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Analysis of Biochemical and Immune Biomarker Abnormalities Associated with Severe Illness and Mortality in COVID -19 Disease

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***Abstract*---**Coronavirus Disease 2019 (COVID-19), a new form of respiratory and systemic disease caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARSCoV2). The inflammation induced by the SARS CoV-2 virus in the host leads to elevation of multiple inflammatory parameters. A raise in PCT, IL-6 and CRP denotes the intensity of inflammation in COVID-19 patients. Similarly, D-DIMER and FIBRINOGEN marks the aberration in the coagulation pathways in COVID-19 patients.COVID-19 positive patients aged between 18 and 65 years were recruited for the study after written informed consent. To confirm all cases according to nucleic acid real time–polymerase chain reaction (RT-PCR) tests. In the present study, a total of 200 COVID-19 patients were assessed. Out of them 100 (50%) were severe cases of COVID-19 and 100 (50%) were non-severe cases of COVID-19. On comparison of various biochemical and immune

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biomarkers, the severe COVID-19 patients demonstrated significantly higher levels of all biomarkers when compared with non-severe group.

***Keywords*---**abnormalities associated, biochemical, COVID-19, immune biomarker, severe illness.

Introduction

The World Health Organization (WHO) has identified coronavirus disease 2019 (COVID19) as a new pandemic during an outbreak survey of the Wuhan fish market in China in December 2019. The outbreak spread to 187 countries worldwide in just three months, showing high morbidity and mortality (1). Coronavirus Disease 2019 (COVID-19), a new form of respiratory and systemic disease caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARSCoV2). The first case of coronavirus disease 2019 (COVID-19) was reported in India on 27 January 2020 when a 20-year-old woman presented to the emergency department at Thrissur General Hospital, Kerala, with a history of cough and sore throat 2. Investigation of local transmission clusters were initiated following identification of the infection. By early March 2020, the observed increase in disease prevalence through surveillance and hospitalizations led to an increase in interventions and promotion of standard barrier measures (3).

The transmission of SARSCoV2 is mainly through the respiratory route through direct or indirect contact (4). These routes of transmission are the same for other respiratory viruses. Therefore, standard precautions should limit the transmission of all these diseases. Indeed, wearing a mask would have an impact on the circulation of these viruses by limiting the spread of droplets (3,4). Infected patients may have any of the following symptoms: fever, high temperature (>37.3°C), cough, muscle pain, sputum production, headache, hemoptysis, diarrhea, dyspnea and in some cases, acute respiratory distress syndrome (ARDS), acute cardiac injury or secondary infection. Onexamination, these subjective clinical symptoms can be more confidently interpreted through the use of biomarkers. These provide objective values throughout disease progression (5). It allows better prediction of patients with mild, severe or critical disease, allowing for earlier intervention (6). The study aimed to explore the role of different biomarkers in the pathogenesis of COVID-19 disease and assess how their levels change depending on disease severity. In doing so, it provides clinicians with a tool for sub grouping patients and predicting prognosis and mortality. The biomarkers analyzed in the study included creative protein (CRP), IL-6, D-dimer, Fibrinogen and Procalcitonin.

Materials and Method

The study was carried out at the Department of Biochemistry in association with Department of General Medicine, Santosh Medical College & Hospital, Ghaziabad from April to June 2021. COVID-19 positive patients aged between 18 and 65 years were recruited for the study after written informed consent. To confirm all cases according to nucleic acid real time–polymerase chain reaction (RT-PCR) tests. COVID-19 Patients having (1) mild, minor symptoms and imaging with no

signs of pneumonia or (2) moderate symptoms with fever, respiratory tract symptoms, and imaging shows pneumonia were classified as Non severe COVID-19.Severe COVID-19 cases were those patients who had any one of the following signs: (1) Increased breathing rate (≥ 30 beats/min), difficulty breathing or cyanosis of the lips; (2) Upon inhalation, oxygen saturation was ≤ 93 %; (3) Arterial blood oxygen partial pressure (PaO2)/oxygen concentration (FiO2) was ≤300 mmHg (1 mmHg = 0.133 kPa); (4) Pulmonary imaging showed multilobular lesions or lesion progression within 50 h of > 50 %; or (5) other clinical conditions requiring hospitalization.

