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ORIGINAL RESEARCH

Studying the correlation of cycle threshold value of RT-PCR and computed tomography score of CT-Scan in covid-19 infection

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ABSTRACT

Background: The COVID-19 pandemic has unfolded as one of the world's worst health crisis. Viral RT-PCR, CRP, and CT scan thorax are the most common tools used for its diagnosis, prognosis and severity assessment. Hence, a parallel between these parameters can aid in better understanding and management of COVID-19 infection.

Methodology: Demographic data, history, cycle threshold values of RT-PCR from nasopharyngeal and oropharyngeal swabs, CRP and computed tomography score were obtained from 108 adult participants. Statistical analysis was performed using python programming (python 3.7) and inbuilt libraries.

Results: Mean age of the study group was 51.05 years. 63.89% were males. The mean CT score was 15.417 indicating severe disease. Men had a higher CRP. Cycle threshold value of N gene was directly proportional to CT score. Lower cycle threshold values were associated with higher CRP. Of the 37 deaths, 62.16% were males. Cycle threshold in non-survivors was significantly higher than survivors indicating lower nasopharyngeal viral load in non-survivors. Diabetes was the most common comorbidity associated with mortality.

Conclusion: Nasopharyngeal load can be low even with severe radiological CT findings probably due to migration of the virus to lower respiratory tract in later stages of the disease. Low nasopharyngeal viral loads cannot negate the possibility of a severe pulmonary infection. CRP values may not always correlate with CT findings in recovering stages of disease. Comorbidities adversely affect the disease outcome. These parameters should be used in conjunction to assess and veer the progression, management and outcome of patients with COVID-19 infection.

Keywords: CT score, CRP, CT value, corona virus, RT-PCR.

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INTRODUCTION

COVID-19, a worldwide emergency, surfaced when its first known case was identified in the city of Wuhan, China in December 2019. The first case in India was detected on 27th January 2020 in Kerala.[1] Since then, COVID 19 has spread as a global pandemic resulting in major health crisis throughout the world. This disease, caused by severe acute respiratory syndrome

coronavirus 2 (SARS-Cov-2) has claimed over more than 6 million lives worldwide, even as the reporting of deaths remains deficient.[2] COVID-19 shows a variable severity ranging from asymptomatic cases to fulminant respiratory failure. It is an airborne disease that spreads via droplet transmission. The corona viruses bind to the cell- surface protein ACE-2 (angiotensin converting protein-2). This protein is expressed on the ciliated epithelium of the nasopharynx, upper respiratory tract, the epithelium of bronchus, type II pneumocytes and other immune cells in the lungs. Since ACE-2 expression is higher in the upper respiratory tract, during the initial phases of the infection, the epithelium of the nasopharynx and upper airways is predominantly involved. Control of viral spread by the person's defence mechanisms determines the severity of the disease in that person.[3]

According to WHO, the method for diagnosis of COVID-19 is collection of upper respiratory sample via nasopharyngeal and oropharyngeal swabs followed by the detection of the SARS CoV-2 RNA by RT-PCR (real-time reverse transcriptase polymerase chain reaction). This test includes a florescent signal which increases with an increase in the amount of nucleic acid present in the sample.[4] Cycle Threshold (CT) value is defined as the number of cycles required for this fluorescent signal to cross the threshold. Cycle Threshold levels are inversely proportional to the amount of target nucleic acid in the sample. RT-PCR can be performed for the detection of various genes of the corona virus like the E(envelope) gene, N(nucleocapsid) gene, ORF1ab gene, RdRp (RNA- dependent RNA polymerase) gene and Spike(S) gene.[5] Since many factors are involved in the testing process like method of sample collection, type of specimen, sampling time, transport and storage conditions, extraction of nucleic acid, viral load, designing of primer, efficiency of RT-PCR and method of determination of cycle threshold value, the relation between cycle threshold value and viral load remains controversial.[6]

High resolution Computed Tomography (CT scan) of chest is one of the imaging tools for diagnosing pneumonia. Since chest radiograph (x-ray) is generally not very sensitive for the detection of pulmonary abnormalities in the early stages of the disease, CT scan proves to be more effective for detection of the same.[7] CT- scans demonstrate typical radiological features of COVID-19 infection like ground glass opacities, linear opacities, crazy-paving pattern, consolidation, air bronchogram, interstitial changes with septal thickening and reticulation showing a peripheral distribution. The CT severity score assesses lung involvement by COVID-19. Each of the five lobes of the lung are visualised and assigned a score from 1 to 5. The cumulative score is then calculated and reported as a score out of 25. Higher the score, more lung volume involved and severe is the disease.[8]

