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Relationship of Age and Viral Load with Clinical and Laboratory Profile in COVID-19 Patients at Presentation

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Abstract

Background: The COVID-19 pandemic caused by novel SARS-COV-2 has caused a sudden and substantial increase in hospitalisations. The incidence and severity are higher among elderly patients, and those with co-morbidities. Its severity has been linked with the viral load of infection. We aim to investigate the link of age and viral load with patients' symptoms and their laboratory findings at the time of presentation to hospital.

Methods: Cross-sectional, observational study of randomly selected COVID-19 RT PCR positive indoor patients.

Results: Patients above 50 years of age were more in number than those less than 50 years of age. Shortness of breath, cough (dry/ sputum), body ache and fever were the main presenting complaints in both age groups. In patients below 50 years of age, those with a high viral load were reported to have a lower SpO2 (p = 0.07) than patients with a low viral load. In patients above 50 years, those with a high viral load were found to have a lower SpO2 (p = 0.009), higher HRCT scores (CT Severity Score) (p = 0.0002) and higher Neutrophil Lymphocyte Ratio (NLR) (p = 0.09), as compared to patients with a low viral load. In patients with a low viral load, the NLR was found to be higher (p = 0.007) in those above 50 years. Finally, in patients with a high viral load, higher HRCT scores (p = 0.08) were found in those above 50 years.

Conclusion: Our results indicate that a higher age can adversely affect some biomarkers and disease outcome, irrespective of the viral load of the patients. At the same time, we found that a higher viral load can also adversely affect the severity of disease, irrespective of the age of patients.

Key words: SARS CoV-2, COVID-19, cycle threshold, CT severity score, NLR, age.

Introduction

The world was hit by the first large pandemic, The Spanish flu, in 1918. It was caused by the H_1N_1 influenza A virus, infected 500 million people (about a third of the world's population at that time) and lasted for more than 2 years.

The COVID-19 pandemic (as declared by WHO on March 11, 2020), hit the world in Dec 2019. As of Jan 16, 2021, more than 94.4 million cases and more than 2.02 million deaths have been attributed to COVID-19. The world has seen the waves of other Corona virus infections like SARS CoV-1 and MERS in the past. The SARS-CoV-2 is a novel Corona virus, thought to have originated from either bats or the animal market of Wuhan, China.

The disease caused by SARS-CoV-2 was named COVID-19. The clinical presentation of the disease is highly variable, ranging from asymptomatic to severe illness involving multiple organs. The virus spreads through the air, when two or more people are at a close distance to each other, in the form of droplets through cough, sneeze, breath, or through contact via contaminated surfaces. Therefore, the preventive measures are wearing masks, washing hands properly, and physical distancing. As the virus is a novel virus, the understanding about its transmissibility, clinical presentation, pathogenicity, and treatment gradually evolved and changed with experience.

The main concerns associated with the virus are asymptomatic carriers and vulnerable elderly population with or without comorbidities. As time passed, it was realised that the disease was affecting elderly patients with comorbidities more severely, who were being hospitalised for prolonged durations. The younger population, who were either affected less severely, or remained asymptomatic, probably acted as carriers.

As this virus spreads through air and contact, another question which was raised was, "How the viral load of infection is associated with infectivity and patient outcome?" High viral load was earlier reported to be associated with severe disease and poor outcome.

Aims and objectives

The severity of COVID-19 has been linked with higher age and viral load in patients. We aim to investigate the link of

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age and viral load with patients' symptoms and their laboratory findings at the time of presentation to hospital.

Data source

This study was planned and executed at the Mahatma Gandhi Medical College and Hospital, Jaipur. Of all the COVID-19 RT PCR positive patients who were admitted in COVID-19 ward at the hospital between October 2020 to December 2020, 106 were randomly selected for this study, after the approval and consent of Institutional ethics committee.

Material and Methods

COVID-19 RT PCR was confirmed by RNA amplification by ABI 7,500 Fast Dx Real-time Polymerase Chain Reaction. We also measured cycle threshold or Ct value; i.e., the number of cycles after which fluorescence of the PCR product is detectable over and above the background signal. Theoretically, the Ct value is inversely proportional to the amount of genetic material (RNA) in the starting sample and lower Ct values generally correlate with high viral load.

