Original Article

Analysis of Correlation between Demographic Data, Inflammatory Markers, and Coronavirus Disease 2019 Illness

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Abstract

Introduction: The coronavirus disease 2019 (COVID-19) is an on-going pandemic caused by severe acute respiratory syndrome coronavirus 2. Majority of people infected with this virus will suffer from mild to moderate respiratory disease and recover without therapy, whereas the elderly and, as well as those who have underlying comorbidities are more prone to have severe infection. Several inflammatory indicators, like procalcitonin (PCT), serum ferritin, C-reactive protein (CRP), and interleukin-6 (IL-6), linked to the increased the risk of development of severe COVID-19 disease. **Objective:** The goal of this research was to see if there was a link between inflammatory markers and the severity of COVID-19 disease, as well as the sociodemographic characteristics that influence COVID-19-positive findings. **Materials and Methods:** This is a cross-sectional at Shri B.M. Patil Medical College, Research Center and Karigoudar Diagnostic Laboratory Vijayapur for a period of 2 months from October to November 2020. This study included 600 COVID-19-positive patients confirmed by real-time polymerase chain reaction (RT-PCR). Investigations included (RT-PCR) and inflammatory markers. The details collected were sociodemographic data and clinical history. Investigations included RT-PCR using throat swab/nasopharyngeal swab and inflammatory markers like CRP, D-Dimer levels, ferritin, IL-6, lactate dehydrogenase (LDH), PCT were performed accordingly. Data were analyzed using the SPSS version 18.0. Results were presented as percentages and mean ± standard deviation. The categorical variables were analyzed using the Chi-square test. **Results:** The mean age of the patients was 43.7 ± 16.7 years with male preponderance. The majority of the patients were between the ages of 21 and 60 (76.7%) years. Increasing age was significantly associated with severity of the disease, similarly CRP levels, D-dimer, ferritin, and LDH levels were significantly higher among those with increasing age and severe disease, i.e., severe acute respiratory infection ($P < 0.05$). **Conclusion:** There was a link between age and inflammatory indicators such as CRP, D dimer, ferritin, and LDH levels, as well as the severity of disease. Hence, measuring these inflammatory markers could help clinicians track and assess the severity and prognosis of COVID-19.

Keywords: Coronavirus disease 2019 disease, C-reactive protein, D-dimer, ferritin

Introduction

A new emergent coronavirus disease 2019 (COVID19) is an infectious pandemic disease caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The outbreak of a novel coronavirus disease in Hubei Province, China, was declared an Internationally Concerned Public Health Emergencies by the World Health Organization in January 2020, and a pandemic was declared on March 11, 2020.[1] On January 20, 2020, Kerala announced the first case of COVID-19 in India.[2]

As per ICMR guidelines, real-time polymerase chain reaction-based assays (RT-PCR) are recommended for the diagnosis of COVID-19.[3] Although the detection of IgM and IgG antibodies against SARS-CoV-2 can be used to provide population-based estimates of infection, they do not typically replace molecular methods as the principal tool for diagnosing SARS-CoV-2 infection.[4]

The majority of people infected with the SARS-CoV-2 develop mild-to-moderate respiratory infection and improve without therapy, whereas the elderly and those with underlying comorbidities are more prone to develop severe infection.[5]

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Inflammatory reactions appear to be important in the evolution of COVID-19 according to the literature.[6] Replication of virus and cellular breakdown trigger inflammatory responses that recruit macrophages and monocytes and cause the release of cytokines and chemokines. Then these subsequently attract cells of the immune system and trigger immunological responses, resulting in cytokine storms and worsening of symptoms. Several inflammatory indicators such as procalcitonin (PCT), serum ferritin, C-reactive protein (CRP), interleukin-6 (IL-6), linked to an increased risk of developement of severe COVID-19 infection. However, these results remain controversial.[7] There is a lack of conclusive data on the relationship between inflammatory indicators and the COVID-19 illness. Patients with severe disease had higher levels of white blood cell count, CRP, PCT, erythrocyte sedimentation rate (ESR), IL-6, and IL-10, according to a meta-analysis.[8] As a result, this research was carried out to see if there was a link between inflammatory markers and the COVID-19 disease, as well as the sociodemographic factors that influence COVID-19 illness.

Materials and Methods

This cross-sectional study was conducted at Shri B.M. Patil Medical College and Research Centre and Karigoudar diagnostic laboratory in Vijayapur City, Karnataka, for a period of 2 months from October to November 2020. A total of 600 study participants included in the study. Ethical clearance was obtained from IEC.

Inclusion criteria

Patients of all ages with confirmed positive COVID-19 disease by RT-PCR in our institution were included in the study.

