MOLECULAR DETECTION OF SEVERE ACUTE RESPIRATORY SYNDROME CORONAVIRUS-2, AN EXPERIENCE OF A TERTIARY CARE HOSPITAL MALIR

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ABSTRACT

Objective: To share our experience related to molecular detection of Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) RNA in COVID-19 suspected patients reported at CMH Malir.

Study Design: Cross-sectional study.

Place and Duration of Study: Department of Microbiology, Pathology Laboratory of Combined Military Hospital Malir, from March to May 2020.

Methodology: Individuals with signs and symptoms of Coronavirus Disease-19 (COVID-19) and asymptomatic patients with history of having close contact to confirmed COVID-19 patients or travelling history were considered for SARS-CoV-2 Polymerase chain reaction (PCR) assay. Total of 1330 nasopharyngeal swabs were collected for qualitative detection of COVID-19 viral RNA by real-time reverse transcription polymerase chain reaction (RT-PCR) assay.

Results: Out of 1330 tests, 74 patients were found to be SARS-CoV 2 PCR positive. Average age of patients was 30.45 ± 31.9 years with predominance of 55 (74.3%) male patients. Within 74 patients, six (8%) died in age group ≥ 40 years. Time duration of positive PCR after initial positive PCR varied between 8 days to 45 days.

Conclusion: In this study, we noticed male predominance as they are more exposed to outside environment and susceptible to acquire the virus. Therefore, they were screened in majority. Also, we need a reliable and globally accepted test like SARS COV-2 RT-PCR for early detection of both asymptomatic and symptomatic cases. This will help us in taking appropriate steps to prevent its spread further.

Keywords: Coronavirus disease-19 (COVID-19), Reverse transcription polymerase chain reaction (RT-PCR), Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2).

INTRODUCTION

Corona virus disease 2019 (COVID-19), caused by novel Severe Acute Respiratory Syndrome Associated Coronavirus (SARS-CoV-2), is a highly contagious disease primarily of zoonotic origin[1]. It resulted in third outbreak of the beta coronaviruses after SARS-CoV and Middle East respiratory syndrome coronavirus (MERSCoV)[2]. First case of pneumonia of unknown etiology was reported on 12 December 2019 from China. Since then this outbreak has almost involved all the continents except Antarctica. Data published on 27 May 2020 showed 5488825 confirmed cases with 349095 deaths worldwide. Therefore, World Health Organization (WHO) has declared it public health emergency of global concern which affected 217 countries/territories including Pakistan[3]. In Pakistan first two cases of COVID-19, one from Karachi and another from Islamabad were reported on February 26, 2020. Both patients travelled back to Pakistan from Iran. These cases kept on rising in our society due to lack of compliance of people in maintaining social distance and wearing mask[4]. Within two months, 69496 confirmed cases were reported with mortality of 1483 individuals. The highest number of cases were reported from Sindh province followed by Punjab[5]. The virus is mainly transmitted from person to person by respiratory droplets within six feet distance and due to direct contact with infected surfaces if exposed to mucous membrane. However, more studies are going on to see other sources of transmission as well. Airborne transmission is observedin health care settings due to aerosol generating procedures[6-8].

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Variable clinical course has been observed from asymptomatic carriers to symptomatic infections, primarily affect the respiratory system which may be mild or severe, either requiring supplemental oxygen or mechanical ventilation support. Certain patients may present with gastrointestinal, ocular, neurological, cardiac and skin manifestations depending upon comorbids[9,10].

Early detection of cases and identification of high risk individuals are essential for prevention of disease transmission and mortality.

Molecular tests including various reverse transcription polymerase chain reaction (RT-PCR) assays are used globally for confirmation of SARS-CoV 2 RNA in upper and lower respiratory specimens[7]. Serological tests are also available but they cannot differentiate between the active and past disease. Several studies have been published internationally but there may be a geographical variations in its clinical course and detection in laboratory[10]. Hence, it is important to know about the trend of this virus in our setup.

The main aim of this study was to share laboratory based experience of a tertiary care hospital about qualitative detection of SARS–COV 2 RNA on respiratory specimens of COVID-19 suspected patients using Real-Time PCR.

METHODOLOGY

A cross sectional study was carried out in the Department of Microbiology of Combined Military Hospital (CMH) Laboratory, Malir, Karachi, from 1st March to 31st May 2020. Permission was obtained from Hospital Ethical Committee (file no: 06/2020/Trg/Adm). Informed consent was taken from all the patients and to maintain their confidentiality, coding was done. Patients with clinical suspicion of COVID-19 while asymptomatic patients with relevant travel history to endemic areas or exposure to confirmed COVID-19 patients within six feet for more than 15 minutes or direct contact with infected material were considered for SARS-CoV-2 PCR. Patients admitted for different surgical purposes were also undergone screening as per hospital policy to ensure infection control measures. Sample collection was done by non-proba-bility consecutive technique. Patients not meeting the above mentioned criteria and duplicate specimen of same patients were excluded from the study.