At presentation, each patient's HRCT chest, baseline investigations, and inflammatory markers were recorded. Along with these, a detailed history was also recorded, and treatment was started as per prevailing protocol of the Ministry of Health and Family Welfare (MOHFW), Government of India.

Data was captured on Google forms and was sent to the investigator. Google sheets were prepared from Google forms and the data was then transferred to Microsoft Excel. Finally, this data was imported into STATA, a statistical analysis software, where it was interpreted and analysed.

Inclusion and exclusion criteria

A patient was included in the study only if:-

- His/her age was more than 18 years
- He/she tested positive for COVID-19 by RT PCR
- Gave informed consent to be a part of the study

Patients who did not meet the inclusion criteria were excluded.

Statistical analysis

All data was analysed using the statistical analysis software, STATA. The analysis started with a calculation of the descriptive statistics of the data: number of patients with co-morbidities, number of patients displaying various symptoms of COVID-19, and a simple calculation of the percentage of patients in various categories (gender, symptoms, co-morbidities and oxygen requirement) according to their age (below or above 50 years of age) and viral load (cycle threshold value of less than and more than 24). The results of this analysis are presented in Figs. 1 and 2, as well as in Table I.

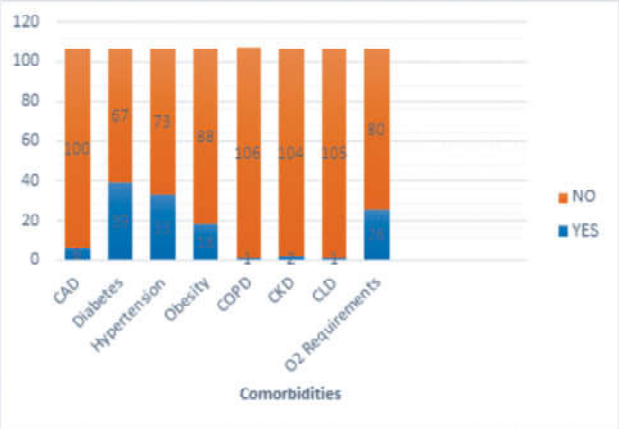
Following this, two analyses were undertaken. In the first, patients were divided into two categories, according to their age, and within each category, were divided according to their viral load. Following this, the mean value of each sub-category was calculated, and independent t-tests for difference in means were undertaken to infer if, given the age category of a patient, his viral load had an effect on the value of his/her investigation variables. The results of this analysis are presented in Table II.

In the second analysis, patients were divided into two categories according to their viral load and then, within each category, were divided according to their age. Following this, the mean value of each sub-category was calculated, and independent t-tests for difference in means were undertaken to infer, if given the viral load of a patient, the age had an effect on the value of his/her investigation variables. The results of this analysis are presented in Table III.

Results

Out of the 106 patients, 29 (27.3%) were females and 77 (72.6%) were males. Patients less than 50 years of age were 30 (6 females, 24 males), and patients more than 50 years of age were 76 (23 female and 53 males).

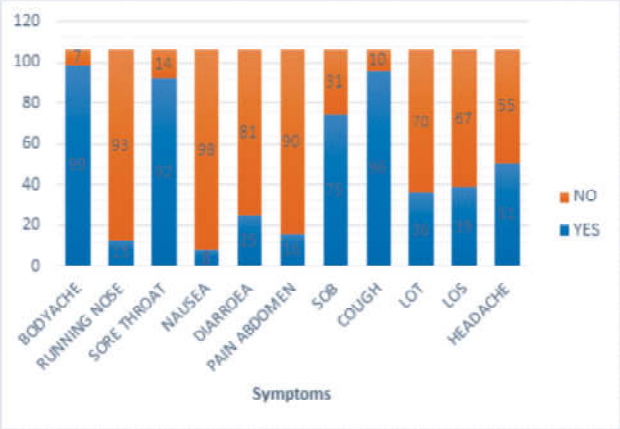
2 patients had coronary artery disease (1.8%), 39 had diabetes mellitus (36.7%), 33 had hypertension (31.1%), 18 had obesity (16.9%), 1 had chronic obstructive pulmonary disease (0.94%), 2 had chronic kidney disease (1.8%), and 1 had chronic liver disease (0.94%) (Fig. 1).



(CAD = Coronary artery disease, COPD = Chronic obstructive pulmonary disease, CKD = Chronic kidney disease, CLD = Chronic liver disease).