Exclusion criteria

Patients of all ages with negative of COVID-19 disease by RT-PCR in our institution were included in the study. The details collected were sociodemographic data, clinical history, and laboratory findings. By interview methodology, sociodemographic data, clinical history such as age, sex, history of presenting illness, and duration of symptoms were collected. RT-PCR using throat swab/Nasopharyngeal swab was performed to detect COVID-19 positivity. Inflammatory indicators such as CRP, D-dimer levels, ferritin, IL-6, lactate dehydrogenase (LDH), and PCT were measured accordingly as below.

C-reactive protein

Determination of CRP was done on the serum sample by Turbidometry principle (kit used: Agappe i3 CRP) on Nephlometry Instrument (Agappe Mispa i plus). This is a latex enhanced turbidimetric immunoassay. CRP samples bind to specific anti-CRP antibodies, which have been adsorbed to latex particles and agglutinates. The agglutination is proportional to the quantity of CRP in the sample. The actual concentration is then determined by interpolation from a calibration curve prepared from the calibrators of known concentrations.

D-dimer

D-Dimer was detected by D-Dimer Exclusion II™ kit which is an automated quantitative test for use on the VIDAS instrument for the immunoenzymatic determination of fibrin degradation products in human plasma (sodium citrate) using the Enzyme-Linked Fluorescent Assay technique (ELFA).

Procalcitonin

B·R·A·H·M·S PCT™ kit which is an automated test for use on the VIDAS instruments was used for the determination of human PCT in human serum using the ELFA technique.

Lactate dehydrogenase

VITROS Chemistry Products LDH inhibitor (LDHI) Slides kits were used for quantitative measurement of LDH activity in serum and plasma using VITROS 250 Chemistry Systems instrument. The VITROS LDHI Slide is a multi-layered, analytical element coated on a polyester support. A drop of patient sample is deposited on the slide and is evenly distributed by the spreading layer to the underlying layers. LDH catalyzes the conversion of pyruvate and NADH to lactate and NAD+. The oxidation of NADH, which is monitored by reflectance spectrophotometry, is used to measure LDH activity.

Feritin

Feritin was identified using VIDAS® Ferritin (FER) kits. VIDAS Ferritin is an automated quantitative test for use on the VIDAS instrument for the determination of human Ferritin in human serum using the ELFA technique.

Interleukin-6

IL-6 was determined on fresh serum samples by immuno-chromatography method using Hotgen instrument UPT-3A and kit IL-6 having principle of Up-converting Phosphor Technology.

Erythrocyte sedimentation rate

ESR was determined on Citrate Whole Blood with Western green pipette mounted on the vertical stand. Reading was taken after 1 h of sedimentation.

Operational definitions

World Health Organization case definition for influenza-like illness

Individuals with an acute respiratory infection, a temperature of 38 degrees Celsius, and a cough that started within the last 10 days.[4]

World Health Organization case definition for severe acute respiratory infection

Individuals with an acute respiratory infection who have had a temperature of 38°C and cough that started within the last 10 days and need to be admitted to the hospital.[3]

Statistical analysis

Data were analyzed using SPSS Inc. Released 2009. PASW Statistics for Windows, Version 18.0. Chicago: SPSS Inc. Results were presented as percentages, mean ± standard deviation. The categorical variables were analyzed using the

Chi-square test. Continuous variables viz., CRP, D-Dimer levels, ferritin, IL-6, LDH, PCT levels were expressed in medians and compared with the COVID-19 disease.

Results

The mean age of the study subjects was 43.7 ± 16.7 years and it ranged between 1 and 94 years. The majority of the patients were between the ages of 21 and 60 (76.7%) years. Males 353 (58.8%) outnumbered females 247 (41.2%). Maximum number of patients 466 (77.7%) had symptoms for 7–14 days on presentation with median of 12 days (range between 1 and 20 days). Majority, i.e., 316 (52.7%) presented as influenza-like illness (ILI) followed by severe acute respiratory infection (SARI) 154 (25.7%), asymptomatic primary and secondary contact 130 (21.1% and 0.5%, respectively) [Table 1].

Among the study subjects who presented with ILI, most of them were between 21 and 40 years 130 (54.9%) followed by 41–60 years, >60 years and <20 years 112 (50.2%), 55 (52.9%) and 19 (52.8%) respectively. Similarly, the study subjects who presented with SARI, most of them were between 41 and 60 years 69 (30.9%), >60 years 32 (30.8%) followed by 21–40 years 44 (18.6%) and <20 years 9 (25%). Among the asymptomatic contacts most of the patients were between 21 and 40 years 63 (26.6%) followed by <20 years 8 (22.2%), 41–60 years 42 (18.8%) and >60 years 17 (16.3%). Which were statistically significant ($P < 0.05$). However higher proportion of males 98 (27.8%) presented with SARI whereas females 139 (56.3%) presented with ILI but it was not significantly associated [Table 2].

