CORRELATION BETWEEN CLINICAL FEATURES AND IMMUNOLOGICAL PARAMETERS WITH VIRAL LOAD IN HOSPITALISED COVID POSITIVE PATIENTS

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Abstract

Background: COVID has caused a worldwide pandemic and brought the countries to a halt. It is not only a disease of the respiratory system but the entire body. RT-PCR remains the main stay of diagnosis for COVID, limited study has been done in terms of usability of cycle threshold (CT) values for understanding the severity of the disease. This study was done to find association between CT values, clinical features and biomarkers

Materials and Methods: this is an observational study done between November 2022 to October 2022. A total of 200 cases were studied. RT-PCR was performed using Quant studio 12K flex system. Biomarker estimation was done on Architect c8000

Results: A total of 200 cases studied. 43.28% cases had low CT value in E gene and 40.79% cases in RdRp gene. The predominant symptom noted in our study is breathlessness. No statistical correlation was made between CT values and clinical symptoms and also no statistical correlation was made between biomarkers.

Conclusion: COVID RT-PCR is the gold standard diagnostic method but the viral load cannot be used as a prognostic marker because the sample collection technique is not standardised across the board and also the quantity of sample obtained during nasopharyngeal swabbing varies. Further studies and establishment of standardised protocols can bring about a usability for the CT values.

Introduction

Infections of the respiratory tract have been part of civilization since ancient times and their etiologies are varied ranging from tiniest of pathogens like viruses to multicellular organisms like moulds.[1] A lot of the viruses either through aerosols, droplets or droplet nuclei make use of the respiratory tract to cause a localized respiratory tract infection but some are also the cause for systemic disease.[2] The Coronaviruses (CoV) are no exception. The CoV are a group of viruses found in avian and mammalian species and are known to cause nearly 20% of common cold infection in humans. They sometimes in their highly virulent state can be the cause for severe and serious illnesses in humans.[3]

Two of the Viruses that belong to this family which in the recent past were the cause for some serious debilitating effects on human life include Severe Acute Respiratory Syndrome-Coronavirus (SARS-CoV) and Middle Eastern Respiratory Syndrome-Coronavirus (MERS-CoV). At present another virus of the same family namely SARS-CoV-2 is the cause of the pandemic since 2019.[3,4]

SARS-CoV-2 has become the focus of worldwide attention. In February 2020, the World Health Organization has declared it a public health emergency of international concern. SARS-CoV-2 has spread around the world with approximately(approx) 4 Crore confirmed cases and approx 11 Lakh deaths and in India there are approx 77 Lakh confirmed cases and approx. 1.1Lakh deaths (as of Oct 2020).[5]

Polymerise Chain Reaction (PCR) is molecular based investigation which can identify the infection easily compared to other direct or indirect investigation. PCR techniques have higher sensitivity and specificity in view of direct detection of genetic material of causative organism. PCR test is most commonly used for the viral infection however the causative organism contains RNA or DNA. For RNA based causative agents required Reverse Transcriptase- PCR (RT-PCR) for genetic material amplification and polymerisation.[6-8] CoV contain RNA base genetic material and RT-PCR was worldwide used for the diagnosis. It is a confirmatory test of the Covid-19 infection in the patients.[4,8,9] RT-PCR reports contain various gene and their Cycle Threshold (CT) value which decide the infection or viral load. Lower CT value was interpreted as high viral load and higher CT valued was interpreted as lower viral load. There were a misconception that lower CT value patient had severe infection and patient required ICU care while higher CT value patient had low grade infection and can be treat by oral medication at home isolation.[10] Current study was planned to check for associations between CT value of real time PCR of COVID-19 and symptoms in a COVID positive patient and study if any other associations are present between CT and biomarkers detected in COVID

Methodology

An observational study was conducted for 2 years between (November 2020 to October 2022) at tertiary care hospital and include 200 patients between 15-65 years age group and Covid RT-PCR positive. Informed consent will be taken in prescribed consent format.

