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Original Article

Correlation of Severe Acute Respiratory Syndrome Coronavirus 2 viral load with clinical characteristics, mortality and laboratory findings among hospitalized Patients with Coronavirus Disease

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Abstract

Background: Patients hospitalized with coronavirus disease 2019 (COVID-19) have high mortality rates and also showed many laboratory abnormalities but the relationship between clinical presentation, mortality and laboratory features with viral load is still under research. **Materials and Methods:** We conducted a retrospective study of patients hospitalized with COVID-19 from 5th August 2020 to 15th December 2020 at TMSS Medical College and Rafatullah Community hospital in Bogura. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) viral load was assessed using cycle threshold (Ct) values from a reverse transcription-polymerase chain reaction assay applied to nasopharyngeal swab samples. We compared clinical presentation, outcomes and laboratory features of patients with high, medium, and low admission viral loads and assessed whether viral load was associated with mortality and other laboratory abnormalities. **Results:** We evaluated 253 patients with COVID-19 confirmed by RT-PCR. In-hospital mortality was 36%, 17% and 7% with high, medium, and low viral loads, respectively ($P < .05$). Viral load was associated with increased neutrophil count, decreased lymphocyte count and elevated CRP. **Conclusions:** Admission SARS-CoV-2 viral load among hospitalized patients with COVID-19 correlates with in-hospital mortality. Viral load was also associated with some laboratory abnormalities. Providing this information to clinicians could potentially be used to guide patient care.

Keywords: SARS-CoV-2; coronavirus disease 2019; viral load; hospitalized patients; outcomes.

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARSCoV-2) is a novel pathogen that has rapidly caused a devastating pandemic of coronavirus disease 2019 (COVID-19). As of 19 January 2021, SARS-CoV-2 had infected more than 138 million people and killed more than 2.92 million people throughout the world [1]. In Bangladesh, from the first case identified in early march 2020, a total of 697,985 individuals tested positive for covid-19, and 9,891 died with case fatality 1.42% up to 12 April 2021[2]. Although the majority of patients who develop COVID-19 have mild presentations[3], up to 20% of hospitalized patients die[4–6]. Investigations of risk factors and mortality with COVID-19 in hospitalized patients have largely focused on patient characteristics, such as older age, obesity, and comorbidities, as well as presenting symptoms and laboratory parameters[7-9]. In contrast, the impact of SARS-CoV-2 viral load on clinical outcomes in hospitalized patients has rarely been thoroughly investigated. Association of viral load with patient's mortality, clinical presentation, hematological, biochemical and infection related parameters is an area of research.

The current standard-of-care test to diagnose COVID-19 is to collect a nasopharyngeal (NP) swab

and use a reverse transcription-polymerase chain reaction (RT-PCR) assay to detect SARS-CoV-2 RNA[10]. These RT-PCR assays provide clinicians only with information on whether SARS-CoV-2 is detected or not detected. However, these assays also contain quantitative information on cycle threshold (Ct) values that are inversely correlated with viral load and are not reported clinically. We hypothesized that assessing SARS-CoV-2 viral load by analyzing Ct values from an initial NP swab sample could be a clinically valuable tool for predicting in-hospital mortality and associated with laboratory abnormalities. We therefore conducted this retrospective analysis of SARS-CoV-2 viral loads on admission to find out any association with patient's clinical presentations, outcomes and laboratory abnormalities at TMSS Medical College and Rafatullah community hospital using a RT-PCR assay.

Materials and Methods

Study Population and Setting

This retrospective, observational study consisted of patients who were hospitalized at TMSS Medical College and Rafatullah community hospital and had an NP swab sample collected and analyzed for SARS-CoV-2 using the XABT RT-PCR Kit (China) between 5th August 2020 to 15th December 2020. The predominant NP swab collection was done from suspected COVID-19 patients and analyzed within 1 day of hospital admission. Patient who were COVID-19 RT-PCR positive enrolled in this retrospective study.

Viral Load Assessment

The Center for disease control, USA provided emergency use authorization approval for the XABT SARS-CoV-2 RT-PCR test, and the test was performed according to the manufacturer's instructions [11]. This assay amplifies 2 targets within the SARS-CoV-2 genome: ORF1ab and N gene for SARS-CoV-2–specific target. For routine clinical care, results are classified as detected if either the ORF1ab or N gene is detected or as not detected if neither target is detected. However, the instrument also generates a Ct value for each target that correlates inversely with quantitative viral load and is not released to clinicians. The Ct value represents the number of replication cycles required for sufficient gene amplification to produce a fluorescent signal that crosses a predefined threshold. For this study, we reviewed Ct values for both gene targets for all initial SARS-CoV-2 tests that were performed on NP swab samples that were collected from study participants for routine clinical care within 1 day of hospital admission. We separated the Ct values for the SARS-CoV-2– specific target (ORF1ab) into terciles based on the quantitative values. We then designated high viral load samples (Ct value <25) as the lowest Ct tercile, medium viral load samples (Ct value 25-30) as the middle tercile, and low viral load samples (Ct value >30) as the highest tercile. Specimens that were designated positive for SARS-CoV-2 but for which only the ORF or N gene was detected were designated low viral load samples.

