Original Article

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Pathology Section

Laboratory Biomarkers as a Predictor of COVID-19 Disease Progression

SANDHYA PITLA[1], RANGA RAO DIDDI[2], NAVEEN CHANDRA RAO DAMERA[3], SAVITHRI BHAVARAJU[4]

ABSTRACT

Introduction: On December 2019, Wuhan city in China, became the epicentre of unexplained cases of pneumonia. Later in January 2020, scientists identified this as a novel coronavirus, temporarily labelled as Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). Its name was then changed to Coronavirus Disease 2019 (COVID-19) by the World Health Organisation (WHO) in February 2020 as the disease spread worldwide.

Aim: To investigate the association of different biomarkers in the COVID-19 disease progression and assess how their levels vary with presence and absence of co-morbid conditions.

Materials and Methods: The present study was a retrospective cohort study conducted in November 2020 on laboratory confirmed 82 COVID-19 positive patients admitted in Dr. Pinnamaneni Sidhartha Institute of Medical Sciences and Research Foundation, Chinnaoutpally, Gannavaram, from July 12, 2020 to September 30, 2020. A confirmed case with COVID-19 was defined as patients getting a positive result to real-time Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) assay for nasal and pharyngeal swab specimens. Only laboratory confirmed cases were included in the study. Suspected cases and clinically diagnosed cases were not part of this study. Data was analysed using IBM Statistical Package for Social Sciences (SPSS) version 21. Independent t-test was used to analyse the data.

Results: The 82 COVID-19 patients (age range (15-80 years), 52 males and 30 females), were confirmed by RT-PCR method. On admission, 57 and 25 were divided into non severe and severe groups, respectively. Severe COVID-19 patients had high leucocyte count (3.54-21.3×10^9/L), neutrophil count (2.59-18.33×10^9/L), D-dimer (0.08-2.55 µg/mL, p-value <0.0001), C-Reactive Protein (1.47-151.84 mg/L p-value 0.001) and they had significantly lower levels of lymphocyte count (0.17-5.79×10^9/L, p-value <0.0001).

Conclusion: To conclude, dynamically monitoring haematological and coagulation parameters association with comorbid conditions such as diabetes may provide a reliable and convenient method for classifying and predicting the severity and outcomes of patients with COVID-19. This information will be a useful tool for physicians to categorise COVID-19 patients and manage critically ill patients.

Keywords: Coronavirus disease-2019, C-reactive protein, D-dimer, Lymphocyte count, Severe acute respiratory syndrome coronavirus 2

INTRODUCTION

Coronaviruses are important pathogens of humans that can cause diseases ranging from the common cold to more severe and even fatal respiratory infections. In the past two highly pathogenic human coronaviruses, the coronavirus responsible for SARS-CoV and the coronaviruses responsible for Middle East Respiratory Syndrome (MERS-CoV), have emerged in two separate events [1,2]. In December 2019, a rapidly emerging new strain of coronavirus, officially named SARS-CoV-2, was first isolated from three patients with coronavirus disease 2019 (COVID-19) by the Chinese centre for Disease control and Prevention, connected to the cluster of acute respiratory illness cases from Wuhan, China [3]. Till now there are no available specific curative medicines and vaccines, and the treatment methods for COVID-19 are largely supportive.

According to the diagnosis and treatment protocol for novel coronavirus (COVID-19) pneumonia published by Government of India Ministry of Health and Family Welfare, there are four severity levels of COVID-19 based on the clinical manifestations: mild, moderate, severe and critical disease. To some extent differentiating severe from non severe patients is helpful and can improve the cure rate of COVID-19. However some criteria used for classification are respiratory factors such as respiratory rate, oxygen saturation and lesion progression in pulmonary imaging. Severe, especially critical cases are complicated by other organ dysfunctions, including septic shock, heart failure and Disseminated Intravascular Coagulation (DIC) [4,5]. Venous thromboembolism is common in patients with severe disease [6]. It was stated that increased D-dimer was associated with severe COVID-19 and high mortality [7]. Coagulopathy is also common complication in patients with critical and fatal disease [5,7]. Therefore, finding efficient and reliable laboratory parameters for risk classifications and predict prognosis is a priority. By doing so, it gives clinicians a tool to group patients and predicts prognosis and mortality of COVID-19 affected patients.