SARS-CoV2 detection is done by checking for the presence of E and RdRp gene; using an ICMR approved and validated kit. The CT values (range 0-38) obtained will be recorded, any CT value above 38 will be regarded as negative. The immunological, haematological and

biochemical parameters are tested for to note the change of values in the parameters, which includes IL-6, Ig-M, Ig-G, CRP, Ferritin, D-dimer, Fibrinogen, LDH, Procalcitonin and hemogram. Architect c8000 (Abbott Park, Illinois, U.S.A) was used to process biomarkers like ferritin, d-Dimer, C reactive protein

Correlation between CT value, immunological and haematological parameters will be studied.

Statistical Analysis

Data were entered in the Excel and analyzed using SPSS v23 (IBM Corp. Released 2015. IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.). Appropriate tests of statistical significance such as Chi-square and paired *t*-test were used.

Ethical Approval

Study approved by institutional ethics sub-committee under reference number I.E.S.C/268/2021

Results

This study has captured the data of 201 patients reported for COVID, further the patient count was distributed with their age and gender as shown in Figure 1. Here, distribution of male and female is shown with different color. Overall distribution pattern for both genders were similar however the count differed for the most frequent age. Two peaks were observed at ≈ 40 and 75 years, at 40 years, males (n=72) has larger number than female (n=16) while at 75 both numbers (n=26, n=21) were comparable.

Figure .1. Gender distribution of 201 patients as per their age.

COVID condition of patients was grouped into three classes based on the CT value reported for the patients indicates the viral load. These classes were (i) high ($CT \leq 25$), (ii) Intermediate ($25<CT<30$), and (iii) low ($CT \geq 30$). Patients with a high viral load are more likely to develop COVID compared to those with a low viral load. Figure 5.2 shows the categorization of these patients on their CT value, envelope (E) gene and RNA dependent RNA polymerase (RdRp) gene both were used to determine the CT value used in this classification.

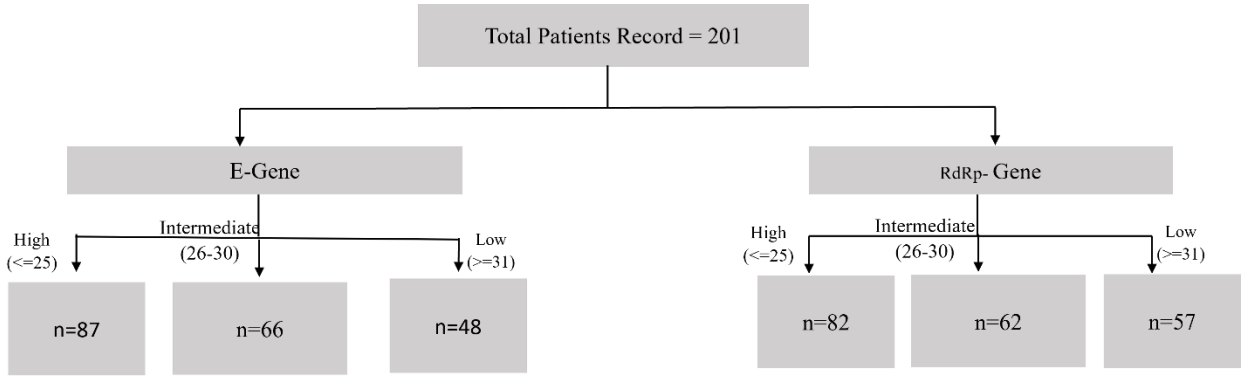

Figure 2. Patients' distribution based on CT values for E-gene and RdRp gene described in study.

Total 201 patients, 87 (43.28%) patients were shown low CT values in E-gene and 82 (40.79%) in RdRp gene. This shows that ≈ 40% of the patients showed low CT value and thus indicated critical COVID condition as per the study. Nearly 62-66 patients (32.8- 30.8%) in both gene showed intermediate level CT values and 48-57 patients (23.8-28.3%) had high CT values >=31 where they considered relatively safer in the study.