A total of 100 non-severe COVID-19 and 100 severe COVID-19 patients were recruited. Drug-resistant TB patients were enrolled in the study following inclusion criteria and were followed up at third and sixth months. Details of patients in the form of demographic information, clinical history, weight, etc. were recorded on a pre-defined proforma. Biomarkers (PCT, D- dimer, IL-6 and CRP) were measured on baseline of blood sample collected. 6 ml of peripheral blood was collected using a disposal syringe under aseptic condition in a plain and sodium citrate vials. The data were analyzed using SPSS software. To compare the mean of the perimeters for the two independent groups: severe illness cases of COVID-19 and non-severe illness cases of COVID-19 , t-test was performed and the difference was found to be significant as the p-value was < 0.05. To identify the correlation between the study parameters Pearson Test was applied. Logistic regression was performed to evaluate the effect of each perimeter on individuals suffering with severe/non-severe illness COVID-19.

Results

In the present study, a total of 200 COVID-19 patients were assessed. Out of them 100 (50%) were severe cases of COVID-19 and 100 (50%) were non-severe cases of COVID-19. On comparison of various biochemical and immune biomarkers, the severe COVID-19 patients demonstrated significantly higher levels of all biomarkers when compared with non-severe group. In severe Covid-19 patients, there was a significant positive correlation found when the biomarker level of D-Dimer was tested against IL-6, CRP against Fibrinogen, and also in PCT against CRP (Table 2). In the non-severe covid patient group, a positive and significant correlation was found for the biomarker CRP when tested against IL-6 and D-Dimer (Table 3).

Table 1
Biochemical parameters in severe and non-severe COVID-19 patients

S.No	Characteristics	Severe patients (n=100)		Non-severe patients (n=100)
	Mean	0.4564		0.2128
PCT	SD	.07867		.06328
	P- value		<0.0001	
	Mean	51.073		19.910
IL-6	SD	5.9646		6.0086
	P- value		<0.0001	
CRP	Mean	103.370		30.810

	SD	14.7906		6.0297
	P- value		<0.0001	
	Mean	3.9602		0.477
D-Dimer	SD	2.73372		.1814
	P- value		<0.0001	
FIBRINOGEN	Mean	1000.440		719.540
	SD	128.0374		40.7132
	P- value		<0.0001	

Table 2
Correlation among the Biochemical parameters for the severe COVID -19 patients

Correlation		PCT	IL-6	CRP	D-DIMER
IL-6	Pearson Correlation	0.123			
	Sig. (2-tailed)	0.223			
CRP	Pearson Correlation	0.209*	0.069		
	Sig. (2-tailed)	0.037	0.496		
D-DIMER	Pearson Correlation	-0.081	0.229*	0.289**	
	Sig. (2-tailed)	0.426	0.022	0.004	
FIBRINOGEN	Pearson Correlation	-0.036	0.141	-0.010	0.284**
	Sig. (2-tailed)	0.722	0.161	0.923	0.004

Table 3
Correlation among the Biochemical parameters for the non-severe COVID -19 patients

Correlation		PCT	IL-6	CRP	D-DIMER
IL-6	Pearson Correlation	0.195			
	Sig. (2-tailed)	0.052			
CRP	Pearson Correlation	0.141	0.259**		
	Sig. (2-tailed)	0.162	0.009		
D-DIMER	Pearson Correlation	0.147	0.069	0.301**	
	Sig. (2-tailed)	0.143	0.492	0.002	
FIBRINOGEN	Pearson Correlation	-0.166	-0.175	-0.049	-0.024
	Sig. (2-tailed)	0.098	0.082	0.630	0.809

A logistic regression was performed to ascertain the effects of PCT, IL-6, CRP, D-DIMER and Fibrinogen levels on the likelihood that individual will suffer severe/non-severe illness COVID-19. The logistic regression model was statistically significant. The model explained 100% (Nagelkerke R^2) of the variance in severity of the COVID-19 disease and correctly classified 100.0% of cases.

Increasing of the PCT, IL-6, CRP, D-DIMER and FIBRINOGEN levels was associated with an increased likelihood of severe condition of the COVID-19 disease.

