Epidemiological Analysis of COVID-19 Patients detected by Real-Time Reverse Transcription - Polymerase Chain reaction in a Tertiary Care Biosafety Level III Laboratory, Rawalpindi

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Abstract

Introduction: Unexpected eruption and global dissemination of Coronavirus disease (COVID-19) has tested the healthcare systems of both developed and developing countries.

Objective: To analyze the spectrum of novel coronavirus infection in a tertiary care setup.

Materials and Methods: All oropharyngeal and nasopharyngeal samples (n=7057) were collected in a viral transport medium (VTM) for qualitative analysis by a real-time reverse transcription-polymerase chain reaction (RT-PCR) machine. Positive and negative controls were applied with each batch. Positive cases were stratified into mild, moderate, severe, and asymptomatic, according to the guidelines of the National Institute of Health, Pakistan. Descriptive statistical tests were applied including percentage, chi-square tests, mean, median, and mode. P < 0.05 was counted as statistically significant.

Results: Average positive test rate was 18.97% (n=1339). The maximum positivity rate (26%) of COVID-19 infection was observed in June 2020. Most of the cases (60%) belonged to Rawalpindi District, were male (n=844, 63.03%), and belonged to age group (20-40 years) and mean of 36 and age range from 2 to 85 years. Forty-nine percent of COVID-19 infected patients were asymptomatic while 9.8% had severe disease. Overall, the mortality rate was 159(11.87%) in RT-PCR confirmed cases.

Conclusion: Average positive test rate was 18.97%. The majority of the participants belonged to the young age group (20-40 yrs.) with a range from 2 to 85 years. Forty-nine percent positive COVID-19 infected patients were asymptomatic while 9.8% had severe disease.

Keywords: COVID-19, RT-PCR, Epidemiological analysis.
Introduction

Unexpected eruption and global dissemination of Coronavirus disease (COVID-19) have challenged the nations. Pakistan is presently suffering through the second wave of infection. The causative agent Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV-2) belongs to the family Coronoviridae, having 79% homology with SARS-CoV-2. The high infectivity has prompted rapid respiratory droplet transmission of SARS-CoV-2 generated by speaking, coughing, or sneezing and direct physical contact, from human to human. Generally, males with SARS-CoV-2 are at higher risk for poor prognosis and mortality, independent of the age variable. Mostly SARS-CoV-2 infected patients remain asymptomatic initial stages of the disease with only chills and respiratory symptoms. However, moderate to very severe complications can be witnessed in many cases. These offered characteristic clinical symptoms for diagnosis. In addition to virological findings, changes in other laboratory parameters such as an increase in neutrophils count with a high neutrophil to lymphocytes ratios (NLR), elevated D-dimer levels, and increase in C-reactive protein, and other inflammatory markers have also been seen in severely infected patients. Early identification, patient isolation, and timely treatment is a fundamental strategy to restrict transmission from human-to-human thus reducing the chances of secondary infections among close contacts and healthcare workers.

The PCR test on upper respiratory tract samples is considered a prime tool of diagnosis. However, cases with negative results, but showing positive findings on HRCT (ground glass opaqueness in peripheral lung fields) are reported. As RT-PCR is considered the most definite laboratory identification method in the pandemic, it provides an adequate and satisfactory way to confirm nCOVID-19 infection within 6-8 hours in case of COVID-19 infection also.

After confirmation of the first two patients with COVID-19 on 25th Feb 2020, a hospital-based surveillance system for SARS-CoV-2 infection was initiated across Pakistan. Various hospitals assessed symptomatic patients and close contacts, collected, and referred specimen to designated PCR laboratories. The diagnostic capability has been judiciously supported by involving both public and private sector laboratories in Pakistan. However, the already scanty resources of Pakistan’s health care system have been further tightened.

Materials and Methods

Objectives:
- To compile and analyze the demographic profile, clinical presentation, contact history of all COVID-19 patients detected by RT-PCR.
- Demographic information for reported COVID-19 cases may offer data on populations that are at increased risk of COVID-19 infection.
- To provide awareness and outreach to high-risk populations and identify any discrepancies that merit further search or focus.
- The data will help in a better understanding of this extensive infection to work in a coordinated way after proper managerial planning and adopt timely effective preventive measures to curtail further spread of disease.

Study design: This cross-sectional descriptive observational study was carried out in Benazir Bhutto Hospital Biosafety level III COVID-19 infection PCR testing laboratory which is the only laboratory with a Biosafety level III facility in the public sector of Rawalpindi district receiving respiratory samples for PCR testing from the whole district including its residential areas, offices, quarantine centers as well as primary and tertiary care hospitals either directly or through district health authorities.