Table 1. Patients' distribution based on their age group with the disorders, admit status, and oxygenation requirement.

		Total	Age Group			
			21-40	41-60	61-80	81-100
Disorder	**DM**	100	5	21	61	13
	HTN	24	2	8	13	1
Admit Status	**ICU**	165	57	28	67	13
	Ward	36	8	19	9	0
Oxygenation	**Intubation**	149	54	22	60	13
	NRBM	43	10	19	14	0

As per Indian Council of Medical Research (ICMR) guideline any CT value < 38 was considered as COVID patients, as pe this benchmarks this study had all COVID positive patients. Table:1 shows the distribution of patients based on their age group in context to the comorbid condition disorders, admit status, and oxygenation requirement. Here, we divided the patients into four age groups starting with 21Yrs. to 100 Yrs. of age; it was found 50% of the total patients had DM while 60% of them fell into 61-80 Years age group. Similarly, HTN also had largest portion 54.1% falls in 61-80 Years group. This indicates the comorbid condition is high in these age group patients. Admission status of these patients had two categories (1) ICU and (2) Ward, study had 82% patients were in ICU and rest 18% were in general ward. Here again, 61-80 years old group had the highest number 67 (40% of total ICU patients) but surprisingly younger age group 21-40 also showed 57 (34.5%) patients. Similarly, in oxygenation these two patients' group (21-40 and 61-80) were most populated in incubation oxygenation support.

This study had taken four symptoms of the patients for the clinical diagnosis; these are (i) fever (ii) breathlessness (iii) loss of taste and (iv) loss of smell. Venn diagram shown in Figure 5.4 illustrates the patients' count distribution based on all four symptoms that were diagnosed. Breathlessness was the most frequent symptoms found 190 cases (94.5%) followed by fever in 97 cases (48.25%) loss of taste and loss of smell were not frequently observed symptoms and they constitute only 6-10% of the cases. There were six cases where all these four symptoms were observed. Table showed in Figure 4 had the list of number of cases where any two symptoms were observed.

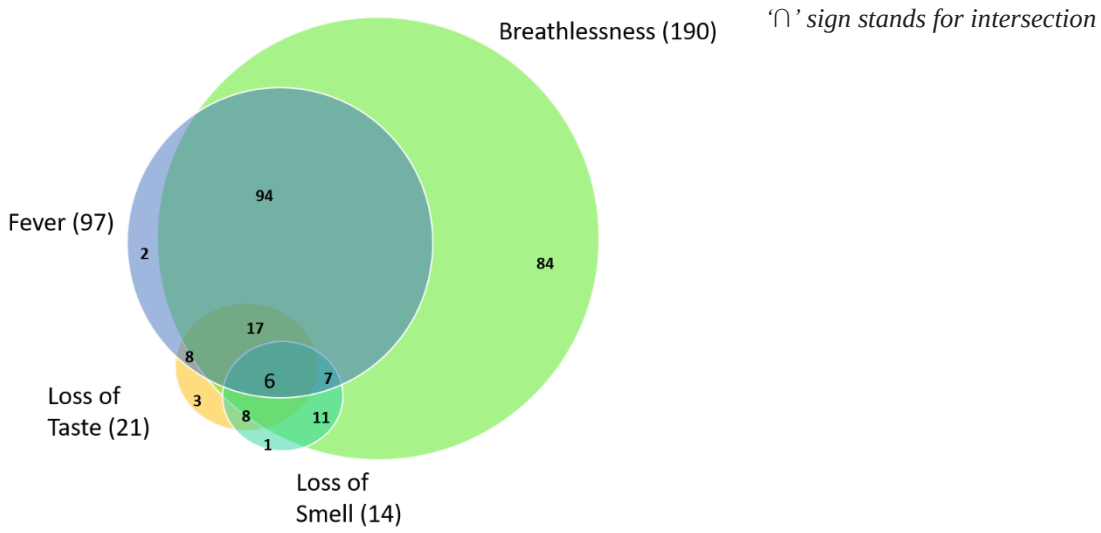

Figure 3. Venn diagram for the patients with one or more COVID symptoms.