The biomarkers included in the study were total leucocyte count, neutrophil count, lymphocyte count, D-dimer, C-Reactive Protein.

The objectives of the study were:

- To study the association of laboratory biomarkers with COVID-19 disease progression.
- To study the association of laboratory biomarkers in non-severe and severely affected COVID-19 patients.
- To study the variation of laboratory biomarkers in COVID-19 patients with and without comorbid conditions like diabetes and hypertension.

MATERIALS AND METHODS

The present study was a retrospective cohort study of laboratory confirmed convenient sample of 82 patients with COVID-19 admitted in Dr. Pinnamaneni Sidhartha Institute of Medical Sciences and Research Foundation, Chinnaoutpally, Gannavaram from July 12, 2020 to September 30, 2020 and the data was analysed in the month of November 2020. This study got approved by Institutional Ethics Committee of Pinnamaneni Siddhartha institute of Medical Sciences and Research Foundation (with IEC No: Faculty/540/20).

Inclusion criteria: The data of RT-PCR confirmed COVID-19 positive patients, aged between 15-80 years, with altered laboratory biomarkers (total leucocyte counts, lymphocyte count, neutrophil count, D-dimer, C-Reactive Protein, Random Blood Sugar levels) and with co-morbid conditions that includes diabetes, hypertension admitted in the study centre during the defined period were included in the study.

Exclusion criteria: COVID-19 patients aged below 20 and above 80 years and were asymptomatic were excluded from the study.

After admission of 82 COVID-19 patients, 57 and 25 patients were grouped into non severe and severe groups which account to 69.52% and 30.48% respectively based on their respiratory rate, peripheral capillary Oxygen Saturation (SpO_2) and presence of pneumonia. A confirmed case with COVID-19 was defined as patients getting positive result to real-time RT-PCR assay for nasal and pharyngeal swab specimens. Only laboratory confirmed cases were included in the present study. Suspected cases and clinically diagnosed cases of COVID-19 were not a part of this study. After admission in the hospital and clinical examination, venous blood was collected and sent to central laboratory where the investigations i.e., total leucocyte count, lymphocyte count, Neutrophil count, D-dimer, C-reactive protein and random blood sugar levels were carried out in automated analysers.

STATISTICAL ANALYSIS

Data was analysed using IBM SPSS version 21. Independent t-test was used to analyse the data.

RESULTS

All the 82 patients were confirmed a positive for COVID-19 by RT-PCR method. Out of these, 82 COVID-19 positive patients, 57 patients came under non severe and 25 patients came under severe groups respectively. All the biomarkers done from the blood samples of these patients in the central laboratory. All the data of the present study was retrieved from the Laboratory Information System (LIS) exclusively which provided information on age, gender, ethinicity and location of the patient. The demographic data was shown in [Table/Fig-1].

Variables		Frequency (n)	Percentage (%)
Age (years)	<30	8	9.8
	30-39	15	18.3
	40-49	17	20.7
	50-59	25	30.5
	>60	17	20.7
Gender	Male	52	63.4
	Female	30	36.6

[Table/Fig-1]: Demographic data of 82 COVID-19 patients confirmed by RT-PCR.

The data showed that total number of female patients was 30 that accounts for 36.6%, the female patients in the non severe and severe groups were 18 and 12, which comprises 21.95% and 14.63%, respectively. The total number of male patients was 52 which accounts for 63.41%. Among the 52 male patients, 39 patients were under non severe group and 13 were grouped under severe group. These comprise 47.56% and 15.85%, respectively among total study sample. Age of the patient ranged between 15-80 years with maximum patients falling in the age group of 50-59 years.

All the 82 patients included in the present study had Complete Blood Picture performed on the second day of their admission. CRP, D-dimer and random blood sugar levels are done on all the patients. The list of laboratory biomarkers analysed is tabulated in [Table/Fig-2]. Analysis of the CBP of all patients revealed that majority of patients had normal total leucocyte counts but most of the severe patients had leukocytosis with a mean of 8.34×10^9/L. Neutrophil count was in the range 2.59-18.33×10^9/L with a mean of 5.803×10^9/L. The range of lymphocyte count among all patients was 0.17-5.79×10^9/L and with a mean value of 1.93. The range of D-dimer values among all the patients was 0.08-2.55 µg/L and with a mean value of 0.347 µg/L. The range of CRP among all patients was 1.47-151.84 mg/L with a mean of 25.31±36.55 mg/L.