Fig. 1: *Number of patients with different comorbidities.*

99 patients presented with bodyache (93.3%), 13 with running nose (12.2%), 92 with sore throat (86.2%), 8 with nausea/vomiting (7.54%), 25 with diarrhoea (23.58%), 16 with pain in abdomen (15%), 75 with shortness of breath (70.7%), 46 with dry cough (43.3%), 50 with cough with sputum (47.1%), 36 with loss of taste (33.9%), 39 with loss of smell (36.7%), 51 with headache (48.1%), and 7 presented with no symptoms (6.6%) (Fig. 2). Oxygen was required in 25 patients (23.5%). 1 patient required ventilation, while 80 (75.47%) did not require oxygen (Fig. 2). We divided symptoms according to age below 50 years and above 50 years and further divided according to low and high viral load in Table I.



(SOB = Shortness of breath, LOT = Loss of taste, LOS = Loss of smell).

Fig. 2: *Number of patients with different symptoms.*

In patients below 50 years of age, those with a high viral load (Cycle threshold value less than 24) were reported to have a lower SpO2 (92.5%) (p = 0.07) than the patients with a low viral load (SpO2 - 94.3%). In patients above 50 years, those with a high viral load (Cycle threshold value less than 24) were found to have a lower SpO2, (91.1% versus 94.4% in the low viral load group; p = 0.009), higher HRCT scores (CTSS) (13.4 versus 9.4 in the low viral load group; p = 0.0002) and higher Neutrophil Lymphocyte Ratio (9.1 versus 5.7 in the low viral load group; p = 0.09) (Table II).

In patients with a low viral load, the Neutrophil Lymphocyte ratio (NLR) was found to be higher in those above 50 years (5.7 versus 3.6; p = 0.007). Finally, in patients with a high viral load, higher HRCT scores were found in those above 50 years (13.4 versus 10.4 out of 25; p = 0.08) (Table III).

We did not find any significant difference in Neutrophil to Monocyte Ratio (NMR), C-Reactive Protein, SGOT, SGPT, D-Dimer, LDH, Ferritin, and Procalcitonin between the two age groups and there was no difference in these markers between high and low viral load groups, too.

Table I: Percentage of patients in various variable categories.

According to their age and Cycle threshold (Ct) value						
Variable	**Age below 50 yrs**		**N = 30**	**Age above 50 yrs**		**N = 76**
	Low viral load (Ct: > 24)	**High viral load (Ct: < 24)**		**Low viral load (Ct: > 24)**	**High viral load (Ct: < 24)**	
Gender			30			76
Female	67%	33%	6	78%	22%	23
Male	63%	37%	24	58%	42%	53
Shortness of breath			30			75
No	100%	0%	7	70%	30%	23
Yes	52%	48%	23	62%	38%	52
Loss of taste			30			76
No	58%	42%	19	63%	37%	51
Yes	73%	27%	11	68%	32%	25
Loss of smell			30			76
No	60%	40%	20	64%	36%	47
Yes	70%	30%	10	66%	34%	29
Cough			28			76
No	67%	33%	3	60%	40%	5
Dry	50%	50%	14	53%	47%	32
Sputum	73%	27%	11	74%	26%	39
Headache			30			76
No	77%	23%	13	74%	26%	42
Yes	53%	47%	17	53%	47%	34
Bodyache			30			76
No	100%	0%	1	50%	50%	6
Yes	62%	38%	29	66%	34%	70
Hypertension			12			46
No	20%	80%	5	33%	67%	15
Yes	57%	43%	7	68%	32%	31
Diabetes			11			49
No	17%	83%	6	33%	67%	15
Yes	60%	40%	5	62%	38%	34
Temperature			30			75
No	83%	17%	6	67%	33%	15
Yes	58%	42%	24	63%	37%	60
Oxygen/ventilator			10			16
Oxygen	37%	63%	8	36%	64%	14
HFNC	0%	100%	1	0%	100%	2
Ventilation	100%	0%	1	-	-	0

(N = Number, Ct = Cycle threshold, HFNC = High flow nasal cannula).

Table II: Mean values of investigations by age and Cycle threshold (Ct) value and associated t-test results.

