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SARS-CoV-2 infection pattern, transmission and treatment: Multi-center study in low to middle-income districts hospitals in Punjab, Pakistan

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Abstract: Pakistan has reported a substantial number of COVID-19 cases since 2020. A multicenter observational study was conducted to identify the pattern of SARS-CoV-2 infection, transmission, and treatment in patients admitted to seven low to middle-income district hospitals in the Province of Punjab, Pakistan from March to June 2020. A total of 102 patients were recruited. 57 patients tested positive and 45 developed moderate-severe COVID-19 disease. About 67% of the patients in March-April and 93% in May-June have contracted the disease from the local transmission. The mean number of symptoms in SARS-CoV-2 positive patients was significantly higher than suspected patients (6.46 vs 5.04, p=0.003). The number of deaths was low (n=8) with 86% recovery rate. Mild COVID patients received acetaminophen (n=102), azithromycin (n=8), and hydroxychloroquine (n=4) in addition to standard medical care. The treatment provided to moderate-severe cases included acetaminophen (45/45), azithromycin (45/45), Ivermectin (14/45) and corticosteroids (13/45). The mean number of antimicrobials was significantly higher in moderate-severe patients than mild cases (1.80 vs 1.12, p=0.001). Low number of deaths with a high recovery rate was reported. Diabetes was the most common comorbid condition followed by hypertension. Many antimicrobials were prescribed in both mild and moderate-severe cases that require careful review.

Keywords: SARS-CoV-2, Punjab, COVID-19, azithromycin, corticosteroids, suspected, confirmed cases.

INTRODUCTION

The world is witnessing the unprecedented challenge of Severe Acute Respiratory Syndrome Coronavirus 2 SAR-CoV-2 (COVID-19) infection after its emergence in a populated city of Wuhan, China in December 2019. Despite employing numerous controlling strategies by World Health Organization (WHO), SARS-CoV-2 has spread across the borders to almost every nation on the globe (World Health Organization, 2020). Up till now, more than 42 million people have contracted the virus with more than 1.2 million people have lost their lives and this has resulted in tremendous social and economic loss across the globe (World Health Organization, 2020)

Coronavirus was believed to cause mild respiratory symptoms in humans and never gained much medical attention until 2002 when severe acute respiratory syndrome (SARS-CoV) was identified in Guangdong Province, China and WHO issued a global alert regarding the disease. SARS-CoV leads to 916 deaths in 29 countries in 2002-2003, with the majority of cases in China. Another severe lower respiratory tract infection occurring from Coronavirus, identified in Saudi Arabia in 2012 was Middle East Respiratory Syndrome (MERS) (Omrani and Shalhoub, 2015). MERS resulted in 858 deaths globally However, both strains. were well contained and did not spread rapidly and caused a pandemic situation. (Hosseiny *et al.,* 2020).

SARS-CoV-2 is a positive sense, single-stranded RNA virus belonging to genus Beta coronavirus (Paul *et al.,* 2020). It spreads from an infected individual to others through the air while sneezing, coughing, touching, and rarely through the orofecal route. The common sign and symptoms of coronavirus are fever, sore throat, cough, shortness of breath, muscle ache, confusion, headache, sore throat, rhinorrhoea, chest pain, diarrhoea, and nausea and vomiting (Chen *et al.,* 2019; Guo *et al.,* 2020). The infants, older people, immunocompromised and

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cardiopulmonary diseased persons are at a greater risk (Center for diseases control and prevention, 2020)

In Pakistan, the very first case of SARS CoV 2 was reported on February 26th and since then an exponential increase in the number of cases has been reported. According to the National Disaster Management Authority, about 195,745 positive cases and 3,972 deaths were reported throughout the country with 328, 602 positive cases and 6,739 fatalities have been reported in Punjab province (National disaster management authority, 2020). A study published regarding clinical characteristics of SARS-CoV2 in the Pakistani population, conducted at a tertiary care hospital in Karachi, Sindh reported dry cough and fever as the most common symptoms in positive patients. The median age of positive patients was 52 years and the most common comorbidities were diabetes and hypertension (Asghar *et al.,* 2020) Another study conducted in Lahore, Punjab reported fever, cough, fatigue, runny nose or nasal congestion and vomiting were more common in confirmed SARS CoV2 patients (Khan *et al.,* 2020). Data on clinical characteristics, transmission, the pattern of illness, and treatment provided, particularly from health facilities in low to middle-income regions, are scarce in Pakistan. The current study aims to assess the SARS-CoV-2 infection pattern, transmission, clinical characteristics, and treatment provided in seven hospitals of Punjab Province in Pakistan.

