ORIGINAL ARTICLE
CLINICAL AND VIROLOGICAL COURSE OF SARS-CoV-2 INFECTED PATIENTS IN A TERTIARY CARE HOSPITAL IN PAKISTAN

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Background: Acute respiratory illness caused by Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) involved the whole globe within no time. Various studies published globally have shown variable severity of disease and mortality. The objective of our study was to describe clinical and epidemiological characteristics of the disease in our setup. **Methods:** in this descriptive case series, individuals with signs and symptoms of Coronavirus disease-19 (COVID-19) and asymptomatic patients with history of close contact to confirmed COVID-19 patients were considered for SARS-CoV-2 Polymerase chain reaction (PCR) assay. Epidemiological and clinical features of only PCR positive cases were recorded. Data regarding hospitalization status, exposure to known COVID-19 patients, clinical feature and clinical outcome of patients was collected and interpreted. **Results:** A total of 266 patients were found to be SARS-CoV 2 PCR positive which were included in the study. Mean age of patients was 39.45±31.9 years and majority of the patients in our study were male, i.e., 238 (89.5%). Most common clinical features among COVID-19 symptomatic patients were fever and dry cough followed by myalgias and sore throat. Eighteen (7%) out of 266 died in our setup. Time duration of viral shedding after initial positive PCR varied between 11 days to up to more than 55 days. **Conclusions:** Coronavirus disease-19 (COVID-19) can present with wide range of clinical spectrum and disease can be life threatening. Severity of disease, requirement of ICU care and mortality were directly related to age of the patient and underlying comorbidities. Rigorous precautionary measures are of utmost importance particularly in this high-risk population.
Keywords: Severe Acute Respiratory Syndrome Coronavirus-2, SARS-CoV-2; Coronavirus disease-19, COVID-19; Polymerase chain reaction

INTRODUCTION

Multiple cases of acute respiratory illness of unknown aetiology occurred in Wuhan, Hubei Province, China in December 2019. Agent of this disease was finally isolated on 12th January 2020 and provisionally named as 2019 novel coronavirus (2019-nCoV) by World Health Organization (WHO).[1,2] Coronaviruses are RNA viruses, having positive sense RNA, belonging to family *Coronaviridae* and are non-segmented and enveloped. 2019-nCoV was categorized in betacoronavirus 2b lineage.[3] Mostly human coronaviruses cause mild infections but previously severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV) caused severe disease outbreaks during last 20 years with 10% and 37% mortality rates respectively. 2019-nCoV was later on named as Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2). In February 2020, WHO labelled the disease caused by SARS-CoV-2 as Coronavirus disease-19 (COVID-19).[4–6]

Exact origin of SARS-CoV-2 is still unknown but it was considered to be originated from wild animals in Huanan Seafood market.[1] After person to person transmission of disease in Wuhan, it rapidly spread to South Korea, Japan, Thailand and United States. WHO declared COVID-19 outbreak a Public Health Emergency of International Concern on 31st January 2020. Cases of disease increased exponentially worldwide and finally it was declared pandemic by WHO on 11th March 2020.[7–10] As of 20th July, 2020 total cases of COVID-19 has risen to 14.34 million globally with total death to 603,691. First case of COVID-19 in Pakistan was confirmed in Karachi on 26th February 2020 and within a period of around 5 months, cases increased to 265,083 with 5,599 deaths till now.[11–13]

There is wide clinical spectrum of disease ranging from asymptomatic infection to severe respiratory illness and can lead to death.[6] Elderly Patients and people with underlying comorbidities are more prone to severe disease. Because of rapid and aggressive transmission of disease, strict preventive measures both in hospitals and community and their implementation at government level are

required. As no vaccine or specific treatment has been established, early detection of cases and high risk individuals are most important steps in prevention of disease transmission and mortality.[7,8,10] Multiple studies have already been published internationally but as there is variation in mortality rates of different regions globally due to various factors there is a need to know the disease characteristics in our setup.[11]

The objective of our study was to describe clinical and epidemiological characteristics of COVID-19 cases, various clinical features, patients' characteristics, severity of disease, underlying risk factors for critical illness and mortality and ascertaining virological course during illness.

