Insight on the pattern of viral clearance in SARS CoV-2 COVID-19 virus - during 2nd Outbreak.

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ABSTRACT
BACKGROUND AND OBJECTIVE: COVID-19 posed a health care emergency putting a lot of strain on the hospitals . This study determined the duration of viral clearance in upper respiratory tract specimens.
METHODS: This retrospective analysis was done in COVID-19 hospitalized patients from a tertiary care hospital, UP. Nasopharyngeal and oropharyngeal swabs were obtained for analyzing the viral shedding pattern by RT- PCR.
RESULTS: During the study period of two months, among the 691 patients admitted to the hospital the median viral clearance time seen for 63% of patients was around 21 days. Followed by 25% in the 4th week, 3% in the 5th and 2% went on till 6th week for viral clearance. Delayed admissions to the hospital showed severe conditions with worse treatment outcomes.
CONCLUSION : Symptomatic patients need to be admitted as early as possible with prompt treatment. Viral RNA shedding throughout the period of infection needs to be understood well to be able to keep the patient from spreading the infection. Exercise and a balanced diet should be kept as a habit during the viral clearence days.
KEYWORDS: *rT-PCR, SARS CoV-2, viral clearance time.*

I.   Introduction

27 cases of pneumonia were reported on Dec 12, 2019 from (Wuhan) China.[1]The world health organization(WHO) soon named this emerging disease as the novel coronavirus disease(COVID-19) in February 2020 [2] . The SARS outbreak spread rapidly posing a worldwide health emergency causing strain on heath care facilities globally. India is among the worst hit countries by COVID-19 pandemic. Coronaviruses are enveloped positive - sense single stranded RNA viruses that cause numerous diseases that affect the respiratory, neurological, hepatic and enteric systems showing varying infections among humans and animals. Its morphology has an envelope spike protein which helps in membrane fusion and promote binding to the receptors. It consists of a lipid bilayer consisting of an Envelope(E), Membrane(M) and Spike (S) structural proteins.[14]

The genome of Coronavirus is one of the largest ranging from 27 to 32kb. The clinical signs of SARS CoV-2 include pneumonia like symptoms such as cough, myalgia and fatigue.[15]Lung damage is seen in the later stages showing ground –glass opacities observed in Chest X-ray and CT images. SARS COV-2 virus infect the epithelial lining of lung alveolar cells via the receptors by endocytosis through ACE-2 (Angiotensin converting enzyme).[17] The control measures used to prevent the spread are contact tracing, hand washing, social distancing, isolation and community awareness. The primary mode of transmission is via the respiratory droplets when the person coughs, sneezes and talks etc.

One of the concerns with individuals who are infected with COVID-19 is to be isolated till undetectable level of SARS-CoV 2 RNA.[4] High viral RNA were detected soon following onset of symptoms. [9] The data on Viral RNA shedding is important as it is often considered to minimize disease transmission. Due to late admission of the affected personal in the hospital it was difficult to determine the negative conversion of SARS-CoV 2.[1]Patients with prolonged viral RNA shedding need repeated testing. The lack of clinical virology laboratories and the shortage of reagents and supplies limited the testing capacity. So, knowledge on viral clearance time will help to eliminate unnecessary testing.[2]

Development of numerous vaccines has taken care of the community transmissions showing the most effective prevention.

This study aimed to determine the duration of viral clearance in upper respiratory tract specimens.

II. Material and methods

This retrospective analysis was conducted at a tertiary care hospital , North UP which included all symptomatic as well as asymptomatic hospitalized patients with RT-PCR positive COVID-19 infection from April 1 to May30, 2021. Following CDC guidelines, Nasopharyngeal and oropharyngeal samples were collected and subjected to SARS- CoV 2 detection. The first sample was collected upon admission of patient. Further samples were taken at an interval of every two days. Viral clearance is defined as the time taken for the first positive test to convert in two consecutive negative RT- PCR result in a period of time. Our laboratory database was used to collect the patients data and patients characteristics that included (Age, sex and wards). During hospitalization the patient who died were not included in this study.
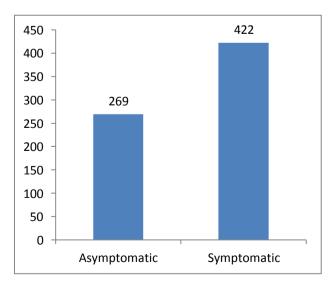
III. Method:

