Identifying The Association Between Inflammatory Markers And Cycle Threshold Of RT-PCR In Paediatric COVID-19 Infection

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DOI: 10.47750/pnr.2022.13.S05. 241

Abstract

Background: Pneumonia caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) poses a significant threat to healthcare and is classified as a global pandemic. This study aims to find the association between the inflammatory marker CRP, LDH, Ferritin, D-dimer, and the cycle threshold of SARS CoV2 in the pediatric population

Methods: Patients admitted with COVID-19 infection (SARS CoV2 Real-Time PCR positive) from November 2020 to October 2021, were included in this study. The cycle threshold (Ct) of SARS CoV2 Real time-PCR test, serum lactate dehydrogenase (LDH), C reactive protein (CRP), serum Ferritin, D-dimer, white cell counts including absolute neutrophil and absolute lymphocyte count were analysed in these patients. Patients were divided into two groups based on the Ct (cycle threshold) value of SARS CoV2 RT-PCR for statistical analysis (Ct<=26 and Ct>26). Statistical analysis using the area under the curve with a Z score (95% confidence interval) was used to detect the significance between the serum parameters and the cycle threshold of RT-PCR in COVID-19 infection. Patients were grouped into asymptomatic, mild, moderate, and severe COVID-19 based on their clinical presentation. Unpaired t-test was used to identify a statistically significant correlation between clinical presentation and the serological parameters

Results: Out of 1125 suspected COVID-19 patients, 47 patients (4% positivity) were identified as COVID-19 positive using RT-PCR. The mean age group of males was 8.74±5.11 years and females was 9.45±5.87 years. 2% of patients had severe and 13% of patients had moderate COVID-19 clinical presentation. The area under the curve analysis using a Z score with 95% CI identified a statistically significant correlation between the cycle threshold of SARS CoV2 RT -PCR and absolute lymphocyte count (p-value 0.04). No statistically significant correlation was identified between D-dimer, serum LDH, serum ferritin, total white cell count, and the cycle threshold of SARS CoV2 RT-PCR. Unpaired t-test showed a statistically significant correlation between clinical presentation and LDH, CRP, and D-dimer.

Conclusion: The cycle threshold of RT-PCR does not show an association with serological parameters such as CRP, LDH, D-dimer, and serum ferritin. Absolute lymphocyte count can be used as an indicator to identify patients with low Ct values in SARS CoV2 RT-PCR.

Keywords: SARS CoV2, paediatric COVID-19, D-dimer, ferritin, LDH, absolute lymphocyte count, C-reactive protein, RT-PCR

INTRODUCTION:

The coronavirus disease (COVID-19) caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV2) has resulted in a worldwide catastrophe affecting millions of people across the globe[(1)]. According to World Health Organisation (WHO) there are 628,694,934 confirmed cases of COVID-19 including 6,576,088 deaths globally as of November 4, 2022[(2)]. The Ministry of Health and Family Welfare (MOHFW) report 14,515 active cases and 4, 41,16,492 discharged cases of COVID-19 in India as of November 7, 2022[(3)]. The symptoms of COVID-19 such as malaise, cough, congested nose, and fever present from 2-14 days after exposure to the virus[(4)]. Serological markers such as interleukin-6(IL-6), C reactive protein (CRP), D-dimer, serum lactate dehydrogenase, serum ferritin, and absolute neutrophil count can help differentiate individuals with severe COVID-19 from mild COVID-19. Previous studies mention that patients requiring ICU admission have lymphopenia and raised LDH[(5)]. SARS CoV2 produces a huge cytokine storm, leading to elevated C-reactive protein (CRP), D-dimer, and dysregulated functioning of the lymphocytes. Interestingly, several studies have analysed the association between the severity of illness in COVID-19 and viral load with differing conclusions. Liu et al stated that viral load of SARS CoV2 was positively associated with disease severity and that CRP, LDH, lymphocyte and neutrophil count could predict injury to the lung[(6)]. Contrary to the above findings, Abdulrahman et al found that there exists no statistical association between Ct values and the severity of COVID-19 on admission[(7)].

This study evaluated and analyzed the correlation between Ct values and serological parameters such as CRP, D-Dimer, serum LDH, serum ferritin, and white blood cell count and its ability to identify the severity of illness in COVID-19.