MATERIAL AND METHODS

This descriptive case series was carried out in the Department of Microbiology in collaboration with Department of Medicine and Intensive Care, Pakistan Navy Ship Shifa Hospital, Karachi, from 26th March to 15th June 2020. Permission was obtained from Hospital Ethical Committee and informed consent was taken from all the patients. Patients with signs and symptoms of COVID-19 and asymptomatic patients with history of close contacts to confirmed COVID-19 patients were considered for SARS-CoV-2 PCR.

Patients who were asymptomatic and some patients with mild symptoms were treated in outdoor settings. Patients with moderate to severe disease were managed as hospitalized patients. Patients who had mild dyspnoea were categorized as having moderate disease while patients who had severe dyspnoea requiring oxygen therapy or respiratory support were categorized as having severe disease.

Nasopharyngeal swabs of all patients were collected and transported to microbiology laboratory in viral transport media. Specimens, which could not be immediately processed, were stored at 2–8 °C for up to 24 hours and at -70 C if not processed within 24 hours. QIAGEN (Germany) was used for real-time reverse transcription polymerase chain reaction (RT-PCR) assay. Extraction of nucleic acid and amplification process of SARS-CoV-2 PCR were done as per manufacturer's protocol. 20µl RNA (Nucleic acid extracted from specimen, positive and negative control) was added to reaction tube with qRT-PCR Mix, making final volume of 40 µl/test. PCR reaction plate was placed into thermal-cycling instrument on which final results were interpreted on detection channels. A cycle threshold value (Ct-value) ≤38 was categorized as a positive test result, and a Ct-value of more than 38 was considered as a negative test.

Clinical and epidemiological characteristics of only PCR positive cases were recorded. Data such as age, gender, hospitalization status of patients, intensive care requirement, and exposure to known COVID-19 cases was collected. Clinical features, underlying risk factors, requirement for respiratory support and clinical outcome of patients were determined. PCR was repeated one week after patient became asymptomatic. Asymptomatic patients with two negative nasopharyngeal swab PCR collected 24 hours apart were considered recovered.

Time at which PCR testing was done after onset of symptoms and time at which PCR testing was done after exposure to known COVID-19 positive patients were recorded. Time duration of viral shedding after initial positive PCR was also recorded.

The data was analysed using SPSS 24.0. Descriptive statistics were used to calculate frequencies and percentages of the qualitative variables.

RESULTS

A total of 1524 SARS-CoV 2 PCR tests were performed in microbiology department over a period of about 3 months. Out of these tests, 266 patients turned out to be positive. Majority of patients (67%) were treated in hospital settings in isolation wards. Mean age of patients was found to be 39.45±31.9 years with minimum age of 17 years to maximum age of 85 years. Maximum patients infected were in age group of 21-40 years. Most of the patients in our study were male, i.e., 238 (89.5%). Eighteen (6.7%) healthcare workers were also infected. There was one pregnant female whose PCR was performed before caesarean section and found to be SARS-CoV-2 positive but remained asymptomatic throughout her PCR positivity period. Only 106 patients had known exposure to confirmed cases of COVID-19 while rest of the patients did not know any exposure. Detailed characteristics of these 266 patients have been shown in Table-1.

Most common clinical features among COVID-19 symptomatic patients were fever and dry cough followed by myalgias and sore throat. The mean time from initial symptoms to development of dyspnea was 7 days. Clinical presentation of PCR positive patients has been shown in Table-2. Patients more than 55 years of age and comorbidities had more severe disease so they had more requirement for intensive care unit and respiratory support which has been depicted in Table 3–9. Eighteen (7%) out of 266 patients died in our study. There was variation in the time period of initial PCR testing after onset of symptoms and exposure because patients presented at different time periods after onset of symptoms and exposure. Time duration of viral shedding after initial positive PCR varied between 11

days to up to more than 55 days. In one of the patients, PCR remained positive for 55 days. This patient initially developed severe disease and put on ventilator. However, he improved and then remained asymptomatic for two weeks and then discharged. PCR patterns have been shown in Table-10.