Sampling technique: We analyzed all samples received from the Rawalpindi District for SARS-CoV-2 RT-PCR testing by using a non-probability convenience sampling technique from 2nd May 2020 to 31st July 2020.

Inclusion criteria: All records of suspects/patients investigated for SARS-CoV-2 by PCR testing in BBH BSL III LAB. COVID-19 positive population of Rawalpindi District whose samples were received for PCR testing of Covid-19 infection on clinical and or epidemiological suspicion by medical teams of the respective designated hospitals was included.

Exclusion criteria: Data of all patients not tested in BBH BSL III laboratory.

Data Collection: The data was collected at the time of sample collection, uploaded on Primary and secondary health information systems on daily basis, and updated by the district health authority and Benazir Bhutto Hospital laboratory personnel as per routine. A total number of 7057 tests were conducted on patients/suspects based on a high index of suspicion for COVID-19 infection due to characteristic respiratory tracts infection symptoms such as fever, cough, and breathlessness, or history of close contact with SARS-CoV-2 patients in three months.
from 2nd May 2020 to 31st July 2020 in Rawalpindi district biosafety level 3 laboratory at Benazir Bhutto Hospital. The epidemiological characteristics of the patients were recorded. Results of real-time PCR for SARS-CoV-2 on respiratory specimens (pharyngeal and/or nasal swabs) were collected from laboratory records. All data was saved by laboratory personnel on HISDU COVID-19 Dashboard and Microsoft excel sheet. The clinical classification of confirmed/suspected cases into asymptomatic, mild, moderate, severe, and critical was done according to the hospital protocol and National Institute of Health, Pakistan guidelines for COVID 19 infection.\textsuperscript{3,15,16}

RNA extraction: RNA was extracted manually using Geneaid extraction kits following the manufacturer’s instructions. COVID-19 was qualitatively analyzed using CFX 96 real-time PCR machine and (nCoV-19) Nucleic Acid Diagnostic Kit (BGI, Inc. China) according to the manufacturer’s instructions. The test was performed on SARS-CoV-2 Nucleic acid detection kit (PCR-Fluorescent Probe method) (CE-IVD), intended for the qualitative dual-target open reading frame 1ab, nucleocapsid protein gene fragments (ORF1ab/N Gene) detection of SARS-CoV-2, the virus that causes COVID-19 disease, in nasopharyngeal and oropharyngeal swab samples from patients (who meet COVID-19 clinical and/or epidemiological criteria for testing) with internal and external positive controls. Conditions for amplification were as recommended by the manufacturer. A medium load required retesting for confirmation. Positive and negative controls were applied with each batch of tests for quality control.\textsuperscript{15,16}

Statistical analysis: Data was analyzed on a Microsoft excel sheet for intergroup differences. Descriptive statistical tests were applied including percentage, chi-square tests, mean, median, and mode. P < 0.05 was counted as statistically significant.

**Results**

Total 7057 PCR tests were performed in three months’ duration in BSL III PCR Lab BBH. Laboratory data of 1339 COVID-19 positive patients were included in the final analysis. The majority of the subjects presented for the COVID-19 test were from Quarantine areas and home isolation (n=3833, 54.3%) followed by RMU and Allied Hospitals. However, the positivity rate was found to be highest (29%, n=845/2916) in patients referred to RMU and Allied Hospitals compared to 12.36% and 6.50% from quarantine area/home isolation and secondary care hospitals, respectively. Additionally, out of total RT-PCR SARS-CoV-2 confirmed cases larger proportion was contributed by RMU and Allied Hospitals (63.1%, n=845/1339) (Table 1). The average positivity rate was 18.97% (n=1339) with a maximum (26% n=915) observed in June (Table 2 and Figure 1). The majority of the subjects (49%) were asymptomatic, while 9.8% had severe disease (Table 3). Though males (63.03%) constituted the predominant gender the difference in infectivity rate among both the sexes was not significant (p=0.074) (Table 4). The overall age of patients was in the range of 2 and 85 years with a median age of 41.5 years. Although a maximum number of subjects tested for COVID-19 belonged to the age group 20-40 years (49.70%, n=3508/7057) with a mean age of 36, the highest positivity rate was observed in the age group 40-60 years. (23.34%, n=396/1704) (Table 5). The statistical difference of the age variable was non-significant. Out of total COVID-19 positive patients tested in BBH BSL III laboratory, 159 deaths were recorded (11.87%) during the study period. Death rate was higher in males (n=108, 67.92%) as compared to females (n=51, 32.07%).