The mean ranks of CRP, D-dimer, ferritin, LDH levels were notably higher among those with SARI (358.69, 356.36, 356.41 and 356.36 respectively) followed by ILI (333.73, 334.44, 334.42 and 334.45 respectively) and asymptomatic contacts (150.80, 151.82, 151.82 and 151.80 respectively) with significant $P < 0.05$. Whereas IL-6 and PCT levels were high among those who presented with ILI followed by SARI and asymptomatic contacts which was not significant statistically ($P > 0.05$) [Table 3].

Table 1: Sociodemographic details (*n*=600)

Particulars	*n* (%)
Age group (years)	
≤20	36 (6.0)
21-40	237 (39.5)
41-60	223 (37.2)
>60	104 (17.3)
Gender	
Males	353 (58.8)
Females	247 (41.2)
Symptoms duration (days)	
<7	25 (04.2)
7-14	466 (77.7)
>14	109 (18.2)
Presenting illness	
ILI	316 (52.7)
SARI	154 (25.7)
Asymptomatic contacts	130 (21.6)

ILI: Influenza like illness, SARI: Severe acute respiratory infection

Discussion

The ongoing worldwide pandemic of COVID-19 has posed a serious danger to the public health around the world. In patients with severe disease, a number of inflammatory markers are higher compared to individuals with less severe illnesses.[8] The National Health Commission of China emphasized the high levels of inflammatory markers like IL-6 and CRP as early alarming indicators of serious disease.[9] In view of understanding the current situation of COVID-19 and to get a better understanding of the possible link between demographic data, inflammatory indicators and COVID-19 illness this study was conducted among 600 study subjects who are COVID-19 Positive by RT-PCR.

In this study, mean age of the study subjects was 43.7 ± 16.7 years (range from 1 to 94 years) with majority of age between 21 to 60 years and male preponderance with median duration of symptoms of 12 days (range between 1 and 20 days). These findings are comparable to the study findings of Tambe *et al.* who noted a mean age of 45.8 ± 17.3 years (ranging from 4 months to 85 years); majority in age group of 31–60 years, male dominance was observed, and duration of symptoms ranged from 1 to 21 days with average time of 3.5 days.[10]

Table 2: Association of various sociodemographic factors determining coronavirus disease 2019 illness

Variable	Category	COVID-19 illness*			χ^2 (*P*)
		Asymptomatic contacts (*n*=130), *n* (%)	ILI (*n*=316), *n* (%)	SARI (*n*=154), *n* (%)	
Age (years)	≤20	8 (22.2)	19 (52.8)	9 (25.0)	13.43 (0.03)*
	21-40	63 (26.6)	130 (54.9)	44 (18.6)	
	41-60	42 (18.8)	112 (50.2)	69 (30.9)	
	>60	17 (16.3)	55 (52.9)	32 (30.8)	
Gender	Male	78 (22.1)	177 (50.1)	98 (27.8)	2.57 (0.27)
	Female	52 (21.1)	139 (56.3)	56 (22.7)	

*<7 days: 25 (4.2%), 7–14 days: 466 (77.7%), >14 days: 109 (18.2%). ILI: Influenza like illness, SARI: Severe acute respiratory infection, COVID-19: Coronavirus disease 2019

Table 3: Comparison of means of inflammatory markers with coronavirus disease 2019 illness

Inflammatory markers	Severity of illness	*n*	Mean rank	*H*	*P*
CRP (mg/L) (*n*=600)	Mild	130	150.80	125.90	<0.001*
	Moderate	316	333.73		
	Severe	154	358.69		
D-Dimer (ng/ml) (*n*=600)	Mild	130	151.82	123.74	<0.001*
	Moderate	316	334.44		
	Severe	154	356.36		
Ferritin (ng/ml) (*n*=600)	Mild	130	151.82	123.74	<0.001*
	Moderate	316	334.42		
	Severe	154	356.41		
IL-6 (ng/ml) (*n*=147)	Mild	10	42.95	5.85	0.05
	Moderate	90	77.28		
	Severe	47	74.33		
LDH (IU/L) (*n*=600)	Mild	130	151.80	123.77	<0.001*
	Moderate	316	334.45		
	Severe	154	356.36		
PCT (ng/ml) (*n*=190)	Mild	14	67.86	4.37	0.11
	Moderate	114	99.24		
	Severe	62	94.87		

LDH: Lactate dehydrogenase, IL-6: Interleukin-6, CRP: C-reactive protein, PCT: Procalcitonin