Variable	Age below 50 yrs N=30			p-value	Age above 50 yrs N=76			p-value
	Low viral load (Ct: >24)	High viral load (Ct: < 24)			Low viral load (Ct: >24)	High viral load (Ct: < 24)		
SpO2	94.3	92.5*	30	0.0674	94.4	91.9***	76	0.0087
HRCT	10.3	10.4	28	0.9308	9.4	13.4****	73	0.0002
NLR	3.6	9.3	29	0.2380	5.7	9.1**	72	0.0861
NMR	79	12.6	3	NA[1]	24.4	27.9	11	0.8139
CRP	33.9	40.8	30	0.7333	47.8	64.1	76	0.2979
SGOT	42.8	53.7	30	0.3187	50.4	43.1	76	0.4104
SGPT	52.9	67.8	30	0.5054	41.1	41.6	76	0.9373
D-Dimer	385.9	557.2	30	0.3280	610	862.1	76	0.4138
LDH	210.1	286.6	30	0.2086	257.9	284.2	76	0.4036
HBA1c	5.8	7	14	0.5463	6.8	6.6	57	0.4547
Ferritin	274	313.8	30	0.6726	303.2	328.2	76	0.7271
PCT	0.108	0.154	16	0.4665	0.207	0.229	46	0.8970

*Notes: 1. *Difference in means (two tailed independent t-test) was significant at 10% significance level; **difference in means was significant at 5% significance level; ***difference in means was significant at 1% significance level; ****difference in means was significant at 5% significance level.*

2. There was only one observation with low viral load so the software was unable to calculate standard deviation.

3. Prior to conducting the t-test for each difference in means, tests of equality of variance were undertaken. Wherever the hypothesis of equal variance was rejected, the t-test was undertaken assuming unequal variance.

(N = Number, Ct = Cycle threshold, HRCT = High resolution computed tomography, SpO2 = Oxygen saturation, NLR = Neutrophil to lymphocyte ratio, NMR = Neutrophil to monocyte ratio, CRP = C reactive protein, SGOT = Serum glutamic oxaloacetic transaminase, SGPT = Serum glutamic pyruvic transaminase, LDH = Lactate dehydrogenase, PCT = Procalcitonin).

Table III: Mean values of investigations by Cycle threshold (Ct) value and age and associated t-test results.

Variable	Age below 50 yrs N = 68			p-value	Age above 50 yrs N = 38			p-value
	Low viral load (Ct: >24)	High viral load (Ct: < 24)			Low viral load (Ct: >24)	High viral load (Ct: < 24)		
SpO2	94.3	94.4	68	0.9376	92.5	91.9	38	0.5367
HRCT	10.3	9.4	68	0.5683	10.4	13.4*	33	0.0800
NLR	3.6	5.7***	66	0.0070	9.3	9.1	35	0.9735
NMR	79	24.4	5	NA[1]	12.6	27.9	9	0.4611
CRP	33.9	47.8	68	0.3835	40.8	64.1	38	0.3346
SGOT	42.8	50.4	68	0.4095	53.7	43.1	38	0.3200
SGPT	52.9	41.1	68	0.1696	67.8	41.6	38	0.2415
D-Dimer	385.9	610	68	0.2581	557.2	862.1	38	0.3356
LDH	210.1	257.9	68	0.1960	286.6	284.2	38	0.9636
HBA1c	5.8	6.8***	49	0.0028	7	6.6	22	0.7945
Ferritin	274	303.2	68	0.7480	313.8	328.2	38	0.8692
PCT	0.108	0.207	37	0.4713	0.154	0.229	25	0.4988

*Notes: 1. * Difference in means (two tailed independent t-test) was significant at 10% significance level; **difference in means was significant at 5% significance level; ***difference in means was significant at 1% significance level; ****difference in means was significant at 5% significance level.*

2. There was only one observation with low viral load so the software was unable to calculate the standard deviation.

3. Prior to conducting the t-test for each difference in means, tests of equality of variance were undertaken. Wherever the hypothesis of equal variance was rejected, the t-test was undertaken assuming unequal variance.

(N = Number, Ct = Cycle threshold, HRCT = High resolution Computed tomography, SpO2 = Oxygen saturation, NLR = Neutrophil to lymphocyte ratio, NMR = Neutrophil to monocyte ratio, CRP = C reactive protein, SGOT = Serum glutamic oxaloacetic transaminase, SGPT = Serum glutamic pyruvic transaminase, LDH = Lactate dehydrogenase, PCT = Procalcitonin).