<table>
<thead>
<tr>
<th>Table 1: Reporting Health Facility</th>
<th>Total tests (n)</th>
<th>Positive (n)</th>
<th>Negative (n)</th>
<th>Positivity rate (%)</th>
<th>Percentage Positive (%)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RMU and Allied Hospitals</td>
<td>2916</td>
<td>845</td>
<td>2071</td>
<td>29.00</td>
<td>63.1</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Quarantine areas and home isolation</td>
<td>3833</td>
<td>474</td>
<td>3359</td>
<td>12.36</td>
<td>35.4</td>
<td></td>
</tr>
<tr>
<td>Secondary Care Hospitals</td>
<td>308</td>
<td>20</td>
<td>288</td>
<td>6.50</td>
<td>1.5</td>
<td></td>
</tr>
</tbody>
</table>
Table 2: Monthly Distribution of COVID 19 RT-PCR Tests

<table>
<thead>
<tr>
<th>Month (2020)</th>
<th>Total tests (n)</th>
<th>Positive (n)</th>
<th>% positivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>May</td>
<td>1294</td>
<td>276</td>
<td>21.00</td>
</tr>
<tr>
<td>June</td>
<td>3563</td>
<td>915</td>
<td>26.00</td>
</tr>
<tr>
<td>July</td>
<td>2200</td>
<td>148</td>
<td>6.70</td>
</tr>
</tbody>
</table>

Figure 1: Monthly Distribution of PCR SARS-CoV-2 Samples

Table 3: Clinical Presentation of COVID-19 Cases

<table>
<thead>
<tr>
<th>Presenting Symptoms</th>
<th>Frequency n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asymptomatic</td>
<td>656 (49%)</td>
</tr>
<tr>
<td>Mild</td>
<td>362 (27%)</td>
</tr>
<tr>
<td>Moderate</td>
<td>189 (14.1%)</td>
</tr>
</tbody>
</table>

Table 4: Gender Distribution of COVID-19 RT-PCR Testing

<table>
<thead>
<tr>
<th>Gender</th>
<th>Total tests</th>
<th>Positive n (%)</th>
<th>Negative n (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>4570</td>
<td>844 (18.47%)</td>
<td>3726 (81.53%)</td>
<td>0.074</td>
</tr>
<tr>
<td>Female</td>
<td>2487</td>
<td>495 (19.90%)</td>
<td>1992 (80.09%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>7057</td>
<td>1339</td>
<td>5718</td>
<td></td>
</tr>
</tbody>
</table>

Table 5: Age-wise Distribution of COVID-19 Cases

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Total Tests</th>
<th>Positive (n)</th>
<th>Negative (n)</th>
<th>Positivity Rate (%)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-20</td>
<td>1156</td>
<td>209</td>
<td>947</td>
<td>18.08</td>
<td></td>
</tr>
<tr>
<td>20-40</td>
<td>3508</td>
<td>582</td>
<td>2926</td>
<td>16.59</td>
<td></td>
</tr>
<tr>
<td>40-60</td>
<td>1704</td>
<td>396</td>
<td>1308</td>
<td>23.24</td>
<td></td>
</tr>
<tr>
<td>61 &amp; Above</td>
<td>689</td>
<td>152</td>
<td>537</td>
<td>22.06</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>7057</td>
<td>1339</td>
<td>5718</td>
<td>18.97</td>
<td>4.35</td>
</tr>
</tbody>
</table>