Table-1: Characteristics of PCR positive patients (n=266)

Characteristic	No (%)
Gender	
Male	238 (89.5)
Female	28 (10.5)
Source of Sample	
Outdoor	88 (33)
Inpatient	178 (67)
Admission in Hospital (n=178)	
Isolation ward	126 (72)
Intensive Care unit (ICU)	52 (28)
Age groups (years)	
< 20	12 (4.0)
21–40	140 (53)
41–55	72 (27)
≥ 56	42 (16)
Health care workers	
Doctors	8 (3)
Paramedics	10 (4)
Contact with known COVID-19 patients	
Yes	106 (40)
No	160 (60)

Table-2: Clinical presentation of PCR positive patients (n= 266)

Clinical Characteristic	No of patients (%)
Symptomatic/ Asymptomatic	
Symptomatic patients	212 (80)
Asymptomatic patients	54 (20)
Clinical features	
Fever	144 (54)
Cough	150 (56)
Dyspnoea	76 (29)
Mild	28 (10.5)
Severe	48 (18)
Sore throat	88 (33)
Myalgias	98 (37)
Gastrointestinal complaints	32 (12)
Headache	76(29)
Flu	38 (14)
Comorbidities	
Hypertension (HTN)	48 (18)
Diabetes mellitus (DM)	36 (13.5)
Cardiovascular disease (CVS)	24 (9.5)
Chronic lung disease	8 (3)
Cancers	2 (0.8)
Chronic kidney disease (CKD)	2 (0.8)
Immunocompromised	2 (0.8)
Respiratory support	
Invasive ventilation	12 (4.5)
Non-invasive ventilation	14 (5.2)
No respiratory support	240 (90.2)
Outcome of patients	
Recovered	248 (93)
Death	18 (7)

Table-3: Relation of age groups to hospitalization status (n= 266)

Age groups (years)	Hospitalization status		
	Outdoor	Isolation Ward	ICU
≤20	8	4	0
21–40	64	70	6
41–55	16	40	16
≥ 56	0	12	30

Table-4: Relation of age groups to respiratory support (n=266)

Age groups (years)	Respiratory support		
	Invasive ventilation	Non-invasive ventilation	No respiratory support
≤ 20	0	0	12
21–40	0	2	138
41–55	2	8	62
≥56	10	4	28

Table-5: Relation of age groups to severity of disease (n= 266)

Age groups (years)	Severity of disease		
	Mild disease	Moderate disease	Severe disease
≤20	10	2	0
21–40	122	14	4
41–55	46	10	16
≥56	12	2	28

Table-6: Relation of gender to severity of disease (n= 266)

Gender	Severity of disease		
	Mild disease	Moderate disease	Severe disease
Male	198	22	38
Female	12	6	10

Table-7: Relation of comorbidities to hospitalization status (n= 266)

Comorbidities	Hospitalization status		
	Outdoor	Isolation Ward	ICU
HTN	0	12	2
DM	0	2	6
Cancers	0	0	2
Immunocompromised	0	2	0
Chronic lung disease	0	2	0
More than one comorbidity	0	4	30
No comorbidities	88	104	12

Table-8: Relation of comorbidities to severity of disease (n= 266)

Comorbidities	Severity of disease		
	Mild disease	Moderate disease	Severe disease
HTN	12	0	2
DM	2	0	6
Cancers	0	0	2
Immunocompromised	2	0	0
Chronic lung disease	0	2	0
More than one comorbidity	4	2	28
No comorbidities	170	24	10

Table-9: Relation of mortality to age groups and comorbidities (n=18)

Comorbidities	No of patients (%)
Hypertension (HTN)	2 (11)
Diabetes mellitus (DM)	2 (11)
More than two comorbidities	14 (78)
No comorbidities	0 (0)
Age groups	No of patients (%)
≤20	0 (0)
21-40	0 (0)
41-55	3 (17)
≥ 56	15 (83)

Table-10: PCR patterns

PCR became positive after onset of symptoms (days)	
Minimum	1
Maximum	20
Mean	6
PCR became positive after exposure (days)	
Minimum	2
Maximum	17
Mean	7
Viral shedding duration after initial positive PCR (days)	
Minimum	11
Maximum	55
Mean	21

DISCUSSION

The transmission of disease within Pakistan and globally has become a grave threat to communities. The outbreak has spread to all the continents and it is a public health emergency of global concern.[14]

In our study, mean age of patients was 39.45±31.9 years with median age of 34 years. Median and mean age in our study is quite less than some studies from China and other international studies.[1,3–5,7,15–18] Out of total 266, 178 (67%) of patients were managed in indoor setting while 52 (17%) patients required intensive care unit (ICU) facility. The finding of inpatients was comparable to other studies from China and US but ICU admissions were lesser as compared to those studies which could be because of more cases in young population in our study.[1,3–6,8,17]

There were 54 (20%) asymptomatic patients in our study which were greater than a study conducted in Japan.[16] There were 26 patients who were asymptomatic at the time of PCR but they developed symptoms later on and they were included in category of symptomatic patients. PCR testing was more focused on symptomatic patients and patients with history of close contact with known COVID-19 patients so we may have missed certain asymptomatic patients.