A pulse oximeter's "SpO2" value indicates the proportion of oxygen in a person's blood. A typical healthy oxygen level is close to 95 percent or greater. Moreover, individuals with persistent lung illness or sleep apnoea might have normal levels as high as 90%. SpO2 level of all 201 COVID positive patients were recorded, and frequency distribution plot was generated.

Figure 5.5.2 gives distribution of patient on type of oxygen supplementation

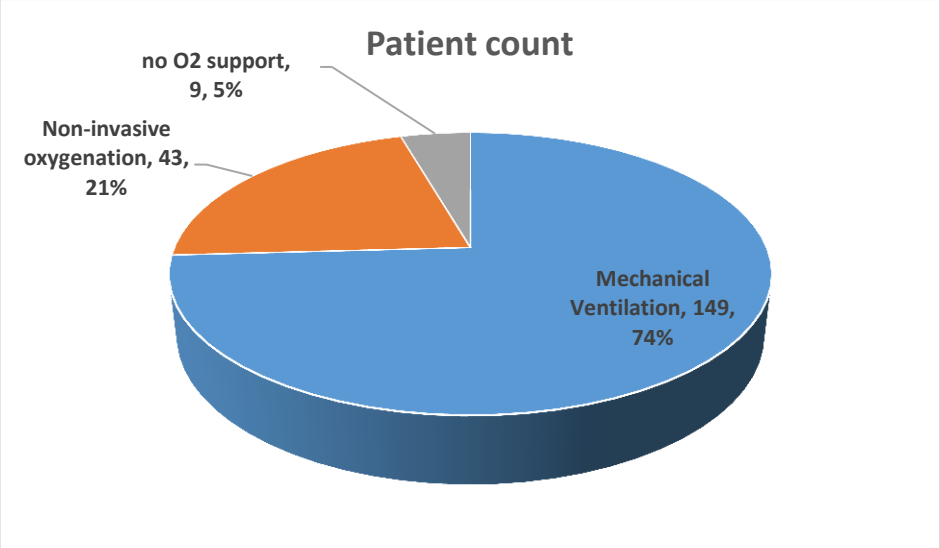

Figure 4 Distribution of oxygen supplementation among patient

Later, various blood markers were diagnosed for all the COVID positive patients to determine the most populated range. Lactate dehydrogenase (LDH) test indicates the damage of body tissue. LDH is an enzyme which normal level in blood for healthy person is 140 – 280 U/L. In this study 90.54%, COVID positive patients showed LDH value between 440 and 760.

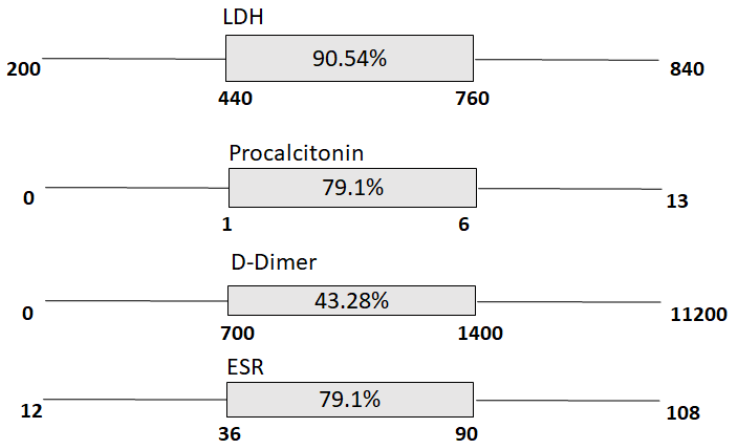

Figure 5. Level of LDH, Procalcitonin, D-Dimer and ESR level inn COVID positive patients.

