicated serious findings. Nevertheless, CRP has been linked to the onset of illness

and is a predictor of severe COVID-19(6). Lactate dehydrogenase (LDH) is an intracellular enzyme that catalyzes the conversion of pyruvate to lactate in anaerobic glycolysis. Clinically, serum LDH is frequently evaluated in a variety of diseases. Elevated serum LDH levels have been linked to a worse prognosis in a range of infections, including malignancies and inflammation. Patients with severe COVID-19 had increased serum LDH levels, according to previously studies(7). The virus has shown a tendency of mutations in various clinical features. There is limited data available from Pakistan showing pattern of hematological, biochemical, coagulation and inflammatory markers. Thus this study was designed to determine these markers on the onset and evaluate association with severity of COVID-19 infection in the area and population of Peshawar, Pakistan.

METHODS

This descriptive cross-sectional study was conducted in Hayatabad Medical Complex(HMC) Peshawar, Pakistan from December 2020 to June 2021. A total of 200 patients were recruited randomly from the hospital ward and intensive care unit. Among these, based on the patient's condition/disease severity, 97 patients admitted to the hospital ward were affected with non-severe disease, while 103 patients admitted to the intensive care unit were suffering from severe disease.

Patients more than 20 years, regardless of gender with PCR-confirmed for Covid-19 and having signed informed consent were included in the study. All COVID-19 RT PCR negative individuals, aged less than 20 years and not willing to sign the consent form were excluded from this study.

Specimen for COVID-19 PCR: Nasopharyngeal swab samples were collected from 200 COVID-19 confirmed patients registered at Hayatabad Medical Complex in Peshawar, while their blood samples were taken in EDTA, Sodium Citrate, and Gel Tubes. Blood samples were preserved at 2 to 8 °C temperature till further processing.

Transportation: Nasopharyngeal swabs were transported in a triple zip begs to the PCR section in the Microbiology department and blood samples in EDTA Tube to the Hematology department, Sodium Citrate, and Gel tube to the Biochemistry department were transported at 2 to 8 °C for further processing.

RNA Extraction: The specimens were directly applied to the TAN Beads extractor kit with proteinase K. The extracted RNA was amplified using the nucleic acid extractor. During the process, the silicon dioxide layer coated with the magnetic beads adsorbs and purifies RNA from the sample. In the final stage, the purified RNA was aliquot in a plain tube for further use. Mic PCR thermal cycler was used for amplification of the RNA sample.

Sample Collection for Inflammatory Markers: The blood samples were collected aseptically in three different tubes, Gel Tubes for Serum Ferritin, CRP, and LDH, Sodium, Citrate for D-dimer, and EDTA tube for CBC. After collection hematological and biochemical tests were performed in the pathology department of HMC Peshawar. Samples for D-dimer and serum ferritin were centrifuged at 3000 rpm for 10 minutes except for CBC samples, to allow for examination.

Biochemical analysis of inflammatory markers: Immuno-turbidimetry utilizing Roche kits on the Cobas 601 clinical instrument used for the measurement of ferritin, LDH, and CRP. An antigen/antibody complex was formed when latex-bound antibodies combine with the analyte in the sample. This is measured turbidly following agglutination. The amount of turbidity generated is proportional to the amount of ferritin available, and it is measured at a wavelength of 700nm (primary wavelength). Considering the normal value of ferritin as 30 – 400 ng/mL, LDH as 140 – 280 U/L, CRP as < 10 mg/L. Besides these to check the accuracy of the results one normal and abnormal sample were used as controls.

Analysis of coagulation and inflammatory markers: Latex particles covered with specific antibodies to human D-dimer fragment D form immunological complexes in the presence of D-dimer from the sample. The immune complexes cause an increase in light dispersion, which is directly proportional to the amount of D-dimer present in the plasma sample. The turbidity at 570 nm is used to measure light scattering. The concentration of a sample D-dimer is measured by comparing it to dilutions of a known concentration D-dimer calibrator. Cobas 601 fully automated machine was used for D-dimer analysis. Centrifuged Sodium citrate samples were processed by selecting the D-dimer test on the machine one by one and obtaining the results in 35 minutes. Normal value of D-dimer was < 0.50 µg/dL. In all these procedures one normal and one abnormal samples were used as controls.