Nasopharyngeal swabs of all patients were collected as per standard protocol and transported to laboratory in viral transport media (VTM). Preferred specimen of patients with lower respiratory system was sputum. Specimens which could not be dealt immediately were stored at 2-8°C for upto 24 hours. Kit manufactured by Primer design Genesig®, was used for qualitative detection of COVID-19 viral RNA assay. Extraction of nucleic acid followed by amplification and detection process of SARS-CoV-2 was carried out as per manufacturer instructions. The reverse transcription polymerase chain reaction (RT-PCR) assay include internal control to identify possible PCR inhibition, to measure extraction purity and to confirm the integrity of the PCR run.

Specimens were processed in biosafety cabinet A2 as per standard laboratory guidelines. 20µl RNA (Nucleic acid extracted from specimen, positive and negative control) was added to reaction tube with qRT-PCR Master Mix, making total volume of 40µl/test.

The reaction plate was sealed and placed in thermalcycler. The final results were interpreted on detection basis ofcycle threshold value (Ct-value). Before interpreting sample results, success of the run was verified as per manufacturer criteria. The tests were repeated if the run were not as desired (invalid). A Ct-value ≤34 was categorized as a positive test result, a Ct-value between 34-38 was considered as indeterminate/ borderline and Ct-value ≥38 was interpreted as a negative test result. All the indeterminate tests were repeated at least twice and reported after clinical correlation. PCR was repeated one week after patient became asymptomatic.

The data was entered on Excel sheets regarding age, gender, indoor/outdoor status of COVID-19 positive patients and analyzed using SPSS-24. Demographic data was assessed by

using descriptive statistics. Mean and standard deviation (SD) were calculated for numerical variables like age. Variables were expressed used using frequencies and percentages. Chi square test was applied to calculate the *p*-value ≤0.05 was considered statistically significant.

RESULTS

In a period of three months, total of 1330 SARS-CoV 2 PCR tests were performed in Microbiology Department. Out of these, 1157 (87%) tests were negative. 74 (5.5%) cases were actual positive tests while 67 (5%) were old cases who remained positive on repeat testing. Out of 32 (2.4%) indeterminate results, 30 were found to be negative while only 2 were found positive on repeat testing. In this study, 55 (74.3%) of the positive patients were males. Majority of SARs-CoV-2 positive 50 (68%) were from indoor settings admitted either for surgery or with sign and symptoms of COVID 19 disease. Most of them 40 (54%) were treated in hospital settings in isolation wards. Few critical patients 10 (13.5%) among them requiring respiratory support like 4 (5.4%) on ventilators and 6 (8%) on oxygen support were kept in Intensive Care Units (ICUs). Only two pregnant females along with their newborns were found to be PCR positive when reported for Cesarean section. Both remained asymptomatic throughout their course of PCR positivity. Other patients, who were screened prior to their surgery, were found negative. Total 6 (8%) patients died with COVID-19 disease during this period. They fell in ≥40 yearsage group which include three females and three males. Among them, four patients were having comorbid conditions such as (hypertension, malignancy, dilated cardiomyopathy and diabetes) while two were otherwise healthy individuals (table). Mean age of patients was found to be 30.45 ± 21.9 years with minimum age noted from few days to maximum age of 74 years as shown in table-I. Maximum infected patients 47 (63.5%) belonged to age group of ≥40 years including 39 males and 8 females. 25 (34%) in ≤39 years age

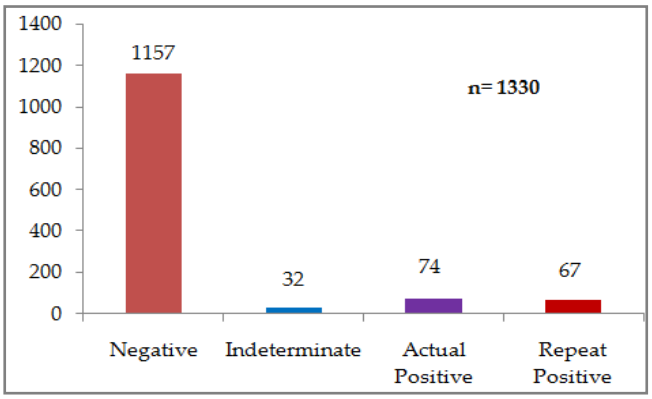

Figure: Total Polymease Chain Reaction (PCR) carried out in a period of three months.

Table: Showing details of 74 COVID-19 Polymerase Chain Reaction (PCR) positive cases during three months period.