CRP is an acute phase reactant produced by the liver and an early marker of inflammation. The normal CRP values in blood are less than 10 mg/dL. CRP is seen to increase in COVID infections reaching a peak within 48 hours of onset of disease.[9] When CRP binds to phosphocholine that is expressed on the damaged cells, it leads to activation of the classical complement pathway resulting in clearance of the both the virus as the damaged cells. CRP levels fall with the resolution of inflammation.[10]

Studies on correlation of Cycle threshold value of RTPCR with Chest computed tomography score on CT scan are limited. Also, studies on significance of cycle threshold value and severity of infection in the patients are also limited. This study aims at bridging the knowledge gap and attempts to seek a parallel between CT value of RT-PCR done for diagnosis of COVID -19 and its influence over the chest computed tomography score. It also attempts to determine the importance of using both these tests in conjunction for appropriate diagnosis and severity assessment.

METHODS

The study included patients above 18 years of age admitted in the COVID isolation wards of a district hospital in North Karnataka over a duration of 1 month. Children and pregnant women were excluded from the study.

Clinical diagnosis of COVID-19 was made based on WHO case definitions which included acute onset of fever and cough or acute onset of any three or more of the following- fever, cough, general weakness, headache, fatigue, sore throat, myalgia, dyspnoea, coryza, anorexia, nausea, vomiting, diarrhoea and altered mental status.

Data including the demographic details and comorbidities of the patients were obtained. Nasopharyngeal and oropharyngeal samples were collected and viral RNA was extracted from the samples with the help of viral nucleic acid buffers. RT-PCR test was performed targeting the E-gene and N-gene of the virus. Cycle threshold values for the corresponding genes were noted. Blood CRP values were also assessed.

Chest CT- scans were taken and the computed tomography score was calculated considering the area of lung involved. In this score, both lungs were divided into five lobes and each lobe was assessed individually. A score of 0 to 5 was awarded to each lobe depending upon the area of involvement.

Table 1 depicts the CT score based on lung involvement.

The total score was obtained by summation of individual scores. The scoring ranged between 0 to 25. Disease is mild if CT score of < 7, moderate if score 8-15 and severe if score >15.

The investigated values were then tested statistically for correlations.

Statistical analysis

Data for this study was structured manually using Microsoft Excel. Statistical analysis such as mean, median, mode and percentage were calculated for each of the descriptive parameters. To study the correlation between the descriptive parameters, tests such as standard deviation, interquartile deviation, Pearson correlation, Spearman rank order correlation and chi-square test were conducted. P value of <0.05 was considered significant. Additionally, Mann- Whitney U test was performed to analyse the correlation between continuous valued functions. Regression plots and GG plots were employed to express the data. The statistical analyses were performed using python programming (Python 3.7) and inbuilt libraries such as Numpy, Scipy. The plots were generated using the libraries Matplotlib, Seaborn.

RESULTS

Of the 108 patients observed, 69 (63.89%) were males and 39 (36.11%) were females. The mean age was 51.05 years with a standard deviation of 13.72. All cases were reported positive for COVID-19 RTPCR. Factors such as age, CRP value, CT- score, Cycle threshold values for E gene and N gene were analysed statistically for mean, median, mode, interquartile range (indicating the spread of data) and standard deviation. (Table 2) The mean of CT score obtained was 15.417 signifying a prevalence of more severe disease in the selected data. Figure 1 shows GG plots to show correlation between sex and various variables.

Table 2 showing descriptive statistics of various parameters.

Further, age, CRP value, CT score, cycle threshold values for E gene and N gene were studied for their variation and correlation with respect to sex. (Table 2). It was observed that CRP value was significantly more in men as compared to females. Variation with respect to age, CT score, cycle threshold values was insignificant between the two sexes.

Table 3 showing statistical analysis and correlation of various parameters with respect to sex.