Analysis of hematological inflammatory markers: The Sysmex XN-1000 is a quantitative fully automated hematology analyzer used to analyze whole blood tests in vitro. Analyzing the quantitative and/or morphological values of a complete blood count, which involves HB (Hemoglobin), WBC, Platelet count, and other parameters, the differential leukocyte count is important in the identification of illness states such as anemia, leukemia, allergic responses, and viral, bacterial, and parasite infections, according to the reports. To conduct hematology tests, the hydrodynamically focused impedance analysis, the flow cytometry technique (using a semiconductor laser), and the SLS-hemoglobin methodology were used. Cytometry is used to investigate the physiological and biochemical characteristics of cells and other biological particles. Flow cytometry is a technique used for recognizing cells and particles moving through very small flow cells. From the results, the differential leukocytes count i-e Neutrophil (Normal Value: 40 – 75%) and lymphocytes (Normal Value: 20 – 45%) were calculated.

Statistical analysis

The results of the study were statistically analysed by using Statistical Package for Social Sciences (23.0 version). The data was initially assessed on frequency distribution. For the correlation of inflammatory markers with COVID-19 severity, the simple t-test and one-way ANOVA were applied for continuous variables and chi-square test was applied for categorical variables. A p-value <0.05 was taken as significant.

RESULTS

In the current study, a total of 200 patients meeting the inclusion criteria were selected. Out of which 138 (69%) were males, and 62 (31%) were females. The more affected age group was observed to be 41 to 60 years with 98 (49%) patients, while 61 to 80 years were 56 (28%), 21 to 40 years were 40 (20%), and 81 to 100 years were 06 (03%) patients (Table 1). Among the 200 COVID-19 positive patients, the inflammatory marker CRP was highly associated with severity of disease, 95 % abnormal in COVID-19 disease, followed by D-dimer and LDH in 92 %, neutrophil in 83 %, lymphocyte in 82 %, ferritin in 79 %, and HB in 20 % (Table 1). The ferritin, D-dimer, LDH and blood inflammatory cells were abnormal in greater proportion of males as compared to females, while higher rate of abnormal CRP was seen in females. The pattern of the markers also showed difference in different age groups. A summary of the pattern of markers according to gender and age distribution is given in Table 2. Statistically, both age and gender showed a significant correlation (p-value <0.001, F=13.16, DF=6, r2=0.6942) with COVD-19 inflammatory markers.

In non-severe disease patients, the mean of ferritin was observed as 518.53 ng/ml (range 11.5-890 ng/ml), while in severe disease the mean ferritin was 1891.84 ng/ml (range 895-16520 ng/ml) (p-value <0.001). In non-severe disease patients, the mean of D-Dimer has observed as 1.06 µg/ml (range 0.2-2.2 µg/ml), while in severe disease the D-Dimer was 9.18 µg/ml (range 2.3-57 µg/ml), (p-value <0.001). The LDH was significantly high in severe cases (mean 857.74 U/L, range 575- 2903 U/L) as compared to non-severe

disease patients(mean 404.9 U/L, range 177-573 U/L) (p-value <0.001). Similarly, mean CRP was significantly high in severe cases (mean 19.03, range 7.9-45 mg/dl) while in non-severe cases it was 2.17 mg/dl (range 0.2-7.3 mg/dl) (p-value 0.037). In non-severe disease patients, the mean of neutrophils was observed at 75.54 % with a range (48-87 %), while in severe disease the neutrophils count was 92.04 % with a range (87-97 %), (p-value <0.001). In non-severe disease patients, the mean of Lymphocytes was observed at 16.84 % with a range (09-41 %), while in severe disease the lymphocytes was 4.07 % with a range (01-08 %) (p-value <0.001). In non-severe disease patients, the mean hemoglobin was observed at 15.08 g/dl with a range (13.08-18 g/dl), while in severe disease the hemoglobin was 11.78 g/dl with a range (8.8-13.5 g/dl) (p-value=0.002). A summary of these parameters is given in Table 3.

Table 1. Demographic characteristics and pattern of inflammatory markers in COVID-19 positive patients

Variable	Number (n)	Percentage (%)
Total COVID-19 Positive Patients	200	100%
Gender wise distribution of COVID-19		
Male	138	69%
Female	62	31%
Age wise (Years) distribution of COVID-19		
21-40	40	20%
41-60	98	49%
61-80	56	28%
81-100	06	03%
Pattern of inflammatory markers in COVID-19 patients		
Inflammatory markers	**Normal n (%)**	**Abnormal n (%)**
Ferritin	42 (21%)	158 (79%)
D-Dimer	16 (08%)	184 (92%)
LDH	16 (08%)	184 (92%)
CRP	10 (05%)	190 (95%)
Neutrophil	34 (17%)	166 (83%)
Lymphocytes	36 (18%)	164 (82%)
HB	160 (80%)	40 (20%)