Discussion

On 26th February 2020, the first laboratory-confirmed case of COVID-19 disease was recorded in Pakistan. Risk awareness communication, early detection of positive COVID-19 cases, proper tracing of contacts, social distancing, creation of quarantine centers, isolation wards, increase in lab diagnostic capability, and donations for the safety of people were the major interventions carried out by the country. The regional strains found on the ORF1-b gene sequence were categorized into clade A3. It showed complete homology with the strains identified in adjacent countries like China and India. Depending on the available data it is evident that the peak was seen in June 2020 in the Rawalpindi region. Overall positivity rate in the Rawalpindi district remained 19% during the study period which is almost as high as in other districts like Faisalabad (17.9%) and Karachi. National Institute of Health, Islamabad, documented an 8.27% positivity rate but a study in Wuhan showed a very high rate of 38.4% based on PCR testing. Evidence has shown the stability of this virus and verified its existence in the air for almost 8 hours and 16–24 hours on various inanimate surfaces like wood and plastics. The respiratory droplets of the virus in the air may spread up to 1 to 2 metres and can easily be transmitted to other individuals by inhalation. Sixty-three percent of our patients presented in tertiary care hospitals and the least showed up in primary and secondary care areas showing the increased patient burden on tertiary care setups. Quarantine and field hospital centers catered 35% of laboratory-confirmed COVID-19 cases. Almost half (49%) of the SARS-CoV-2 positive cases were asymptomatic at the time of testing and the remaining study population presented with a different presentation.
range of symptoms. Similar results were documented by Badar et al showing 68% asymptomatic cases whereas only 32% were symptomatic.15 Contrary to this Tahir et al in Karachi recorded 91% symptomatic lab-confirmed patients presenting with at least one symptom with higher fatality over the age of 80.3 This can be due to the presentation of only symptomatic patients in the catchment isolation hospitals. Similarly, in a study in Hubei, only 12.3% of patients developed symptoms, and most were asymptomatic (71.2%) having only a contact history with COVID-19 disease positive cases. The major proportion of the symptomatic patients in the mentioned study presented with mild symptoms only as in our study (29%) like fever (84.9%) and cough (21.9%).26 COVID-19 infection is mainly associated with respiratory complications so, routine radiological assessments and high-resolution Computerized Tomography scan tests are also crucial for diagnosis.10,17

The male gender was affected almost twice as much (63.03%) as females (36.96%). Similar results were documented in local studies carried out in Karachi and Faisalabad showing 64.6% and 78.3% males in total SARS-CoV-2 positive patients, respectively.3,17 This proportion is endorsed by another study carried out in Wuhan where 56% population affected were males.1,21 A similar study of approximately 150 COVID-19 cases from Wuhan documented 50% male.13 Generally, more males are affected and tested but statistically, the positivity rate was almost the same in both genders as verified by P-value as documented in all study setups mentioned here. The majority of the participants belonged to the age group 20-40 years (43.46%) followed by 40-60 years (29.6%) with a mean and median age of 36 and 41.5 years, respectively. The age of COVID-19 patients was in the range of 2 and 85 years. One of the factors affecting it can be the younger median age of the Pakistani population (21.5 years) as documented in 2015. The age-wise difference in the positivity rate was also not statistically significant (P-value > 0.05) showing that age is not the main defining factor in COVID-19 disease. Similarly, age groups 37-47 (45%) and 21-40 (43%) years were the most infected with this pandemic virus in Faisalabad and Karachi study population, respectively. The majority of the patients belonged to the same age group 30-39 years (n = 1097) in a study in Wuhan.1,15 Similarly, no significant statistical difference (p-value > 0.05) was found based on the age among COVID-19 infected patients in the studies carried out in Faisalabad, Karachi, and Wuhan.1,3,17

Male gender has emerged as a substantial risk factor of mortality in the COVID-19 pandemic.23,24,25 According to Jin et al., males are more prone to progress to severe disease. In their retrospective study, even though, men and women had a similar predisposition, the death rate was quite higher in males and causing almost 2.5 times higher mortality as compared to the female population.3,4 Similar results were documented in our study with 67.9% versus 32.07% female to male death rate23 among the patients tested for COVID-19 infection.

There is an ongoing increase in diagnostic services in the public sector on a massive scale. Enhanced surveillance and improvement in infrastructure for the delivery of services is the top priority of our Government.

### Conclusion

1. A high prevalence rate (18.9%) of COVID-19 infection was observed. The majority of the participants belonged to the young age group (20-40 yrs.). The mild disease was found in 59%, while 9.8% had severe disease. The death rate was almost twice in males as compared to the female sex.
2. Pakistan is struggling hard to control the pandemic by adopting various precautionary measures such as isolation and quarantine facilities, lockdown, and community awareness programs, to face the continuous rise in the COVID-19 cases.
3. Scaling-up of public health measures and upfront efforts to strengthen diagnostic and treatment services, most importantly in public sector health facilities, reflects the resilience of our healthcare system.
4. Prevailing uncertainty, about COVID-19 disease, behavior, and the possible emergence of new variants, needs more vigilance.

### Limitations

A few limitations are there in our study. Patients included in this study belong to only one district (Rawalpindi) of Pakistan. A review on a larger scale from various areas of the country may further reflect accurate statistics regarding the patient’s demographics. Moreover, the study duration was only three months. Comprehensive scrutiny of the laboratory parameters, clinical presentations,
Complications, and predictive markers is endorsed to acquire an improved vision of the infection and its accompanying matters, which can support the government to enhance its fight against the pandemic.

References