The correlation between various parameters is depicted in Table 3. With increasing age, both CRP and CT score significantly decreased. CRP and cycle threshold value for E gene saw an inverse relation with respect to CT score. However, cycle threshold value of N gene increased with increasing CT score. Cycle threshold values of both the genes showed an inverse association with CRP. Cycle threshold values increased with increasing age. Covariance values depicted the extent of correlation. Parameters showed significant correlations with Chi square test and Mann- Whitney U tests. However, the factors were not found to be statistically significant when studied under Spearman's rank order correlation and Pearson correlation.

Table 4 showing correlation between various parameters.

Table 1: depicts the CT score based on lung involvement.

Score	Percentage of involvement in each lobe
0	0
1	Less than 5%
2	5% to 25%
3	26% to 49%
4	50% to 75%
5	Greater than 75%

Table 2: showing descriptive statistics of various parameters.

SR. No	Parameter	Mean	Median	Mode	Interquartile range	Standard deviation
1	Age	51.046	51.0	38	20	13.724
2	CRP value	65.088	70.0	45	48.5	33.547
3	CT-score	15.417	16	18	6.5	4.487
4	Cycle threshold for E gene	24.879	24.355	27.64	7.435	4.668
5	Cycle threshold for N gene	25.068	24.55	18.001	7.061	4.615

Table 3: showing statistical analysis and correlation of various parameters with respect to sex.

SR. No	Parameter 1	Sex	Mean	Median	Mode	Interquartile range	Standard deviation	P value
1	Age	Male	49.812	50.0	38.0	18.0	14.580	0.996
		Female	53.231	53.0	60.0	12.5	11.748	
2	CRP value	Male	69.054	70.0	45.0	36.0	29.951	$7.335*10^{-12}$
		Female	58.072	68.0	82.0	66.5	38.109	
3	CT score	Male	15.754	17.0	18.0	6.0	4.425	0.967
		Female	14.821	16.0	10.0	7.5	4.534	
4	CT for E gene	Male	24.990	24.43	27.64	7.654	4.658	0.996
		Female	24.683	24.25	15.145	5.937	4.680	
5	CT for N gene	Male	25.133	24.43	18.001	7.454	4.603	0.997
		Female	24.954	24.67	15.252	6.32	4.633	

Table 4: showing correlation between various parameters.

SR. No	Parameter 1	Parameter 2	Co-variance	Chi square test		Mann-Whitney U test	
				Correlation	P value	Correlation	P value
1	Age	CRP	-47.791	1390.360	<0.001	3827.0	<0.001
2	Age	CT score	-1.496	242.033	<0.001	11503.5	<0.001
3	CT score	CRP	-17.091	848.358	<0.001	1307.5	<0.001
4	CT score	Cycle threshold E gene	-0.272	133.579	0.042	701.0	<0.001
5	CT score	Cycle threshold N gene	0.016	131.623	0.053	650.0	<0.001
6	Cycle threshold E gene	CRP	-3.759	891.406	<0.001	2044.0	<0.001
7	Cycle threshold N gene	CRP	-3.980	892.54	<0.001	2062.5	<0.001
8	Age	Cycle threshold N gene	6.555	197.997	<0.001	11358.0	<0.001

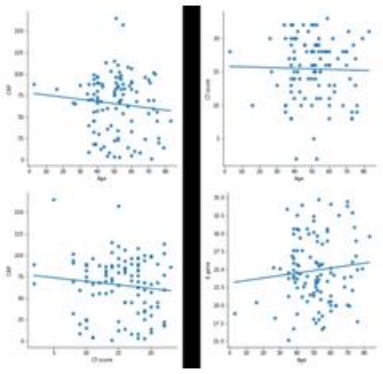

Figure 1: shows GG plots to represent correlation between various variables and sex.

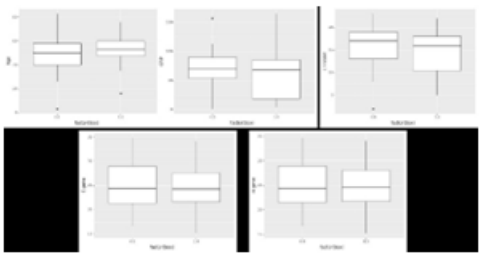

Figure 2: shows regression plots of correlations between age, CT score, and CRP.