Level of LDH, Procalcitonin, D-dimer, and ESR in blood sample is shown in Figure 5. In continuation, another four markers were also studied for these patients as shown in Figure 6.

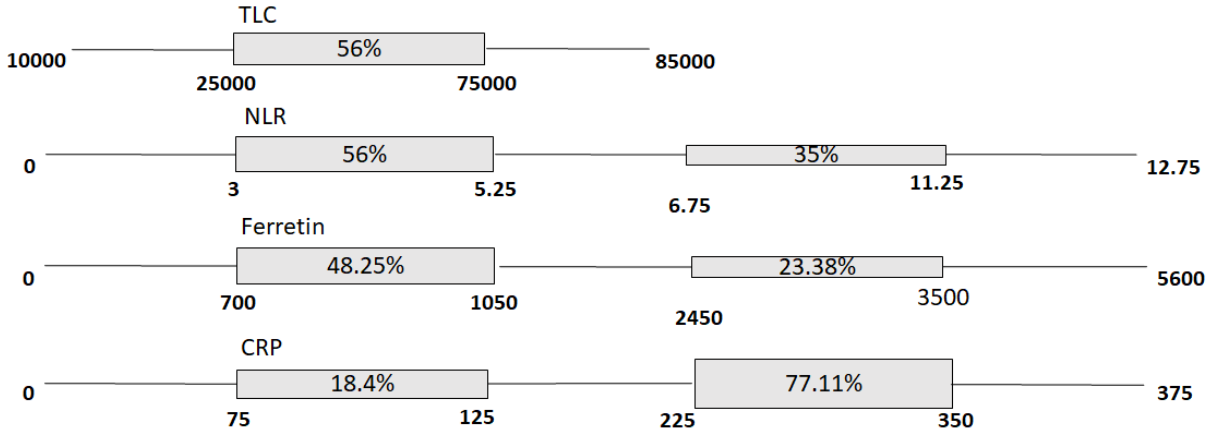

Figure 6. Level of TLC, NLR, Ferritin and CRP level inn COVID positive patients.

Discussion

COVID19 caused havoc worldwide and many countries were not prepared adequately to tackle a pandemic scenario. It was novel experience for many when it came to drafting plans and protocols to battle the situation. Now after wading through the pandemic we have gained experience to tackle similar scenario further into the future.[11]

SARS CoV-2 affects everyone but certain gender, age group and person with pre-existing condition are at a higher risk of disease and complication. Studies show that though anyone among the population can contract SARS-CoV-2 but certain age groups, gender or people with pre-existing conditions/immunosuppressed state suffer from COVID infection more than their counterparts.[12] COVID pandemic though spread wide is not lethal enough when compared to other flu variants belonging to the same family.[13]

Among the 201 patients involved in this study 61% accounted for male and 39% were female patients, we found patients aged from 20 to 90 years, but maximum numbers of patients were found to be either middle aged or old aged. In the middle age group majority of the numbers were made up by males (35.82%) rather than females (7.9%) but in comparison at old age numbers of both males (12.9%) and females (10.45%) were comparable. Similar to our study Kushwaha *et al* 2021, Khan M *et al* 2020, Li Q *et al* 2020, Chen N *et al* 2020 in their studies found higher proportion of males with COVID than females.[12,14-16]

Our study included only COVID-19 positive patients admitted at the hospital, their COVID RT-PCR was done and based on their CT value the patients were categorised as follows, CT values ≤25 were categorised as high, ≥30 values as low and anything in between as intermediate.[17] The kit being used in our hospital tests for E and RdRp genes. About 40% of the patients showed low CT values, according to study a study by Aranha *et al*, low CT indicated critical COVID condition [18] but we did not find any significant statistical association between CT value and their symptomology or area to which they were admitted. Between 32-38% of patients' CT value fell in the intermediate category and about 23-28% of the patients had high CT value. Similarly to ours, in studies done by Arons *et al* 2020, He *et al* 2020, Kimball *et al* 2020, Zou *et al* 2020, no correlation was found between CT value and patients symptoms or severity of the disease, the average CT value in their studies ranged between 25.5-29.4.[19-22].