MATERIALS AND METHODS

Study design and population

This observational study assessed the COVID-19 infection pattern, clinical characteristics, transmission of SARS-CoV-2 virus, and treatment provided to the patients admitted to seven hospitals included District Headquarters (DHQ), Tehsil Headquarter (THQ), and Tertiary health care hospitals of Punjab Province. The hospitals comprised of DHQ hospital Vehari, DHQ Pakpattan, DHQ Layyah, THQ Haveli Lakkah, THQ Arifwala, THQ Wazirabad and Jinnah Hospital, Lahore. The study was conducted by the team of independent researchers working as frontline healthcare professionals in the above-mentioned health facilities and University staff.

Data collection process

The data of patients suspected of SARS-CoV-2 admitted to seven DHQ hospitals from 25th March 2020 to 14th April 2020 and from 28th May to 14th June 2020 was extracted. The data collection form was prepared that was based on the questions included in the previously published research articles on SARS CoV 2 patients (Chen *et al.,* 2020; Guo *et al.,* 2020). Consenting patients in district hospitals in the province of Punjab, Pakistan were selected using convenience sampling. An interviewer-administered data collection form contained questions that were used to assess demographics, clinical presentation, transmission, and treatment of SARS CoV 2 patients.

Inclusion and exclusion criteria

Patients suspected of SARS CoV2 infection who were quarantined in hospital and the samples were sent for testing were included in the study. Patients suspected of SARS CoV2 infection whose samples were sent for SARS CoV2 testing and discharged were excluded from the study.

Study variables

In the current study different variables including demographic details like age, gender, traveling history, date of appearance of symptoms, hospital admission date was recorded. The presence of clinical symptoms like fever, cough, sore throat, body ache, flu, gastrointestinal disturbances, breathing difficulty, blood pressure, vomiting, hemoptysis, co-morbidities and treatment was noted. A suspected case was defined as a patient who presented with symptoms of fever, cough, and myalgia. Patients with positive PCR results were labeled as confirmed cases. The SARS CoV2 infection pattern in various vicinities of Punjab was analyzed. The suspected and confirmed cases were analyzed for demographics, clinical symptoms, and treatment. The confirmed cases were analyzed for therapies and outcomes.

Ethical approval

Ethical approval was obtained from the Human Ethical Review board (TDR/PRD/ETHICS/ 2000.1) of Lahore College for Women University Lahore. Moreover, permission to gather data was obtained from each health facility prior to data collection.

STATISTICAL ANALYSIS

Microsoft Excel was used to record all variables and records. The statistical analysis was performed using SPSS version 24 with 0.05 as the level of significance. The descriptive statistics were used to present variables as percentages, frequencies, means, and standard deviations. Chi-Sq test was used to compare the categorical variables, for example, gender (male vs female) and type of case (suspected and confirmed cases). In the case of continuous variables, an independent t-test was used to compare the differences, for example, type of case (suspected and confirmed) vs number of symptoms reported by the patients. GraphPad Prism 6 was used to create graphs of categorical variables like transmission and number of suspected and confirmed cases across seven hospitals of Punjab.

RESULTS

A total of 102 patients tested for SARS-COV 2 were recruited in this study: 32 patients in March-April and 70

patients in May-June. The patients who presented with symptoms of SARS-CoV 2 (suspected cases) were admitted to the isolation wards created in the hospitals until the test results were received.

After the test results were received out of 32 patients, 12 (35.48%) patients in March-April, and 45 (64.3%) out of 70 in May-June were tested SARS-CoV-2 positive. In March-April, 67% of the people were found to have contracted the disease from the local transmission and 33% of patients had contracted the illness from someone travelled from abroad whereas, in May-June, 92.9% transmission was local as presented in fig. 1.