MATERIALS AND METHODS:

A cross-sectional study, prospective, single center study was conducted at SRM Medical College Hospital and Research Centre between November 2020 to October 2021. COVID-19 was diagnosed according to the WHO interim guidelines [(8)]. A confirmed case of COVID-19 was a patient who was positive to SARS CoV2 using Real time RT- PCR (Saragene COVID-19 RT-PCR, COSARA Diagnostics) with Ct value less than 35. The study included only laboratory-confirmed cases of COVID-19. Patients were grouped into asymptomatic, mild, moderate, severe and critical COVID-19 based on their clinical presentation according to the WHO interim guidelines [(8)].

Table 1: The Grading Of Symptoms Of Covid 19

S. no	Severity of disease	Clinical presentation
1	Asymptomatic	➢ No symptoms ➢ Nasopharyngeal and Oropharyngeal swab – positive for SARS CoV2 RT-PCR ➢ Chest X-ray- normal
2	Mild	➢ Fever, Sore throat, dry cough, body ache ➢ Vomiting, nausea, stomach pain, diarrhea ➢ No dyspnea or abnormal chest imaging
3	Moderate	➢ Lower respiratory tract infection (diagnosed by clinical examination and chest imaging) ➢ SpO2 ≥94% on room air ➢ High-resolution CT chest- lesions
4	Severe	➢ SpO2 <94% on room air ➢ Ratio of arterial partial pressure of oxygen to fraction of inspired oxygen (PaO2/FiO2) <300mmHg ➢ Respiratory rate: >30 /min ➢ Lung infiltrates: >50%
5	Critical Illness	➢ Respiratory failure ➢ Septic Shock ➢ Multiple organ dysfunction

The serological parameters such as serum lactate dehydrogenase (LDH), C-reactive protein. serum ferritin, D-dimer, white cell counts including absolute neutrophil and absolute lymphocyte count by flowcytometry were analyzed in these patients.

The Ct value of SARS CoV2 real time RT-PCR was used to divide the study patients into two groups (Ct<=26 and Ct>26) for statistical analysis. The statistical significance of the association between the various serological parameters and Ct value of SARS CoV2 real time RT-PCR was performed using area under the curve analysis with Z score (95% confidence interval). Unpaired t-test was used to identify a statistically significant correlation between the various clinical presentation and the serological parameters.

RESULTS:

Among the 1125 COVID-19 suspected patients, only 47(4%) were confirmed to have SARS CoV2 infection using RT-PCR. Among the confirmed COVID-19 patients, 27(57%) were female and 20(43%) were male. The mean age group of males was 8.74±5.11 years and females was 9.45±5.87 years. The clinical presentation of the COVID-19 confirmed paediatric patients is mentioned in Table 1. Among the study patients, 25/47(53%), 15/47(32%), 6/47(13%), 1/47(2%) had asymptomatic, mild, moderate, and severe COVID-19 presentation respectively.

Table 2: Clinical Presentation Of COVID-19 Confirmed Paediatric Patients.

Characteristics	Male (n=27)	Female (n=20)
Age (mean- years)	8.74±5.11	9.45±5.87
Signs and symptoms		
Asymptomatic	13±4.276 (48.15%)	13±4.454 (65%)
Fever	12±4.276 (44.44%)	8±4.454 (40.01%)
Cough	6±4.276 (22%)	4±4.454 (20%)
Vomit	2±4.276 (7.40%)	0
Diarrhoea	5±4.276 (18.5%)	2±4.454 (10%)
Muscle ache	2±4.276 (7.40%)	1±4.454 (5%)
Headache	5±4.276 (18.5%)	2±4.454 (10%)
Anosmia	3±4.276 (11%)	1±4.454 (5%)

Cycle threshold (Ct) of SARS CoV2- RT PCR was more than 26 in 35/47 patients (74%) and less than 26 in 12/47 patients(26%). C-reactive protein (CRP) was positive in 19/47(40%) patients. D-dimer was positive in 17/47 patients (36%). Serum ferritin was normal in all COVID confirmed patients. Serum LDH was positive in 10/47 patients (21%).

Unpaired t-test showed statistically significant correlation between clinical presentation of the COVID-19 confirmed paediatric patients and the laboratory parameters such as LDH, CRP and D-dimer (Figure 1,2 and 3).