Data Collection

Data were retrospectively abstracted manually from the electronic medical record using a quality-controlled protocol. Data included age and sex of the patient, presenting symptoms on arrival to the hospital, laboratory parameters, and in-hospital mortality. Clinical data after hospital discharge were not consistently available, thus only outcomes that occurred during the hospital admission were analyzed. The TMSS Institutional Review Board approved the study.

Statistical Analyses

We compared baseline characteristics which includes age and sex, clinical presentation, laboratory parameters and mortality of hospitalized patients with COVID-19 who had high, medium, and low initial viral loads. Continuous variables were represented with medians and interquartile ranges and categorical variables were represented as proportions.

A 2-sided P value of ≤ .05 was used to designate statistical significance. Using spearman method, we correlated the Ct value (Viral load) of the 2019-nCoV virus with hematological, biochemical and infection related parameters.

Results

Cycle Threshold Values and Establishment of Viral Load Categories

A total of 253 NP swab samples were available for analysis from unique hospitalized patients who met the study inclusion criteria. Ct values for the ORF1ab locus ranged from 14.3 to 38, and 10 samples were considered positive for SARS-CoV-2 based on detection of the ORF1ab gene even though N was not detected. The median Ct value was 27.9. For simplicity, we designated high viral load samples to have Ct values <25 (n=42), medium viral load samples to have Ct values between 25 and 30 (n=66), and low viral load samples to have Ct values > 30 or for which only ORF gene was detected(n=145).

Table-I: Patient characteristics, presentations and mortality stratified by viral load

Variable	High Viral Load (Ct <25) n =42	Medium Viral Load (Ct 25-30) n = 66	Low Viral Load (Ct >30) n = 145	*P-value*
Age, years	55(32 -100)	56 (29 -96)	55(20 -85)	.795
Male gender	26(62%)	46(69.7%)	113(77.9)	.306
Female gender	16(38%)	20(30.3%)	32(22.06)	.306
Symptoms				
Fever	26(62%)	47(71%)	105(71.7%)	**.045**
Cough	22(54%)	35(53%)	75(51.7%)	.54
Headache	8(19%)	7(10.6%)	15(10.3%)	.95
Dyspnoea	21(46.7%)	33(50%)	74(51%)	.12
In-hospital mortality	15(3 6%)	11(17%)	10(7%)	**.023**

The median age of patients with high, medium and low viral loads was 55, 56 and 55 respectively. Among 253 admitted patient male were 73%. Fever (74%) and cough (57%) were most common symptoms, followed by shortness of breath (54%) and headache (12%). The average Ct values of ORF1 ab gene of SARS-CoV-2 were lower in deceased patients. High and medium viral load (Ct values <30) were observed in 71% (25/35) of non-survivor patients, suggesting that viral load of SARS-CoV-2 was an important feature for deceased patients.

Table-II: Viral load with hematological characteristics

Variable	High Viral Load (Ct <25) n =42	Medium Viral Load (Ct 25-30) n = 66	Low Viral Load (Ct >30) n = 145	*P-value*
Laboratory values				
Leukocytosis: white blood cell cou nt >11 × 109 cells/Lb (n = 253)	11(26%)	19(28%)	25(17%)	.56
Lymphopenia: absolute lymphocyte count < 1 × 10^9 cells/L (n = 253)	13(30%)	20(30%)	32(22%)	**.008**
Neutrophillia(>6×10^9 cells/Lb (n = 253)	19(45%)	35(38%)	29(20%)	**.03**
Anemia: hemoglobin<11 g/dL (n = 253)	12(28%)	22(33%)	42(29 %)	.84
Thrombocytopenia: platelet count < 150 × 10^9 /L (n = 253)	3(7 %)	5(8 %)	8(6 %)	.38

Leukocytosis, lymphopenia, Neutrophilia, anemia and thrombocytopenia was found higher in rate with High and medium viral load (Ct value <30) in comparison to low viral load (Ct value>30) which was (27% vs 17%); (30% vs 22%); (50%vs 20%) ; (31% vs 29%) and (7% vs 6%). Elevated neutrophil count and reduced lymphocyte count was significantly associated with viral load (p value <0.05).