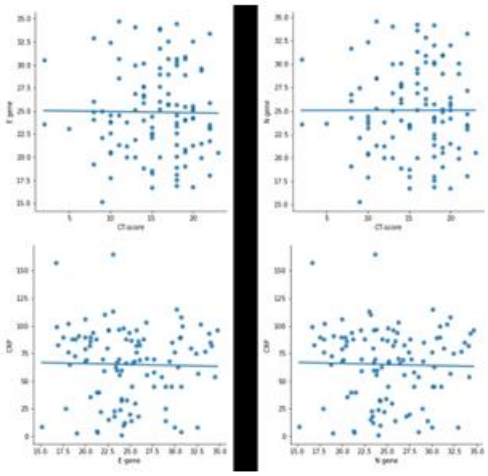

Figure 3: shows regression plots representing correlations between cycle threshold values for N gene and E gene, CRP, and CT score.

71(65.74%) patients were cured and discharged. A total of 37 (34.26%) deaths were reported of which 14(37.84%) were females and 23(62.16%) males. The mean CRP of non-survivors (73.81) was significantly higher than that of the survivors (60.54). The mean cycle threshold for N gene in survivors was 24.81 while the non-survivors had a mean cycle threshold of 25.20. Radiological CT score in survivors (14.43) was significantly lower than patients who died (17.3).

20(18.52%) patients had comorbidities, diabetes and hypertension being the most common ones accounting for 16 and 13 cases respectively. Other comorbidities included history of cerebro-vascular accident (1 case) and asthma (1 case). Of the patients with comorbidities, 7(35%) died. Diabetes alone, or with hypertension was the most common co-morbidity noticed accounting for 6 out of the 7 deaths (85.71%). 1 death was attributed to a co-existing asthma in the patient. Deaths due to comorbidity was slightly higher in females (57.14%) as compared to males (42.86%).

DISCUSSION

The COVID-19 pandemic appeared as a global health crisis which was unprepared for. With an increasing incidence of this disease, studying its demographic factors, pathophysiology, indicators of severity, effect of comorbidities on the disease incidence and progression, etc. become vital to prevent the occurrence, control the spread and find treatment options for COVID-19. In our study, we attempted to compare and draw correlations between the same.

Our data group saw a higher incidence of COVID in males as compared to females. This finding is consistent with various studies including an epidemiological data from 38 countries which showed a male preponderance in COVID-19 infections. The possible reason suggested was the location of the ACE-2 receptor on the X chromosome. This receptor is seen to get downregulated with increasing estrogen levels. Other factors like sex hormones, epigenetic modifications and genetic polymorphisms are also implicated in the varied immune response between the two sexes, females being less susceptible to various viral infections.[11] A study by Emily S. Lau et al. on the sex differences in inflammatory markers in patients hospitalised with COVID-19 infections showed a more aggressive inflammatory activation in males with greater peaks of inflammatory markers and poorer prognosis.[12] In our study the higher CRP levels seen in men were similar to the previous findings. However, there was no significant difference in the CT score and hence the disease severity between the two sexes. The cycle threshold values for viral genes also showed no significant association with sex. Deaths were more common in males (62.16%).

An increase in severity of disease is reported with increasing age.[13] The mean age of 51.05 years in our study implicated a middle-age preponderance. A study by Shimaa Farghaly and Marwa Makboul studied the correlation between age, sex and severity of COVID-19 disease based on CT severity scoring system. They observed the highest CT severity score in patients above 70 years of age indicating a more severe disease in the elderly.[14] The aging immune system with dysfunctional recognition and destruction of viral particles, immunosenescence and inflammaging (chronic increase in systemic inflammation arising from an overactive and ineffective alert system to viral particles) are implicated in increased mortality due to COVID-19 infection in the elderly.[15] However, in our study, CRP values, CT scores, and viral loads were seen to be on a lower side in the elderly. This could be a Berksonian bias (because of different probability of admission to a hospital).

A study by B Rajyalakshmi et al. on the correlation between the cycle threshold value of reverse transcriptase polymerase chain reaction (RT-PCR) test in confirmed COVID-19 patients and the severity of disease showed an inverse relation of cycle threshold value to disease prognostic factors like ICU admission, high mortality, shock and increased length of ICU stay.[16] Although controversial, studies have shown that cycle threshold values for viral genes can be a raw estimate of viral loads.[17,18]

The relation between disease severity assessed by various clinical and biochemical markers like CRP, and cycle threshold values have shown variable responses. While some studies showed a positive relation between these parameters, some found no correlation.[19,20] In our study, considering that the lower cycle threshold values represent a higher viral load, we drew a correlation between viral loads and CRP. We found patients with higher viral loads (low cycle threshold values) to have increased CRP. Since, CRP is an acute phase reactant and a marker of systemic inflammation; higher viral loads tend to produce more bodily damages and hence higher CRP. Sharply increasing CRP values can also indicate an accentuation of inflammation predisposing the patient to a cytokine storm.[21] We observed a higher mortality in patients with higher CRP and higher CT scores.