Figure 1: Association Between CRP And Clinical Presentation Of COVID-19 Positive Patients

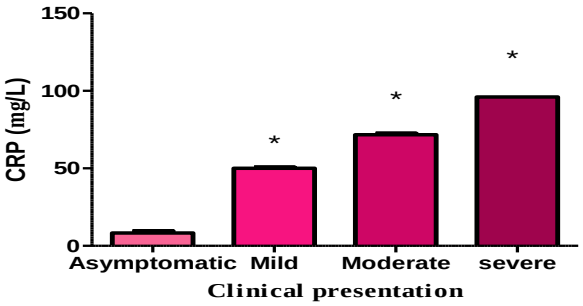


Figure 2: Association Between LDH And Clinical Presentation Of COVID-19 Positive Patients

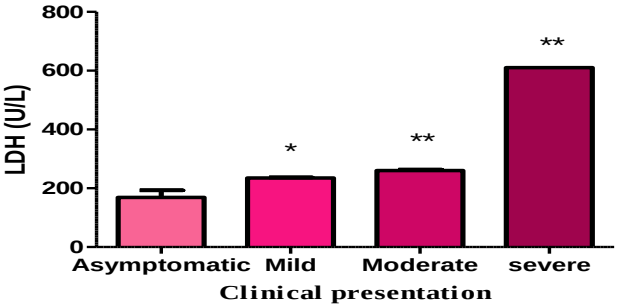
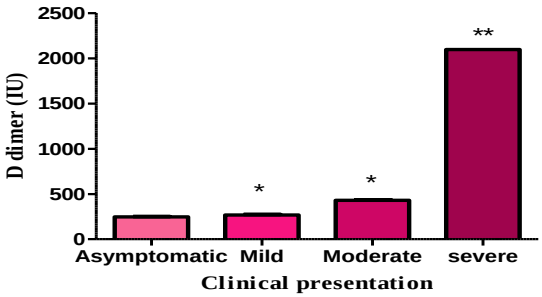


Figure 3: Association Between D-Dimer And Clinical Presentation Of COVID-19 Positive Patients.



Area under the curve analysis using a Z score with 95% CI identified a statistically significant correlation between the cycle threshold of SARS CoV2 RT -PCR and absolute lymphocyte count (p-value 0.04). No statistically significant correlation was identified between D-dimer, serum LDH, serum ferritin, total white cell count, and the cycle threshold of SARS CoV2 RT-PCR (Figure 4)

Figure 4: Diagrammatic Representation Of Area Under The Curve Analysis Of Ct Value And Laboratory Parameters In COVID-19 Positive Patients.

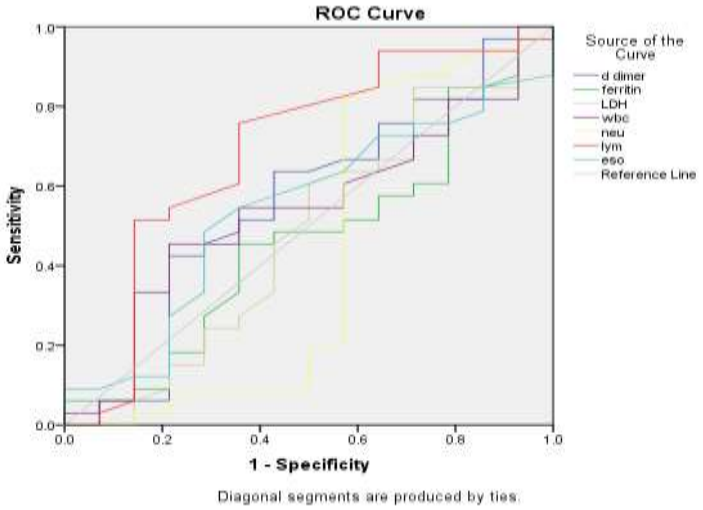


TABLE 3: The Area Under The Receiver Operating Characteristic (ROC) Curve (AUC) Of The Studied Parameters

TEST PARAMETER	AUC	p value	95% CI	
			LOWER BOUND	UPPER BOUND
D-dimer	.558	.530	.369	.748
Serum ferritin	.464	.701	.281	.647
LDH	.481	.834	.285	.676
Total white cell count	.543	.642	.366	.721
Neutrophil count	.427	.436	.203	.652
Lymphocyte count	.689	.042	.507	.871
Eosinophil count	.541	.659	.362	.720

DISCUSSION:

Coronavirus infectious disease (COVID-19) pandemic which is rampant across the world is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Previous studies indicate the direct correlation between clinical presentation of COVID-19 patients and laboratory parameters such as CRP, serum ferritin, LDH, D-dimer, total leukocyte count, procalcitonin and liver function tests while others contradict the same. Cycle threshold (Ct) value of RT-PCR can be taken as a direct indicator of viral load though it does not identify the accurate number of viral copies in a given sample. The presence of a high Ct value indicates a lower viral load and vice versa. Ct value can help clinicians make decisions about infection control within the hospital. The measurements based on RT-PCR and Ct value can depict the viral dynamics in COVID-19 infection. Ct value can provide an insight into the prognosis of the infection [1,9,10]. The present study aimed to identify the association between Ct value of RT-PCR and laboratory parameters such as CRP, D-dimer, LDH, serum ferritin and white blood cell count.