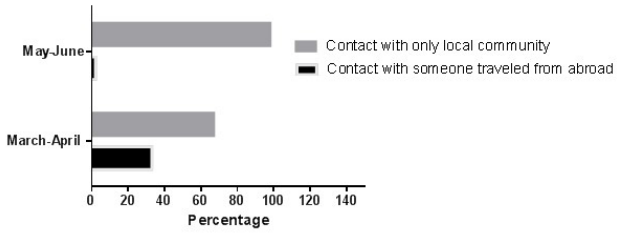

Fig. 1: Comparison of SARS-CoV-2 transmission (Local/From Abroad) in confirmed cases

Out of the total suspected cases admitted to hospitals in March-April, the highest percentage of suspected cases was in DHQ Vehari (31%). Regarding the confirmed cases, 32% of the confirmed cases were in DHQ Vehari whereas only 22% of the confirmed cases were in THQ Arifwala. The remaining 3 hospitals (Pakpattan, Layyah, and Haveli Lakha) had no confirmed cases. In May-June out of the 70 suspected cases admitted to three hospitals (Jinnah hospital Lahore, DHQ Pakpattan, and THQ Wazirabad) 45 (64.3%) were tested SAR-CoV-2 positive (confirmed cases). In Jinnah hospital out of 46 suspected cases, 34 (73.9%) were confirmed for SARS-CoV-2 infection. The results have been presented in fig. 2.

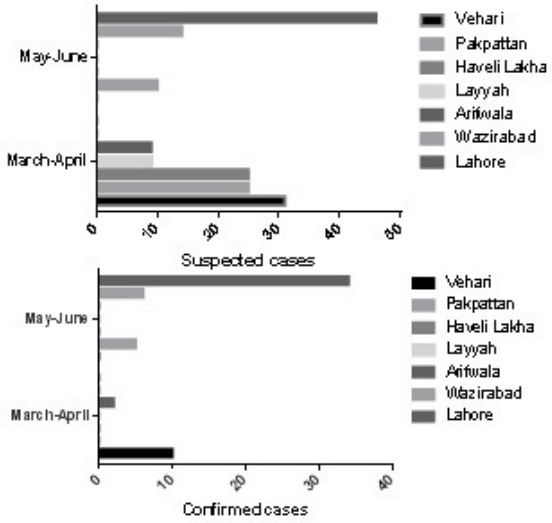

Fig. 2: Number of Suspected and Confirmed cases in the Seven Hospitals of Punjab.

SARS-CoV-2 cases were more common in males and adults aged 31 to 50 years. The mean time from the onset of illness to hospital admission was higher in SARS-CoV-2 positive cases than suspected cases (3.72 vs 3.20, p=0.061). The mean number of symptoms in SARS-CoV-2 positive patients was significantly higher than suspected patients (6.46 vs 5.04, p=0.003). In the current study a total of 12 symptoms (range: 3-12) in SARS-CoV-2 positive and suspected patients. More SARS-CoV-2 positive patients developed a sore throat, severe cough, severe myalgia and difficulty of breathing (table 1).

Of 102 patients, 27 were having DM, 17 having hypertension, and 13 having airway diseases. The majority of these patients (diabetes = 22/27, hypertension = 10/17 and Airway disease = 8/13) were found to have SARS-CoV-2 positive results.

Both SARS-CoV-2 positive and negative patients received several antimicrobials (1.60 vs 1.42, p=0.327). Antivirals and corticosteroids were mainly prescribed to SARS-CoV-2 positive cases. SARS-CoV-2 positive patients had higher mortality (10.5% vs 4.4%, p=0.257) and length of stay than suspected cases (19.49 vs 9.32, p=0.001) (table 2)

Diabetes and hypertension were more common in the moderate-severe form of COVID-19. All the patients received acetaminophen irrespective of disease severity. For the treatment of mild cases, 8 patients received azithromycin (500mg, once a day), 4 patients received Hydroxychloroquine (200mg, twice a day) and three patients received mechanical ventilation (table 3).