Table 2. Gender wise and Age wise distribution of abnormal inflammatory markers of COVID-19 infected individuals

Inflammatory markers	Gender wise		Age wise			
	Male	**Female**	**21-40**	**41-60**	**61-80**	**81-100**
Ferritin	81.1%	74.1%	85%	77.5%	78.5%	33.3%
D-Dimer	92.7%	90.3%	95%	91.8%	85.7%	100%
LDH	97.1%	80.6%	75%	93.8%	100%	100%
CRP	94.2%	96.7%	90%	95.9%	100%	66.6%
Neutrophil	88.4%	70.9%	20%	87.7%	89.2%	100%
Lymphocytes	86.9%	70.9%	50%	91.8%	89.2%	100%
HB	13%	35.4%	35%	10.2%	28.5%	00%
p-values	<0.001		<0.001			

Table 3. Covid-19 non-severe and severe diseases patient's Inflammatory Markers

Inflammatory markers	Non-severe disease n=97 mean (range)	Severe disease n=103 mean (range)	Standard Deviation	p-Values	Normal reference range
Ferritin	518.53(11.5-890)	1891.84 (895-16520)	1728.201	<0.001	30—400 ng/ml
D-Dimer	1.06(0.2-2.2)	9.18 (2.3-57)	8.452	<0.001	<0.5µg/ml
LDH	404.9(177-573)	857.74(575-2903)	368.219	<0.001	80—235 U/L
CRP	2.17(0.2-7.3)	19.03(7.9-45)	88.418	0.037	<0.5 mg/dl
Neutrophils	75.54(48-87)	92.04(87-97)	11.360	<0.001	40--75 %
Lymphocytes	16.84(09-41)	4.07(01-08)	15.329	<0.001	20--45 %
Hemoglobin	15.08(13.8-18)	11.78(8.6-13.5)	1.972	0.002	11.5--17.5 g/dl

DISCUSSION

COVID-19 pandemic is a significant general health threat now a days that requires a quick action plan for its treatment and control. Notwithstanding the extreme endeavors to discover novel medications for SARS-CoV2, this procedure is tedious with constrained advancement to date. Hence, medicate repurposing has been recognized as the quickest method of figuring out restorative specialists for COVID-19 to meet the desperation of the situation. Males had a greater mortality rate than females in all age groups older than 20 years in Spanish, German, Swiss, Belgium, and Norway(8).

The current study was based on the epidemiological study of COVID-19 and its correlation with hematological, biochemical, and coagulation markers. The current study findings revealed that male patients were predominant as compared to female positive patients. Our study is in close agreement with the study reported previously according to Guan et al. , males were more (58.1%) infected than females (41.9%)(9). Similarly, another study reported from Sahiwal Pakistan by Pervaiz et al. also reported the high prevalence (74.2%) of COVID-19 in male subjects as compared to female subjects (25.8%)(10). In our present study the predominant COVID-19 infected patients age group was 41 to 60 years (49%), followed by 61 to 80 years (28%), 21 to 40 years (20%), and 81 to 100 years (3%). However, the study reported previously showed the age group 51-60 was highly infected(11), this age group is also included in the high prevelant group seen in our study.

nfection with COVID-19 affects entire body causing derangment of biomarkers, such as inflammatory markers. As reported previously COVID-19 patients have higher ferritin levels in their blood. This excess could lead to secondary bacterial infection and intensify COVID -19 infections(12). In the current study the levels of ferritin in the COVID -19 infected patient's serum, was significantly high (79%), while in non-severe disease the mean of ferritin was observed at 518.53 ng/ml and in severe disease was 1891.84 ng/ml. These findings are in agreement with previous studies. According to the study of Arshad et al., the ferritin was high in 51.26% of COVID-19 infected patients(13). In another study, they found that patients infected by bacterial disease had higher ferritin levels compared with infection by the virus the increase of ferritin levels in serum predicts a poor outcome in the hospital with infection by influenza(14).

Biomarkers of inflammation and coagulopathy can help identify hospitalized patients of COVID-19. Ayanian et al. stated in their study that inflammatory marker D-dimer was high in COVID-19 infected individuals(15), the present study results showed agreement with their findings, as per our study, the inflammatory marker D-dimer was 92% high and abnormal in infected individuals of COVID-19, while in non-severe disease the mean of D-Dimer was observed 1.06 µg/ml and in severe disease was 9.18 µg/ml.