Variables	Range	Mean±SD
Age	15-80 years	48.75±14.23
Total leucocyte count	3.54-21.3×10^9/L	8.34±3.88
Neutrophil count	2.59-18.33×10^9/L	5.803±2.34
Lymphocyte count	0.17-5.79×10^9/L	1.93±1.30×10^9/L
D-dimer	0.08-2.55 µg/mL	0.347±0.415 µg/mL
CRP	1.47-151.84 mg/L	25.31±36.55 mg/L
RBS	70-431 mg/dL	148.07±88.91 mg/dL

[Table/Fig-2]: List of laboratory biomarkers analysed in COVID-19 patients with their range and SDs.
CRP: C-reactive protein; RBS: Random blood sugar; SD: Standard deviation

The mean of total leucocyte count among severe patients and non severe patients were 7.55 and 8.69×10^9/L respectively shown in [Table/Fig-3]. The mean values of lymphocyte counts among severe and non severe patients were 1.16 and 2.28×10^9/L with p-value of <0.0001 which is statistically significant. The mean of D-dimer among severe and non severe patients were 0.73 and 0.17 µg/mL with a p-value of <0.0001 which shows high statistical significance. The average means of CRP among severe and non severe patients were 45.39 and 16.35 mg/L, respectively with a p-value of 0.001 which is statistically significant.

Variables	Total cases	Mean±SD	t value	p-value
Total leucocyte count (×10^9/L)	Severe (25)	7.55±3.15	-1.24	0.22
	Non severe (57)	8.69±4.14		
Neutrophil count (×10^9/L)	Severe (25)	5.66±2.29	-0.67	0.51
	Non severe (57)	2.4±1.73		
Lymphocyte count (×10^9/L)	Severe (25)	1.16±2.29	-3.88	**<0.0001**
	Non severe (57)	2.28±1.16		
D-dimer (µg/mL)	Severe (25)	0.73±0.56	7.0	**<0.0001**
	Non severe (57)	0.17±0.13		
CRP (mg/L)	Severe (25)	45.39±35.49	3.53	**0.001**
	Non severe (57)	16.35±33.59		
RBS (mg/dL)	Severe (25)	144.96±71.36	-0.21	0.84
	Non severe (57)	149.46±96.27		

[Table/Fig-3]: Laboratory parameters among severe and non- severe patients with their mean, SD and p-values using Independent t-test.

Random blood sugar test was done on all patients shown in [Table/Fig-4]. Out of 82 patients, 31 were diabetic and 51 were non diabetic patients. Among the 31 diabetic patients, 15 were severe COVID-19 patients and 16 were non severe patients. The range of random blood sugar levels among all the 82 patients was 70-431 mg/dL. The average mean of random blood sugar among severe and non severe patients were 144.96 mg/dL and 149.46 mg/dL. In diabetic patients, d-dimer showed statistical significance with p-value of 0.002, CRP showed p-value of 0.003 and RBS showed p-value of <0.0001.

Among the 82 patients, hypertensive patients were 16 which accounts for 19.52% and 66 were non-hypertensive patients accounting for 80.48% in [Table/Fig-5]. Among the hypertensive patients, all the laboratory biomarkers didn't showed any statistical significance.

Variables	Diabetes	Mean±SD	t-value	p-value
Total leucocyte count (×10⁹/L)	Present (31)	9.16±4.43	1.51	0.16
	Absent (51)	7.82±3.44		
Neutrophil count (×10⁹/L)	Present (31)	6.77±4.06	-0.76	0.34
	Absent (51)	57.86±372.05		
Lymphocyte count (×10⁹/L)	Present (31)	1.79±1.13	-0.81	0.42
	Absent (51)	2.02±1.41		
D-dimer (µg/mL)	Present (31)	0.572±0.59	4.22	**0.002**
	Absent (51)	0.208±0.13		
CRP (mg/L)	Present (31)	42.89±45.56	3.21	**0.003**
	Absent (51)	14.41±24.37		
RBS (mg/dL)	Present (31)	234.16±91.93	10.63	**<0.0001**
	Absent (51)	94.7±11.62		

[Table/Fig-4]: Laboratory parameters variation among COVID-19 patients with diabetes and their p-values using independent t-test.