Table 4
Logistic regression model for various parameters in classifying COVID-19 patients based on severity

	B	S.E.	Wald	df	Sig.	Exp(B)	95% C.I.for EXP(B) Lower	Upper
PCT	12.079	39663.370	.000	1	1.000	176137.761	.000	.
IL6	.149	550.889	.000	1	1.000	1.161	.000	.
CRP	.631	502.339	.000	1	.999	1.880	.000	.
D-DIMER	.249	1396.788	.000	1	1.000	1.282	.000	.
FIBRINOGEN	.027	70.827	.000	1	1.000	1.027	.000	1.995E+60
Constant	-69.570	43883.612	.000	1	.999	.000		

The overall model is statistically significant, $x2(5) = 277.259$, $p < .05$ and the variation in the dependent variable i.e. Individual will suffer severe/non-severe illness covid-19 based on our model ranges from 75.0% to 100.0%, depending on whether you reference the Cox & Snell R^2 or Nagelkerke R^2 methods, respectively. Also we applied Wald test to determine statistical significance for each of the independent variables. In this model all the independent variables showing statistical significance in the model.

Discussion

D-dimer values below 0.5 g/mL are generally considered normal, and these values increase with age and during pregnancy. D-dimer levels increased with increasing severity of community-acquired pneumonia 7. Following the outbreak of the COVID-19 pandemic, D-dimer was identified as a potential marker of its prognosis in COVID-19 patients. D-dimer on date of admission has shown promise in predicting disease severity in several studies 8. Fibrinogen is a glycoprotein complex that is enzymatically converted to thrombin to fibrin at the time of tissue injury. COVID-19 patients were shown to present with pro-thrombinolysis with significantly elevated fibrinogen levels in critically ill patients (9).

The systemic inflammatory response known as cytokine release syndrome (CRS) can be triggered by a variety of factors such as infection, toxins, or a specific response to a drug, which is characterized by high numbers of cytokines. Pro inflammatory (tumor necrosis factor) (TNF), IL-6, IL1 β, etc) IL-6 contributes to host defense by inducing acute phase, hematopoietic, and immune responses. It develops rapidly and is transient in response to infection and tissue damage. IL-6 has pathological effects on systemic inflammation and autoimmunity although its expression is tightly regulated by transcriptional and post-transcriptional pathways. IL-6 is a major cytokine whose development is implicated in a number of inflammatory diseases. Elevated levels of IL-6 have been observed in patients

with SARSCoV2, and these concentrations are associated with pneumonia and severe lung injury (10).

Procalcitonin is a calcitonin prohormone glycoprotein released by the parafollicular cells of the thyroid gland. In microbial infections, PCT levels are significantly increased because it is released from all parenchymal tissues under the influence of endotoxins and proinflammatory cytokines. Therefore, at physiological state, serum PCT was recorded as significantly less than 0.05 ng/mL. In addition, given the risk-stratified timelines, PCT follows a rapid process with its degree of inclination detected 2-6 hours after stimulation. Although PCT is used as a biomarker of bacterial infection, conflicting opinions exist regarding the effectiveness of PCT as a prognostic tool for COVID19 (11). In our study, we observed that PCT, IL6, CRP, D-DIMER and Fibrinogen levels were significantly raised in severe cases as compared to non-severe COVID-19 patients. On regression analysis, we observed that the increase of the PCT, IL-6, CRP, D-DIMER and FIBRINOGEN levels was associated with an increased likelihood of severe COVID-19.

Conclusion

The inflammation induced by the SARS CoV-2 virus in the host leads to elevation of multiple inflammatory parameters. A raise in PCT, IL-6 and CRP denotes the intensity of inflammation in COVID-19 patients. Similarly, D-DIMER and FIBRINOGEN marks the aberration in the coagulation pathways in COVID-19 patients. Our Studies indicate that D-dimer elevation is more consistently elevated in COVID-19 patients with thrombosis as compared to the other coagulation parameters. The aforementioned markers cater to two major determinants of the severity of COVID-19 viz., inflammation and coagulopathy. Hence the combined evaluation of these markers would give insight into the probability of patient having COVID-19 going into a more severe phenotype.The identification of parameters that can distinguish severe COVID-19 cases from non-severe COVID-19 cases would help in early detection of severe cases. Research shall be continued in the field of treatment plans including prophylactic and therapeutic anticoagulants along with anti-inflammatory drugs to help in devising optimum treatment strategies and appropriate allocation of resources at hand for the treatment of COVID-19 patients. However further studies are required to in the field of prevention and treatment due to emergence of Omicron against which the efficiency of the vaccines is not clear.

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