Demographic Details	Hospital Details	PCR Pattern	*p*-value
Gender distribution Male: 55 (74.3%) Female: 19 (25.7%) Age group distribution ≤1 month: 2 (2.7%) 1 month - ≤39 years: 25 (34%) ≥40 - ≤75 years: 47 (63.5%)	Source of Sample Total indoor patients: 50 (68%) COVID-19 suspected: 48 (65%) Screening before surgery: 2 (2.7%) In Isolation: 40 (54%) In intensive care: 10 (13.5%) On ventilator support: 4 (5.4%) On Oxygen support: 6 (8%) Outdoor patients: 24 (32%) Travel history: 16 (21.6%) Exposure to COVID-19 positive patients: 8 (10.8%) Outcome of patients Total deaths: 6 (8%)	Time duration after initial positive PCR days Minimum: 8 days Maximum: 45 days Mean: 18 ± 2 days	*p*-value ≥0.02 in relation to age and gender

category, 14 males and 11 females were observed. Time duration after initial positive test result was found to vary between 8 to 45 days. Two clinically stable patients remained PCR positive even after 45 days. The *p*-value ≤0.02 in relation to gender and age was found to be significant.

DISCUSSION

This study was the first case series from CMH Malir of 74 COVID-19 patients with 1330 samples of RT-PCR tests for SARS-CoV-2 detection. Our preliminary results were note worthy for providing substantiation of SARS-CoV-2 dynamic profile in infected patients.

We collected series RT-PCR test results from 70 COVID-19 patients and investigate the epidemiology of disease. Mean age of patients was found to be 30.45 ± 31.9 years with average age of 35 years. This was quite less as compared from China (50 years) and other international data (60 years in Italy, 62.2 years in USA)[11,12,15]. In this study, out of total 74 PCR positive patients, fifty (68%) were from indoor and 24 (32%) from outdoor settings. Percentage of in-patients was comparable to other studies from China and USA but ICU admissions were lesser as compared to those studies which could be because of more cases in young population in our study[13-15]. We showed majority of patients got positive results of RT-PCR test for SARS-CoV-2 within week after the onset of symptoms. The negative results of RT-PCR test for SARS-CoV-2 varied widely ranging from 8 to 45 days. The positive rate of RT-PCR test results kept waning in 6 weeks. The above findings suggested that SARS-CoV-2 viral replication has quite extensive phase in infected patients[16,17]. Findings of this study was consistent with Dowd *et al*, Covid *et al* and Richardson *et al*, that elderly patients (≥65 years old) were more likely to have severe type of COVID-19 with increased mortality rate (14.8% in China, 27.7% in Italy, 21% in New York)[11,18,19]. But we observed less mortality rate which was 8% as compared to their data. We also found that men have more tendency to acquire infection than women, this is probably because men have more exposure to outside environment and as this particular study was conducted in military settings therefore total number of tested people were mostly soldiers.

We found that there was extreme diversity in age group who was infected with disease starting from new born to maximum age of 74 years, however mortality was observed in patients with age >40 years and majority with underlying co morbidities. Previous studies suggested that coronavirus is more likely to infect older individuals, for whom the immune pathogenesis and induction of a pro-inflammatory cytokine storm might be the culprit[20,21]. Older patients with impaired immune function might have a prolonged period of viral elimination.

In our study, patients who are scheduled for surgery and were screened for SARS COV 2 were also included and only 2 pregnant females were tested positive whereas no case from general surgery or any other surgical department was found to be positive. These findings were consistent with another study by M.A. Al-Muharraqi which concludes that screening with gold standard RT PCR in all surgical units can also be a part of a pandemic suppression campaign leveraged to move the current crisis closer to the ideal situation, especially in the absence of therapeutics or vaccines[22].

Evidence suggested that the outbreaks of COVID-19 may be correlated to its rapid person-to-person transmission ability. Since specific treatment has not been validated for COVID-19, traditional public health tactics isolation, quarantine and community containment are critical to control the spread[5]. This preliminary study has found evidence of the dynamic profile of SARS-CoV-2 in non-ICU COVID-19 patients during disease course. According to the results in our study, we suggested prolonged observation and repeat confirmation of RT-PCR test from respiratory specimens for safe discharges and discontinuation of quarantine. This will not only assist in better clinical outcome and improving infection control measures but also reduce hospital burden.

CONCLUSION

In this study, male predominance is prevalent because of their exposure to outside world thus making them more susceptible to acquire the virus. Real time SARS-CoV 2 RT-PCR is a useful tool to provide a rapid and timely confirmation of SARS-COV 2 viral RNA for early diagnosis, prompt treatment and reduce mortality. Since COVID-19 can be present with variety of clinical spectrum and diseases that can be fatal, therefore, we need a reliable and globally accepted test with better sensitivity and specificity. This will help us to break its chain of transmission particularly due to asymptomatic carriers.

CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any author.

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