Variables	Hypertension	Mean±SD	t-value	p-value
Total leucocyte count (×10⁹/L)	Present (16)	9.42±3.74	1.28	0.21
	Absent (66)	8.07±3.89		
Neutrophil count (×10⁹/L)	Present (16)	6.98±3.21	-0.96	0.34
	Absent (66)	46.03±326.28		
Lymphocyte count (×10⁹/L)	Present (16)	1.61±1.08	-1.27	0.21
	Absent (66)	2.02±1.35		
D-dimer (µg/mL)	Present (16)	0.517±0.49	1.85	0.06
	Absent (66)	0.31±0.387		
CRP (mg/L)	Present (16)	40.67±113.02	1.91	0.13
	Absent (66)	21.53±33.32		

[Table/Fig-5]: Laboratory parameters variation among hypertensive patients and their significance using Independent t test.
CRP: C-reactive protein

DISCUSSION

Novel coronavirus in 2019 was transmitted by droplets on coughing and direct contact with patients body fluids, other ways of its transmission was unclear [8,9]. Until 16 February 2020, the number of COVID-19 patients was more than 70,000, yet the number of infections was still rising all around the world [10]. However, there are still no effective treatment for COVID-19, the main treatment is symptomatic and supportive therapy to alleviate symptoms and maintain organ function in COVID19 affected patients of severe disease [11]. Although some studies have recently reported that some drugs can be used against the COVID-19, these drugs still in the research stage [12,13].

This study compared the level of inflammatory and haematological parameters between two different groups of disease severity. This study participants of 82 patients contacted the infection with neighbours or colleagues at work place who after testing with RT-PCR showed positivity. In these 82 patients, fever was present in 47 (58.75%), cough in 48 (58.53%) patients. Though sore throat was a rare finding, it was present in 23 (28.04%) patients. Chest computed tomography scan showing ground glass opacity/haziness of diagnosed patients account for 45 (54.87%), where as severe pneumonia was seen up to 31 (37.80%). The data suggest that gender may not be an influencing factor in COVID-19 infection, yet advanced age was a risk factor for viral infection and poor prognosis.

On comparison of haematological markers with other studies [Table/Fig-6], in the present study, lymphocyte count showed statistically significant association with other studies done by Qin C et al., and Wang D et al., [14,15] with a p-value of <0.0001. It shows that among the RT-PCR positive COVID-19 patients, severe cases present with lesser lymphocyte counts when compared with non severe patients. It shows that it has positive association to attend to the critical patients with lesser lymphocyte counts.

Parameter	Severity	Qin C et al., [13] 2020	Wang L et al., [14] 2020	Present study 2020
Total leucocyte count (×10⁹/L)	Severe	5.6 (4.3-8.4)	6.6 (3.6-9.8)	7.5 (3.65-15.6)
	Non severe	4.9 (3.7-6.1)	4.3 (3.3-5.4)	8.69 (3.54-21.3)
Neutrophil count (×10⁹/L)	Severe	4.3 (2.9-7.0)	4.6 (2.6-7.9)	5.66 (2.77-11.94)
	Non severe	3.2 (2.1-4.4)	2.7 (1.9-3.9)	2.4 (2.59-18.33)
Lymphocyte count (×10⁹/L)	Severe	0.8 (0.6-1.1)	0.8 (0.6-1.1)	1.16 (0.17-5.79)
	Non severe	1.0 (0.7-1.3)	0.9 (0.6-1.2)	2.28 (0.38-5.58)
p-value		<0.001 for all	WBC=0.003 NC <0.001 LC=0.03	WBC 0.22 NC 0.51 LC <0.0001

[Table/Fig-6]: Comparison of haematological markers of the present study with other studies.

D-dimers are very tiny multiple peptide fragments produced as a result of degradation of crosslinked fibrin, mediated by plasmin [16,17]. The presence and identification of D-dimers indicates the production and degradation of crosslinked fibrin, reflecting coagulation and fibrinolysis processes occurring concomitantly. In healthy subjects D-dimer is measurable in smaller amounts, because 2-3% of fibrinogen is converted to fibrin and enters the fibrinolytic pathway under normal physiological conditions [18]. The comparision of D-dimer with other studies on COVID-19 patients and the statistical significance was shown in [Table/Fig-7].