The treatment provided to the moderate-severe cases included acetaminophen (1g, three to four times day, 45/45), azithromycin (250 mg, twice a day, 45/45), ivermectin (6 mg, once a day, 14/45) antihistamines (32/45), corticosteroids (13/45) and bronchodilators (21/45). Antiviral therapy oseltamivir was given to 9 patients. The mean number of antimicrobials was significantly higher in moderate-severe patients than mild cases (1.80 vs 1.12, p=0.001). Oxygen therapy and mechanical ventilation were given to 34 and 13 patients respectively. Of 16 patients who were shifted to mechanical ventilation, four patients did not survive.

The mean duration of stay in the hospital was not significantly different between mild and moderate-severe patients (15 vs 18, p=0.488). The number of patients who died was insignificantly higher in mild cases then moderate-severe cases as presented in table 3.

DISCUSSION

Pakistan and the whole world are currently facing the SARS-CoV-2 pandemic. Pakistan is a developing country

Table 1: Demographics and symptoms of the suspected and SARS-CoV-2 positive patients admitted to seven health care hospitals

Variables	Confirmed/ Positive n (%)	Suspected/ Negative n (%)	p-value
Gender			
Male	43 (75.4)	28 (62.2)	0.150
Female	14 (24.6)	17 (37.8)	
Age (Years)			
10-30	9 (15.8)	21 (46.7)	0.001
31-50	33 (57.9)	21 (46.7)	
>50	15 (26.3)	3 (6.7)	
Symptoms onset to admission (days)	3.72 ± 1.22	3.20 ± 1.56	0.061
No of symptoms	6.46 ± 2.74	5.04 ± 1.64	0.003
Fever (°F)			
<99°F	11 (19.3)	3 (6.7)	0.001
99.1-100.4	5 (8.8)	5 (11.1)	
100.5-102	29 (50.9)	11 (24.4)	
>102	12 (21.1)	26 (57.8)	
Sore Throat			
Yes	44 (77.2)	18 (40.0)	0.001
No	13 (22.8)	27 (60.0)	
Cough			
Mild	16 (28.1)	16 (35.6)	0.016
Moderate	19 (33.3)	23 (51.1)	
Severe	22 (38.6)	6 (13.3)	
Flu			
Rhinorrhea	18 (31.6)	11 (24.4)	0.159
Stuffy	18 (31.6)	9 (20.0)	
Myalgia			
Mild	22 (38.6)	23 (51.1)	0.028
Moderate	14 (24.6)	16 (35.6)	
Severe	21 (36.8)	6 (13.3)	
Vomiting			
Yes	38 (66.7)	37 (82.2)	0.077
No	19 (33.3)	8 (17.8)	
Difficulty Breathing			
Yes	38 (66.7)	19 (42.2)	0.014
No	19 (33.3)	26 (57.8)	
Headache			
Present	43 (75.4)	24 (53.3)	0.020
Absent	14 (24.6)	21 (46.7)	
Expectoration			
Yes	33 (57.9)	8 (17.8)	0.001
No	24 (42.1)	37 (82.2)	
Hemoptysis			
Yes	12 (21.1)	2 (4.4)	0.016
No	45 (78.9)	43 (95.6)	
Loss of smell			
Yes	8 (14.0)	1 (2.2)	0.049
No	49 (86.0)	44 (97.8)	
Loss of taste			
Yes	8 (14.0)	2 (4.4)	0.057
No	49 (86.0)	43 (95.6)	

Table 2: Comparison between SARS-CoV-2 positive and suspected cases for treatment and outcomes

Variables	Confirmed/ Positive n (%)	Suspected/ Negative n (%)	p-value
No of antimicrobials	1.60 ± 1.05	1.42 ± 0.62	0.327
Antivirals			
Yes	11 (19.3)	1 (2.2)	0.008
No	46 (80.7)	44 (97.8)	
Corticosteroids			
Yes	13 (22.8)	2 (4.4)	0.009
No	44 (77.2)	43 (95.6)	
Oxygen Therapy			
Yes	34 (59.6)	5 (11.1)	0.001
No	23 (40.4)	40 (88.9)	
Ventilator Therapy			
Yes	16 (28.1)	0 (0.0)	0.001
No	41 (71.9)	45 (100.0)	
Diabetes			
Yes	22 (38.6)	5 (11.1)	0.002
No	35 (61.4)	40 (88.9)	
Hypertension			
Yes	10 (17.5)	7 (15.6)	0.789
No	47 (82.5)	38 (84.4)	
Airway diseases			
Yes	8 (14.0)	5 (11.1)	0.660
No	49 (86.0)	40 (88.9)	
Mortality			
Yes	6 (10.5)	2 (4.4)	0.257
No	51 (89.5)	43 (95.6)	
Length of stay	19.49 ± 4.86	9.32 ± 8.36	0.001