In our study, age was significantly associated with COVID-19 illness ($P < 0.05$) i.e., study subjects who presented with SARI, most of them were in the age group of 41–60 years 69 (30.9%) and >60 years 32 (30%) when compared to findings of Saluja *et al.* patients over the age of 60 were the ones having SARI and more complications ($P < 0.05$).[11] Similarly according to the Xu K *et al.* the median age was higher among those who were critically ill.[12]

In the current study the mean ranks of CRP levels, D dimer, ferritin, LDH levels were significantly higher with severe disease i.e., SARI followed by ILI compared to asymptomatic contacts ($P < 0.05$) whereas IL-6 and PCT levels were high among those who presented with ILI followed by SARI and asymptomatic contacts. Zeng *et al.* in his meta-analysis to study the association of inflammatory indicators with the severity of COVID-19, observed significantly lower levels of CRP, PCT, IL-6, serum ferritin and ESR in the nonsevere group.[7] Manson *et al.* found that COVID-19-associated hyper inflammation, as a CRP levels more than 150 mg/L, a 24 h doubling of CRP levels from a level of >50 mg/L, or ferritin levels >1500 g/L and these findings were linked to the need for respiratory support escalation.[13] Another study by Petrilli *et al.* found that increased levels of inflammatory biomarkers are more strongly related with severe disease than age or comorbidities.[14] In a study, Xu K *et al.* found that the seriously sick groups had a considerably larger proportion of patients with significantly increased levels of CRP, PCT, and D-dimer than the moderately ill group ($P = 0.05$).[12] When compared to non-COVID group, the mean serum levels of CRP, ferritin, LDH, and D-Dimer were considerably higher in COVID-19 patients. Except for D-Dimer, mean CRP, ferritin, LDH, and IL-6 levels among COVID-19 patients were linked to the severity of SARS-CoV-2 infection.[15] Similarly our study showed the higher mean values for CRP levels, ferritin, LDH levels with severe disease, i.e., SARI followed by ILI and asymptomatic contacts with significant $P < 0.05$. In contrast, our study also showed the association of higher mean values for D-dimer with SARI cases followed by ILI cases and asymptomatic contacts with significant $P < 0.05$. PCT values in all severely ill patients were >0.05 ng/mL, indicating the possibility of many infections in these patients.[16] Keeping an eye on the inflammatory indicators can provide early notification for the advancement of COVID-19. Monitoring CRP, and PCT levels at the same time can help to detect bacterial infections early, decreasing antibiotic abuse and allowing for early treatments to prevent septicemia and some dangerous disorders.[17] In contrast to Zhu *et al.*, who found that IL-6 is raised in COVID-19 patients and that its level is strongly connected with the severity of symptoms in COVID-19 patients, our study found no such link between IL-6 and illness severity.[18] This may be due to less samples included in our study for IL-6.CRP is increased in acute inflammations/infections as acute phase reactants, hence correlate accordingly with clinical signs and symptoms. In COVID-19 patients, raised D-Dimer indicates microthrombi formations. In covid-19 infections, increased LDH indicates severity of Cellular damage or destruction. Ferritin is increased in acute inflammations/infections as acute phase reactants, hence correlate accordingly with clinical signs and symptoms. PCT above 0.1 ng/ml indicate bacterial infections and above 0.5 ng/ml indicates risk of developing sever sepsis/shock. IL-6 is a soluble mediator that affects inflammation, immunological response, and hematopoiesis in a pleiotropic manner. Continuously dysregulated IL-6 production has a detrimental influence on chronic inflammation and autoimmunity, resulting in cytokine storm. COVID-19 disease progression to a critical stage should be closely monitored and can be avoided. COVID-19 severity is linked to inflammatory markers. Monitoring inflammatory markers, according to our findings, could serve as an early warning system for severe disease advancement.

Limitations of the study

This study is inherent to its small sample size and hospital based. The outcome was not studied because of the lack of data. Further studies with higher sample sizes and population-based study are recommended to establish the association.

Conclusion

Severity of the COVID-19 illness was significantly associated with higher age groups, i.e., >40 years. Inflammatory markers such as CRP levels, D dimer, serum ferritin, and LDH levels were significantly higher among those with severe disease,

i.e., SARI. As a result, measuring these inflammatory markers could help clinicians track and assess the severity of illness and prognosis of COVID-19.

Authors' contributions

All authors designed the experiments. Rashmi M. Karigoudar, Mahesh H. Karigoudar, Sanjay M. Wavare, Praveen R Shahapur, Smitha Bagali, Lakshmi kakhandaki performed the experiments, analyzed the data and wrote the manuscript. All authors read and approved the manuscript.

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Conflicts of interest

There are no conflicts of interest.

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