Table-III: Viral load with biochemical characteristics

Variable	High Viral Load (Ct <25), n =42	Medium Viral Load (Ct 25 – 30), n = 66	Low Viral Load (Ct >30), n = 145	P-value
Alanine aminotransferase elevation	12(28%)	25(37%)	43(29%)	.265
Elevated S. creatinine	10(23%)	1(27%)	24(16%)	.577
Inflammatory makers				
CRP	20(48%)	25(38%)	54(37%)	**.026**
Serum ferritin	18(43%)	23(66%)	51(35%)	.757
D-dimer	7(16%)	10(15%)	26(17%)	.953

Elevated ALT, serum creatinine, serum ferritin, and d-dimer was found higher in rate with high and medium viral load (Ct value <30) in comparison to low viral load (Ct value >30) which was (34%vs 29%); (25%vs 16%) ; (41%vs 37%); (37%vs 35%) and (28%vs 17%) respectively. Elevated C-reactive protein was significantly associated with high viral load (p value <0.05)

Discussion

In this study we demonstrate that patients who were admitted to the hospital with high SARS-CoV-2 viral loads, as assessed by Ct values of NP swab samples, were more likely to die during their hospitalization. Prior studies have indicated that viral load correlates with severity of COVID-19 presentation and this study of hospitalized patients adds to this knowledge base by identifying that admission viral load has important prognostic implications. Reporting SARS-CoV-2 viral load based on Ct values from admission NP swabs could therefore may benefit from intensive monitoring[12]. Identifying patients with high viral loads could also be helpful for allocating scare therapeutic interventions such as antiviral agents[13]. It is also

possible that viral load could be used along with other factors, such as age, comorbidities, and severity of symptoms and hypoxia, to decide on the need for hospital admission[14].

The current study has no statistically significant difference in viral loads between age and sex groups. Similar findings were observed in a retrospective study in Saudi arabia where viral load in COVID-19 patients did not differ by age group[15].

Abnormal complete blood count was a notable feature of COVID-19 patients with decreased lymphocyte, hemoglobin, platelet and increased neutrophil count and these features was observed. In addition, the count of lymphocyte was significantly negatively associated with viral load in our study. In agreement with this finding Hung et al reported that 308 COVID-19 positive patient cases had lower Ct median with lower lymphocyte percentage than cases with higher median Ct value suggesting damage to T lymphocyte[16].

Present study showed significant positive correlation between viral load and neutrophil count. In relation to this finding a study in china about 12 COVID-19 cases showed positive correlation between viral load and neutrophil count[17].

In agreement with the study findings a study conducted in china reported that higher rate of anemia and thrombocytopenia was found among patient with high viral load (Ct value <30) in comparison to low viral load group (Ct value >30) [16].

In biochemical analysis elevated ALT and serum creatinine was found more among high and medium viral load cases (Ct value <30) in comparison to low viral load cases (Ct value >30) which was 34% vs 29% and 26% vs 16 % respectively. In agreement with this findings isan et al reported that SARS-Cov-2 viral load is significantly associated with in hospital Acute kidney injury and can be detected in multiple organs with selective tropism for kidney, liver and heart[18].

There are many potential contributing etiologies to elevated liver enzymes in patients with SARS-Cov-2 including direct liver injury, associated inflammatory response, congestive hepatopathy, hepatic ischemia and drug induced liver injury[19].

Inflammatory markers like C-reactive protein, serum ferritin and D-dimer was found elevated more frequently among COVID-19 positive cases whose ct value was <30 in comparison to cases who had ct value >30. C-reactive protein levels was negatively correlated with ct value (p=.026). In agreement with this study liu et al reported that there was a negative correlation with Ct value among hospitalized COVID-19 positive patient and raised CRP suggesting systemic inflammation. Jing et al also reported that hospital admitted COVID-19 patient's CRP was significantly associated with high viral load (Ct value <30)[17].

Our study has limitations. We evaluated only the viral load of a single NP swab sample per patient at the time of hospital admission and thus could not assess viral load dynamics over time or the infectious burden at the time of infection onset. Another limitation is that our study was retrospective and relied on data that were documented in the electronic media record and thus could have misclassified patient characteristics or outcome. Last we focused on in-hospital mortality and did not capture deaths that occurred after discharge from the hospital.

Conclusion

In conclusion, we found that admission SARS-CoV-2 viral loads, as determined by Ct values are associated with death among hospitalized patients with COVID-19. These findings highlight the critical role of viral load in SARS-CoV-2 pathogenesis and suggest that Ct values should be reported to assist clinicians in identifying patients at high risk for adverse COVID-19-related outcomes.

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