Fever and cough were most common symptoms in our patients. Our findings are consistent with other international studies.[4,17,18] however fever was less common in our study as compared to some other international studies.[1,3,5,6] Dyspnoea was found in 76 (29%) of patients which was similar to some international studies[1,4,9,10] but less than some other studies because those studies just considered hospital admitted patients[3,5]. The mean time from initial symptoms to development of dyspnoea was 7 days which was very much similar to other studies.[1,4] Sixteen patients (12%) had gastrointestinal symptoms in our study which were also common manifestations of the disease in some other studies.[1,4,17]

Major comorbidities in our patients were Hypertension and Diabetes mellitus followed by cardiovascular diseases which was comparable to international studies.[3,5,8–10] Twelve patients (4.5%) required invasive ventilation in our patients which was comparable to some studies[2,9] but lesser than other studies.[1,5,6] Comorbidities were also associated with more severe disease, ICU admission and greater mortality. Severity of disease and mortality was even more common in people with more than one comorbidity. This finding was also comparable to other international studies.[1,3,5,6,8,9]

Eighteen patients (7%) died out of total 266 patients. Mortality rate was quite variable in other international studies as well depending upon certain factors e.g age of patients and comorbidities of patients.[1,3,4,6,9,18] Increasing age was directly linked to severity of disease, requirement of ICU admission and ventilator support in our study. People who were more than 55 years of age had more severe disease, there was more requirement for respiratory support and mortality was also more common than younger population. These findings are consistent with other international studies.[1,3,5,6,15.17]

In our study, female patients had more severe disease but as we had very a smaller number of female patients so this finding cannot be generalized.

Mean time period between in initial onset of symptoms to PCR testing in PCR positive cases was 6 days which correlated with other studies.[19,20] Time period between patient exposures to known COVID-19 positive patients to PCR testing in PCR positive cases was 2 to 17 days. Although in most cases exposure was not known so PCR testing was done after onset of symptoms.

Mean viral shedding duration after initial positive PCR was 21 days in our study which was comparable to other international studies.[6,21–24] Variation in duration of viral shedding between 11–55 days could be related severity of disease.[21,24] Moreover viral shedding does not correlate with infectivity duration of patient which was recently found out to be 8 days from symptom onset to PCR test by a recent study.[23]

We concluded that SARS-CoV-2 can cause disease with wide range of clinical spectrum and disease can be fatal as well. Severity of disease, requirement of ICU care and mortality were directly linked to age of the patient and underlying comorbidities. Strict preventive measures including hand hygiene, cough etiquettes and wearing of masks are of utmost importance particularly in this high-risk population. More studies are required to

ascertain the infectious period of virus and prevent its transmission. Strengths of the study are that rigour was applied in carrying out laboratory work. Generalizing it towards population at large would need caution as it was conducted on a limited population.

Ethics approval and consent to participate

Ethics approval and consent to participate Approval to conduct this study from was obtained from Institutional Ethical Committee, PNS SHIFA hospital.

Conflict of interest

The authors have no financial or other interests with regard to the submitted manuscript that might be construed as a conflict of interest.

AUTHORS' CONTRIBUTION

AH: Conceptualization, literature search, Study design, Data collection, Data analysis, Data interpretation, Write up and proof reading. LS: Conceptualization, Data analysis, Data interpretation and proof reading. FH: Conceptualization, Data analysis, Data interpretation and proof reading. MS: Conceptualization, Data analysis, Data interpretation and proof reading. MAG: Data collection, Data analysis, Data interpretation and proof reading. GAKN: Data collection, Data analysis, Data interpretation and proof reading. MNK: Data collection, Data analysis, Data interpretation and proof reading. FS: Conceptualization, literature search, Data collection and Write up. FY: Conceptualization, literature search, Data collection and Write up.

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