Discussion

Starting as a new disease, the SARS-CoV-2 outbreak soon engulfed the world and turned into a pandemic. As this was a novel virus with little understanding about how it presents and spreads, it posed many challenges, including difficulty in early recognition of infection and the difficulty in diagnosing patients who were asymptomatic. Gradually, an understanding of pathogenesis, investigations, and treatment protocol developed, along with some repurposed drugs.

COVID-19 was deemed to be more severe with a higher risk mortality in elderly patients and those with co-morbidities, compared to younger patients and those without co-morbidities. On the other hand, children and younger patients have been less severely affected by the disease. Men have been found to be at higher risk of mortality due to COVID-19[4] and different age groups were found to have different outcomes.

In this study, we compared patients' symptoms, severity and laboratory investigations according to their age group

(below and above 50 years of age) and according to their viral load (cycle threshold value of less than and more than 24). It was found that patients above 50 years of age were more in number in the randomly selected sample. Main presenting complaints in both the age groups were shortness of breath, cough (dry/sputum), body ache, and fever.

In patients above 50, a high viral load was found to be associated with a lower SpO2, higher HRCT scores (CT Severity Score) and higher Neutrophil Lymphocyte Ratio (NLR). Additionally, in patients with low viral load, those above 50 years were reported to have a higher NLR. These two observations show that patients above 50 years had higher biomarkers and an advanced disease, irrespective of their viral load.

In a study, done in 16 countries, persons age 65 years or older had significantly higher COVID-19 mortality rates compared to younger individuals; men had a higher risk of COVID-19 death than women[1].

Pence *et al* found age related poor outcome and related it to pathological monocyte responses in COVID-19 as monocytes and pulmonary monocyte derived macrophages play an early and key role in the progression to severe COVID-19 by promoting cytokine storm, ARDS and other tissue damage[2].

Our results are corroborated by the hypothesis advanced by Chen *et al* that age-related decline and dysregulation of immunological functions, immunosenescence and inflammation play a major role to increase vulnerability to severe COVID-19 infections and poor outcome[3]. Our results are also in line with Moreno *et al*, who discussed the role of damaged/aged mitochondria responsible for oxy-inflammaging, immunosenescence, chronic inflammation, deficient antiviral response mainly responsible for severe disease in the aged population[4].

While patients with a high viral load had higher HRCT scores in above 50 years age group, even patients who were below 50 years were found to have lower SpO2 if they had a high viral load. These observations show that patients with a higher viral load are likely to have advanced disease, irrespective of their age group.

Our results are supported by the findings of Rao *et al*, who undertook a systematic review of the clinical utility of cycle threshold value in the context of COVID-19 and found that lower cycle threshold values are potentially associated with worse outcomes, severe disease and increased morbidity and morality[5]. However, Singanayagam *et al* showed no significant difference in cycle threshold values or culture positivity for upper respiratory tract samples across different age groups[6.] Karahasan *et al* showed that viral load was not a critical factor for hospitalisation and mortality – outdoor patients also had considerable viral load in their nasopharynx and were contagious[7]. Yang *et al* concluded that elevated age and NLR can be considered independent biomarkers of poor clinical outcomes[8]. Liu *et al* concluded that NLR is an independent risk factor of the in-hospital mortality for COVID-19 patients, specially for males[9].

There was no significant difference in other markers like, Neutrophil to Monocyte Ratio (NMR), C-Reactive Protein, SGOT, SGPT, D-Dimer, LDH, Ferritin, Procalcitonin between the two age groups, and high and low viral load groups.

Limitations

Our study was undertaken at a single centre and the patients and doctors were not blinded. A large multi-centre study with a higher number of subjects may be helpful in determining the role of age, gender, viral load and different biomarkers in the severity and outcome of COVID-19 disease.

Conclusion

Our results indicate that a higher age can adversely affect serum biomarkers and disease outcome, irrespective of the viral load of COVID-19 patients. At the same time, we have found that a higher viral load can also adversely affect the severity of disease, irrespective of the age of patients.

While our results are corroborated by and support many existing findings in the literature, a multi-centre study with a higher number of patients would be able to shed more light on the relationships we have sought to explore by the means of this study.

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