and because of the limited resources available in the health care sector, the Government of Pakistan decided to take early preventive steps and announced a lockdown in the province of Punjab on March 22nd, 2020. The early spread of infection was reported in the last week of February and by the end of March in Punjab 1493 confirmed cases with fifteen deaths were reported (Waris *et al.,* 2020)

In March-April, the data of confirmed cases showed a lower percentage (35.38%) of local disease transmission. This suggests that preventive measures taken by the Government of Punjab decreased the rate of transmission. Earlier reports from Pakistan showed transmission from abroad and as seen in our study 33% of patients contracted the disease from abroad. (Waris *et al.,* 2020). However, after May 9th, 2020, the government of Pakistan decided to remove the lockdown and most of the public places were opened. The data acquired in May- June showed 93% of local transmission.

In the current study, the majority of the confirmed cases were males suggesting that the male population was at higher risk of contracting SARS CoV-2 infection. Our results are comparable to previously published results who also reported that the male population was affected by SARS-CoV-2 illness. (Khan *et al.,* 2020; Huang *et al.,* 2020; Xu *et al.,* 2020). Women are less susceptible to acquire viral infections based on the factors related to X chromosomes, steroid hormones, and innate immunity. It has been reported that women have high expression of TLR7 responsible for the recognition of single-stranded RNA virus, production of antibodies against virus and expression of interleukin 6 and 1 (Conti and Younes, 2020). The average days from the onset of symptoms to hospital admission was 3.72±1.22 days. The early detection of the SARS-CoV-2 infection limits the spread of the illness. About 58% of confirmed patients were between 31- 50 years. None of the patients was above 60 years. The median age of the confirmed patients was 34 years suggesting that people aged between 31-50 years are at higher risk of contracting the illness in Punjab.
This finding is different from the previous studies conducted in China (median age: 49 yrs) (Huang *et al.,* 2020; Xu *et al.,* 2020) and in Karachi, Pakistan (median age: 53.8 years) (Asghar *et al.,* 2020). Davies and his colleagues reported that the incidence of clinical cases in low middle countries with younger population structure would be lower, however, comorbidities will influence the disease severity. Our study complies with these findings as Pakistan has a high ratio of the young population (Davies *et al.,* 2020; Khan *et al.,* 2020).

Table 3: Comparisons between mild and moderate-severe cases for treatment and outcome

Item	Mild cases n (%) (n=32)	Moderate-Severe n (%) (n=45)	p-value
Diabetes			
Yes	8 (19.0)	19 (31.7)	0.155
No	34 (81.0)	41 (68.3)	
Hypertension			
Yes	6 (14.3)	11 (18.3)	0.589
No	36 (85.7)	49 (81.7)	
Airway disease			
Yes	7 (16.7)	6 (10.0)	0.320
No	35 (83.3)	54 (90.0)	
Oxygen Therapy			
Yes	0 (0.0)	34 (73.9)	0.001
No	32 (100)	11 (23.9)	
Mechanical ventilation			
Yes	3 (7.1)	13 (21.7)	0.047
No	39 (92.9)	47 (78.3)	
Treatment			
Acetaminophen.	12 (37.5)	45 (100)	
Acetaminophen + Azithromycin	1 (3.1)	45 (100)	
Acetaminophen + Oseltamivir + Azithromycin	1 (3.1)	-	
Acetaminophen + Azithromycin + Hydroxychloroquine	2 (6.2)	5 (11.1)	
Acetaminophen + Azithromycin + Hydroxychloroquine + Antihistamine	2 (6.2)	-	
Acetaminophen + Azithromycin + Antihistamine	2 (6.2)	32 (71.1)	
Acetaminophen + Azithromycin + Corticosteroids	-	13 (28.9)	
Acetaminophen + Azithromycin + Ivermectin	-	14 (31.1)	
Multivitamins	-	12 (26.7)	
Bronchodilators	-	21 (46.7)	
Oseltamivir	-	9 (20.0)	
Days from the onset of symptoms to hospital admission	3.52 ± 1.68	3.47 ± 1.16	0.555
No of antimicrobials	1.12 ± 0.83	1.80 ± 0.82	0.001
Hospitalization in days (mean ± SD)	15.4 ± 11.8	18.0 ± 3.5	0.488
Death			
Yes	5 (11.9)	3 (5.0)	0.202
No	37 (88.1)	57 (95.0)	