According to the MoHW India, children (<18 years of age) account for 8% of COVID-19 infections in India [11]. The statistics are much lesser worldwide, with children accounting 5% of total COVID-19 infections. The present study showed a 4% positivity of SARS CoV2 among the 1125 suspected COVID-19 patients. This was similar to the study conducted in England and Brazil by Ladhani S et al 2020 and V. Cavalcante Pinto Júnior et al respectively [12,13]. In the present study, majority of the patients were female (57%) and the mean age of the study patients was 8-9 years. The distribution of clinical presentation amongst COVID-19 patients in the present study were as follows, 25/47(53%) asymptomatic, 15/47(32%) mild, 6/47(13%) moderate and 1/47(2%) severe COVID-19. Sahi et al, states the mean age of paediatric COVID-19 patients as 3.3-11 years with a male predominance. The study found majority of paediatric COVID-19 patients were asymptomatic with mild respiratory or gastrointestinal symptoms[11]. Unlike the present study, Singh et al found a slight male predominance of paediatric COVID-19 patients and two-thirds of their patients, were asymptomatic or had mild symptoms (56%) similar to the present study and required only outpatient treatment [14].

Previous studies indicate a correlation between inflammatory markers and D-dimer [6,15,16,17]. The present study's findings identified that CRP, LDH, and D-dimer, have a positive association with the disease severity of COVID-19 and can be used as predictors of paediatric COVID-19 infection. CRP is an inflammatory marker which rises during COVID-19 infection. LDH is a marker for poor prognosis in COVID-19 infection. LDH is released in the event of lung infection and is responsible for tissue destruction. D-dimer is produced as a by-product of fibrinolytic activity. It is found to have a positive correlation with inflammatory cytokines and is elevated in critically ill COVID-19 patients. Hyperferritinemia is seen in patients with immune dysregulation and is often associated with COVID-19 complications, especially in diabetic patients [18].

Mardani et al identified that CRP, LDH and neutrophil count were higher in COVID-19 RT-PCR positive (AUC >0.8) patients [5]. Yang et al found that 94% patients in their study had a positive association between CRP values and disease severity [19]. Atique et al, determined that CRP was consistently high in patients with high viral load of SARS CoV2. The study demonstrated a downward trend of CRP from high viral load to low viral load patients. It found high LDH and D-dimer values in all patients with COVID-19 infection. LDH was consistently high in patients irrespective of their viral load and D-dimer showed a downward trend from higher viral load to lower viral load in this study [18]. Patients with low Ct value had high LDH in the study conducted by Huang et al [20]. Azzi et al report no statistical significant association between CRP, LDH and Ct value [21]. There was no association between CRP, LDH, D-dimer and Ct value in the present study.

Chen et al and Atique et al[18,22], report elevated ferritin levels in patients with SARS CoV2 infection unlike the present study. This may be due to the asymptomatic clinical presentation of most patients in the present study. Huang et al report that patients with low Ct value (high viral load) had higher neutrophil count and low lymphocyte count similar to the present study [20]. La Scola et al reveal high LDH and low lymphocyte count in patients with high viral load of SARS CoV2[23]. The study also found high total white cell count and high neutrophil count in patients with low Ct value. Fajnzylber et al found high viral load was positively associated with lower lymphocyte count, critical respiratory disease and increased inflammatory markers among COVID-19 patients [24]. The limitation of the present study is that majority (74%) of the paediatric patients with COVID-19 infection had low viral load (Ct>26). This may be the reason for no statistically significant association between laboratory parameters and viral load (Ct value) of COVID-19 patients.

CONCLUSION:

RT-PCR technique is extremely useful for the diagnosis of COVID-19. Cycle threshold (Ct) value of RT-PCR which is considered as an indicator of viral load may be used as predictor of disease severity. The absolute lymphocyte count is

diretly related to Ct value in SARS CoV2 infection. Inflammatory markers such as CRP, LDH and D-dimer are inversely related to Ct value and can be used to identify COVID-19 disease severity. It can be implied from the study that Ct value is extremely useful for clinicians to make apt decisions on COVID-19 management, and for administrators on occupational and public health measures.

ACKNOWLEDGEMENTS: Nil

FUNDING SUPPORT: Nil

CONFLICTS OF INTEREST:

The authors declare no conflicts of interest related to this study

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