In this study we noted patients ages ranged from 20years to 90years, we grouped them into four age groups as follows 21-40years, 41-60years, 61-80years, 81-100years. Among the various age groups ≈61% of the patients had diabetes, belonging to 61-80years age group and ≈54% of the patients had hypertension, belonging to 61-80years age group.

We also studied requirement for oxygen support, where we found that age groups 21-40years and 61-80years had about 36.2-40.3% of patients each individually requiring mechanical ventilator support. The age group 61-80years having high numbers of patients requiring mechanical ventilation can be attributed to severe disease in patients afflicted with co morbid and also due to regression in immunity. Similar numbers were found in the age groups 21-40years, this age group having such high numbers can be hypothesised as them having ill-advised habits. We also studied patients requiring non-invasive oxygenation support and found that majority of the patients belonged to 41-60years group; this may be due to the patients in this age group having co morbid conditions but in tolerable range.

Studies show that patients with the disease suffer from low oxygen levels in blood. This has been noted in our study too [23,24], oxygen levels in blood are measured as percentage SpO2. In this study, we observed that the percentage SpO2 levels of the patients ranged between 65-94% and many of the patients had SpO_2 levels of 70, 76, 82, 86 respectively. Highest number of patient count had oxygen saturation 82-86%, this is as such because many of the patients we studied were requiring mechanical ventilator support. Statistical significance between CT value and SpO_2 levels were inconclusive. Similar like our study, Borges do Nascimento IJ *et al* 2020, Ramirez-Hinojosa JP *et al* 2021, noted patients requiring oxygen supplementation but the percentage of patients needing oxygen supplementation were fewer, this is due to them including non-hospitalized patients unlike in our study where 74% required mechanical ventilation and 21% required non-invasive oxygen support, and all were admitted patients.[25-26].

Patients with COVID-19 disease also had other deranged parameters as many studies show. These parameters include but not limited to LDH, CRP, Procalcitonin, ferretin, D-dimer, ESR. These parameters are considered as biomarkers to evaluate the prognosis of the disease. Patients of this study had LDH values ranging from 200-840U/L, where ≈90.5%, Procalcitonin levels ranging from 0.1-13ng/mL and about 79.1% had values ranging 1-6ng/mL, D-dimer levels ranging from 700-11200, but majority had values of 700-1400ng/mL, ferretin values ranged from 700-5600ng/mL, we observed two clusters; one at 700-1050ng/mL range and the other at 2450-3500ng/mL range. The statistical association between biomarkers and CT value was insignificant, association between biomarkers and symptoms was carried out, we found a mild to moderate association but statistically insignificant. In studies by Borges do Nascimento IJ *et al* 2020, Shi F *et al* 2020, Khadim AS *et al* 2021, Huan I *et al* 2020, noted increased levels of biomarkers mentioned above.[25,27-29]

Conclusion

In this study we observed that patients presented with varied symptoms but majority suffered from breathlessness, this coincides with the pathogenesis of the disease. Although detection of COVID-19 is done by RT-rPCR and the result is reported in terms of CT value, we did not find any significant correlation between CT value and the symptoms of the patients. The

patients biomarkers that were studied also did not share any significant correlation with the CT value but moderate correlation was observed with symptoms of the patients.
In the course of the study no significant correlation was found between CT value and blood oxygen saturation of the patient, moderate correlation was noted between oxygen saturation, D-dimer and LDH. Majority of the patients studied had comorbidities implying that such people are more prone to contracting COVID, based on our study we cannot decisively conclude due to lack data.

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