The nasopharyngeal and oropharyngeal flocked swabs were collected and transported to the lab using viral transport medium (3ml). They were processed immediately and stored at 2-4 degrees for no further than 24 hours. Viral RNA was extracted by taking 200µl of sample, using the nucleic acid extraction system (Himedia) according to manufacturer's instruction. Finally, 60 µl elution buffer used to elute the RNA. RNA was used for detection by real-time RT-PCR assay targeting the E, N and Rdrp gene of the virus. Ct (Cycle threshold) values and the amplification curves of FAM,HEX and ROX gene were used for qualitative detection of SARS CoV-2. It was evaluated by a S-shaped amplification curve on all the channels(FAM,HEX and ROX) and CT value below 40 resulting as a positive test, while no detection in FAM, ROX channels or Ct values of more than equal to 45 and CT <40 in ROX channel as negative test. Re-testing was needed in samples showing no flouresence in all channels. It was evaluated using CFX96 Real-Time PCR detection systems-Biorad.
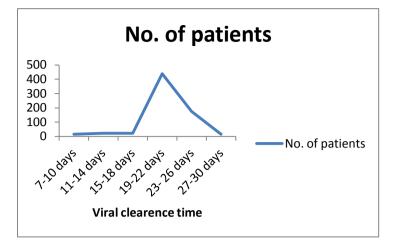
Statistical analysis:

Firstly descriptive data analysis was done , median was used for continuous and percentage used for categorical variables.

IV. Results:

Out of 702 patients that were admitted to the Covid ward of the hospital during the study period of two months while the second outbreak in India. 2 patients lost to follow up. 9 patients died during the study so they were excluded. So, 691 patients were included in the study. Mortality was seen in patients who were old, who were severely symptomatic and were delayed in admission. In this study, 22 severe cases were there where the patient were monitored closely, given the required treatment and were discharged after clinical follow up. 16.5% patients were asymptomatic . Most patients at admission had dry cough(83%), Chills(46%), fever(71%) and fatigue(21%). Viral cure achieved in (63.6%) of Covid-19 patients within first three weeks of admission, leaving the 2% that remained positive for more than four weeks following the first positive swab test. Hypertension was the underlying disease that was most common followed by diabetes. 67.5% of patients were male with hypertension. At the time of admission the proportion of severe symptoms was higher in males than in females. In our study the median time of viral clearance was 21 days (19-22 days) for 63% of patients.

No. of patients

V. Discussion:

Prolonged viral shedding was seen in patients who had comorbidities, older age group people and the severity of disease. Our study showed comorbities as a factor associated with viral clearance. Age played an important predictor of morbidity.

The present study was conducted to establish to focus on the viral clearance time of covid 19 patients.

In this study a clinically recovered patient having persistence of viral RNA shedding has minimal chances of transmitting the infection after recovery of symptoms and a proper isolation. The Viral RNA was still detectable in 4% patients > 30 days of onset of symptoms. In our study the duration of infectivity varied, showing viral clearance of 63.6% in 3rd week of hospitalization and proper care followed by 25% in 4th week, 3% in 5th and 2% took even till 6th week for clearance. In 1 particular patient viral clearance time was 52 days without any symptoms . The asymptomatic group took much more time for viral clearance as compared to symptomatic. Even after the resolution of symptoms in most of the symptomatic patients viral positivity was seen for upto 20 days . In a study done by Zhou B et al. showed that the median of viral shedding in their study was 31 days. Numerous studies showed that increased age is a vital predictor of mortality in COVID-19 infection.[10] In our present study , the number of patients who were discharged early were more in age group less than 60 years of age as compared to 60 years or more.

Carmo et al reported the viral clearance time ranged from 24± 9 days showing only 30.2% achieved negative test in 20 days. A study done by Benoni R showed median recovery time of 24 days.[6]

A study on a total of 157 patients found that abnormal chest x-ray and symptoms are predictors for viral shedding showing VCT of 13 days.[2]

In patients who delayed admission showed severe conditions with worse treatment outcomes. So, this study indicates that patients who are symptomatic need to be admitted as soon as possible after confirmation of SARS CoV-2 infection. While doing this study we noticed that a person who was vaccinated had fewer chances of spreading the disease as compared to the people who are not vaccinated, this point needs to be further studied.

VI. Conclusion:

The results show that symptomatic patients with COVID-19 should be hospitalized and treatment should be started as soon as possible. Knowing the viral clearance time will be helpful in the clinical management of patients mostly in symptomatic patients. It can take a longer time in viral clearance most commonly in people who are hospitalized. Proper diet and exercise will help the person recover during the viral shedding days as seen in most of our patients.

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