We tried to correlate CRP, an early marker of inflammation with CT score on radiology. We observed lower CRP values in patients with higher CT scores. This can partially be explained by the fact that in patients recovering from COVID-19 infection, even with decreasing inflammation, CT scores can show greatest severity around 10 days after onset of symptoms followed by a gradual resolution.[22]

K Y Aysegul et al. studied the relationship of the cycle threshold values of COVID-19 RT-PCR and Total Severity Score (TSS) of CT scan. This study demonstrated an inverse relation between nasopharyngeal viral load (attributed to cycle threshold value) and TSS in both inpatient and outpatient groups. The study speculated that in the early stages of the diseases, the nasopharyngeal viral load is higher and not related to changes in the chest CT. However, in the later stages of the disease nasopharyngeal viral load decreases as the virus migrates to the lower respiratory tract and CT scan changes become evident in the lungs. They also suggested the use of sputum sample or other LRT specimens in severe COVID-19 disease.[23]

Our study drew a correlation between the cycle threshold values of E gene and N gene of the corona virus with CT score on lung HR-CT. Cycle threshold for E gene showed a negative association thereby signifying a higher CT score for increasing viral loads. However, an opposite association was observed with the N gene. Since the sensitivity and specificity of N gene testing is higher compared to the E gene, we chose to consider the findings of N gene.[24] The finding of an inverse relation between the viral load (cycle threshold of N gene) with CT score was consistent with the previous study. Higher viral load obtained from nasopharyngeal samples (lower cycle threshold values) was associated with lower mortality.

A study by Yu F at al. on quantitative detection and viral load analysis demonstrated a higher

viral load in sputum samples compared to the nasopharyngeal swabs, thus postulating that lower respiratory tract samples can be a better reflection of disease severity.[25]

A meta-analysis of comorbidities in COVID-19 infection by Subodh S. P. identified hypertension, cardiovascular and cerebrovascular conditions, and diabetes as the most common comorbidities.[26] A cohort study of 7337 patients with COVID-19 infection observed a higher need for interventions and hospital stay in patients with type 2 diabetes in contrast to a better outcome in non-diabetics.[27] In our study, diabetes was found to be the most common comorbidity overall and also the most common comorbidity associated with deaths. The cause of increased morbidity and mortality in diabetics is attributed to a chronic, low level systemic inflammation resulting in multiple dysfunctions at cellular levels thereby exaggerating the response to COVID-19 infection, and changes in expression of ACE2.[28] Precipitation of asthmatic attacks, pneumonias and acute respiratory distress can be factors of concern in patients with moderate to severe asthma. Asthma was seen only in 1 patient, who unfortunately succumbed to a precipitated attack. Thus, comorbidities were seen to adversely affect the disease prognosis in COVID-19 infection.

Conclusion

In our setting, COVID-19 infection had a male and middle-aged preponderance with a higher mortality in the male gender. CRP values can be an early prognostic marker for mortality. CRP levels may not always correlate with radiological CT findings in the later recovering stages of the disease and this aspect needs further research. Nasopharyngeal viral load as roughly indicated by cycle threshold values of viral genes can be low even with severe radiological CT findings, probably owing to the migration of the virus from the nasopharynx to the lungs with disease progression. Higher nasopharyngeal viral loads associated with lower mortality can further substantiate the aforementioned hypothesis. Hence, low nasopharyngeal viral loads cannot negate the possibility of a severe pulmonary infection. Comorbidities, especially diabetes, hypertension, and CVA can adversely affect the disease outcome. Thus, these parameters should be used in conjunction to assess and veer the progression, management and outcome of patients with COVID-19 infection.

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Individual contribution of authors- Dr. Prashantakumar contributed for data collection and statistics. Dr. Neha Patil- statistics and writing the manuscript. Dr. Giridhar P.- study idea, manuscript writing.

The manuscript is read and approved by all the authors, requirements for authorships has been met, authors believe that the manuscript represents honest work.

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