Studies	severe	Non severe	p-value	Comments
Zhou F et.al [4] 2020	5.2	0.6	<0.001	D-dimer >1 µg/mL can guide clinicians to identify patients with poor prognosis at earlier stage
Zhang L et al., [16] 2020	4.76	0.41	<0.001	D-dimer value >2.0 µg/mL could effectively and accurately predict in hospital mortality with COVID-19 and could be early and helpful marker to improve management
Present study 2020	0.7	0.1	<0.0001	D-dimer with >0.5 µg/mL is associated with progression to severe and critical stages

[Table/Fig-7]: Comparison of D-dimer (µg/mL) with other studies in COVID-19 patients.

CRP is a protein which was discovered in the 1930s by Tillett and Francis and is an acute phase reactant. It is a pentameric protein that is synthesised by the liver under the action of cytokine Interleukin 6 (IL-6). A very high level of CRP >50 mg/dL is mostly associated with bacterial infections, injuries, tuberculosis, cardiovascular processes and other inflammatory states. Elevated CRP levels in a person not only suggest a pro-inflammatory state but also can be used as a prognostic marker for the underlying disease processes [19]. [Table/Fig-8] shows the comparison of CRP with other studies and their significance [20,21].

Studies	Severe	Non severe	p-value	Comment
Li H et al., [20] 2020	66.04±44.89	33.22±32.21	0.001 for all	Significantly high percentage of patients in the severe group experienced higher CRP levels than non severe groups
Wang L et al., [21] 2020	54.15±1.06	1.52±1.56	Severe p=0.947 Non severe p=0.007	Greater CRP are more prominent in the severe group
Present study	45.39±35.49	16.35±33.59	0.001 for all	Significantly patients in severe group had higher CRP than non severe group

[Table/Fig-8]: Comparison of CRP (mg/L) of COVID19 patients with other studies.

Limitation(s)

First as a retrospective cohort study in a single centre of 82 patients. Second, the present study did not test other significant haematological, biochemical and inflammatory biomarkers. The ideal biomarkers for COVID-19 disease progression should be easily reliable accessible, inexpensive and correct. Tests or techniques that cause unnecessary exposure need careful consideration of risk and benefit.

CONCLUSION(S)

The study aimed to find meaningful and reliable COVID-19 biomarkers out of conventional haematological examinations for disease severity classification and early warning of progression. In conclusion, dynamically monitoring haematological and coagulation parameters, such as total leucocyte count, neutrophil count and lymphocyte count, D-dimer, CRP in association with co-morbid conditions such as diabetes and hypertension may provide a reliable and convenient method for classifying and predicting the severity and outcomes of patients with COVID-19. This information will be a useful tool for physicians to categorise COVID-19 patients and manage critically ill patients. A multicenter, prospective research with comparative analysis(including availability, reasonability and exposure consideration to harmful rays) amongst more biochemical markers is needed to further clarify the significance of these laboratory biomarkers in COVID-19 disease progression.

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PARTICULARS OF CONTRIBUTORS:

1. Assistant Professor, Department of Pathology, Dr. Pinnamaneni Siddhartha Institute of Medical Sciences, Gannavaram, Vijayawada, Andhra Pradesh, India.
2. Professor and Head, Department of Pathology, Dr. Pinnamaneni Siddhartha Institute of Medical Sciences, Gannavaram, Vijayawada, Andhra Pradesh, India.
3. Professor, Department of Pathology, Dr. Pinnamaneni Siddhartha Institute of Medical Sciences, Gannavaram, Vijayawada, Andhra Pradesh, India.
4. Lecturer in Statistics, Department of Community Medicine, Dr. Pinnamaneni Siddhartha Institute of Medical Sciences, Gannavaram, Vijayawada, Andhra Pradesh, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Sandhya Pitla,
Flat No: SF-A3, H. No: 48-13-3B, Srirama Residency, Beside Ayush Hospital, Sriramachandranagar, Vijayawada-520008, Andhra Pradesh, India.
E-mail: sanju.pitla@gmail.com

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