Lactate dehydrogenase is an intracellular enzyme that catalysis the anaerobic glycolysis reaction of lactate to pyruvate. Serum LDH is commonly tested in the clinical practice for assessment of a range of disorders. Serum LDH levels that are elevated have been associated with a poor prognosis in a variety of illnesses, including malignancies and inflammation. According to one study, patients with severe COVID-19 had elevated serum LDH levels (7). In agreement with these previous findings, the current study finding also revealed, that LDH was found to be high in COVID-infected individuals, while in non-severe disease patients the mean of CRP was observed at 2.17 mg/dl and in severe disease was 19.03 mg/dl. This finding is also in agreement with the study of, which stated in their study that LDH was high in COVID-19 infected patients(13).

In the current study, the inflammatory marker CRP was also found to be deranged in COVID-19 patients. Besides these and in agreement with our study, during the 2002 SARS outbreak, CRP overexpression was discovered and was connected to respiratory dysfunctions and patient death. Based on these findings, other studies on COVID-19 patients were conducted, hypothesizing CRP as one of the probable biomolecules positively linked to the mortality. However, the conclusions of the publications remained contradictory. The CRP marker was shown to be significantly raised in the early stages of infection in COVID-19 patients, even before CT scans revealed worrisome abnormalities. CRP, which has been associated with the start of the disease, is also a predictor of severe COVID-19(16) (16). Ayanian et al. also reported that CRP was high in COVID-19 infected individuals and their correlation was significant(15).

Neutrophils are the most frequent immune cells in human blood. Neutrophils have key homeostatic roles and are involved in chronic inflammatory illnesses, as well as being the first line of defense against many infections. Polymorphonuclear cells provide a protective role during bacterial or fungal infections, but their role in viral infections is unknown. Neutrophils have been seen in several lung disorders associated with acute respiratory distress syndrome (ARDS), including influenza and SARS-CoV-1 infections. A bioinformatics study of the SARS condition indicated that neutrophil activation and degranulation are very active processes. Recently, the recruitment of PMN cells in the immune response to SARS-CoV-2 was revealed. Furthermore, in COVID-19 patients, neutrophilia has been associated with substantial respiratory symptoms and a poor prognosis (4). The current study results are also similar to these previous findings, the neutrophil was found abnormal in COVID-19 infected individuals.

According to the current findings, lymphocytes were found abnormal in COVID-infected individuals, while in non-severe disease patients the mean of lymphocytes was observed at 16.84 %, and in severe disease was 4.07 %. In agreement with this finding, Yang et al. (17) reported that lymphopenia was seen in 80 % of severely ill adult COVID-19 patients, whereas Zhao et al. (18) reported only 25% of individuals with moderate COVID-19 infection were found to have the virus(17,18). These findings show that lymphopenia may be linked to the severity of the illness. Furthermore, in the current study the hemoglobin was found abnormal at 20% and 80% normal, while in non-severe disease patients the mean of hemoglobin was observed at 15.08 g/dl and in seere disease was 11.78 g/dl. In this regard, Hassan et al. indicated that both the open reading frame (ORF8) and the surface glycoprotein bind to porphyrin(19). ORF1ab, ORF10, and ORF3a proteins are expected to act at once to break the iron in hemoglobin's 1-beta chain, producing porphyrin and lowering hemoglobin's ability to transport O2 and CO2. The virus's method blocks the normal heme metabolic pathway, resulting in disease symptoms(20).

The study has provided baseline information regarding indicators of the severity of COVID-19, however the small sample size is the limitation. More advanced immunological studies need to explore the mechanism of COVID -19 with inflammatory markers. Genetic level studies of COVID-19 need to

explore the mode of action, the activation of the human immune system, and its correlation with inflammatory markers.

CONCLUSION

The current study concluded that among the COVID-19 positive patients, CRP, LDH, D-dimer, and neutrophil-associated inflammatory markers are associated with COVID-19 infection. This study also showed that because an increase in inflammatory markers correlates with disease severity, continuous monitoring utilizing these variables might improve disease outcome and hence could be utilized as important disease prognostic indicators.

ETHICAL CONSIDERATION: The study was approved by ethical committee No. AWKUM/Biochem/Dept/Commit/eth/20 of Abdul Wali khan University Mardan, Pakistan. Informed consent was obtained from all the patients or their attendants in case of severe patients.

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