In this study clinical manifestations like fever, sore throat, myalgia, and flu presented in table 1 complies with previous studies reported from China (Tian, Hu and Lou, 2020; Guan *et al.,* 2020) and also predicts the genome sequence SARS-CoV-2. The genomic sequencing of SARS CoV 2 provides an aid in the investigation of disease, prevention, and treatment (Wang *et al.,* 2020) Olfactory and gustatory dysfunctions have been reported in some confirmed cases (table 1). These dysfunctions were more common in patients in Western countries, where SARS CoV 2 existed with a different genome sequence (Lechien *et al.,* 2020). The difference in the clinical picture of various patients suggests that coronavirus with varying genome sequences may exist in the communities in Punjab. Foster and his colleagues indicated that first of the case identified in Mexico, Canada and Brazil carried the genome network of SARS CoV 2 virus brought from the countries they had travelled (Foster *et al.,* 2020). Approximately 11.29 million Pakistanis live abroad in various countries (https://beoe.gov.pk/reports-and-statistics) supporting the notion that SARS CoV 2 of various genome sequences might exist in the Pakistani community. Vomiting reported in 67% confirmed cases may occur due to direct viral damage to the intestine and the primary underlying mechanism reported so far is the expression of angiotensin-converting enzyme 2 expressed on the surface of gastrointestinal epithelial cells (Smyk *et al.,* 2020).

Diabetes had a higher prevalence (table 2) among confirmed cases in our study. Hypertension was more common than airway diseases. About 32% of the diabetic patients developed moderate to severe illness. Several mechanisms have been proposed to explain the increased severity of SARS CoV-2 illness in diabetic patients. These many include reduced viral clearance, efficient entry into

the host cells, reduced T-cell function, and increased susceptibility of cytokine storm and hyperinflammation. Most of the diabetic patients prescribed with angiotensin-converting enzyme inhibitors or angiotensin receptor blockers, thiazolidinediones, glucagon-like peptide 1 receptor agonists and with high levels of furin, immune dysfunction could have a high prevalence of severe SARS Cov-2 illness (Azar *et al.,* 2020).

The treatments of SARS-CoV-2 presented in table 3 showed that all moderate to severe patients received acetaminophen with azithromycin and about 11% of patients received acetaminophen, azithromycin, and hydroxychloroquine. This treatment has been reported to reduce the SARS-CoV-2 viral load. In molecular docking studies, azithromycin was found to be directed against the virus and hydroxychloroquine works against the attachment of the virus with the plasma membrane (Million *et al.,* 2020). There is a synergistic anti-viral effect of hydroxychloroquine and azithromycin therapy on SARS-CoV-2 infection if it is administered before SARS-CoV-2 complications develop, however, the combination has shown to be of no benefit in severe COVID-19 infections (Million *et al.,* 2020; Molina *et al.,* 2020) Both drugs prolong the QT interval thereby reducing their use in moderate to severe patients (Fatini, Chahinian and Yahi, 2020). Ivermectin in combination with azithromycin was administered to 31% of moderate to severe patients. Ivermectin is a broad-spectrum antiparasitic drug with anti-viral properties. The mechanism reported by Rizzo suggests that ivermectin is an ionophore that could enter the host cells and exploit their biochemical pathways to inhibit the production of other viral particles (Rizzo, 2020). About 20% of moderate to severe patients received oseltamivir. Oseltamivir in combination with ritonavir and lopinavir have shown high effectiveness against SARS-CoV-2 proteases in computational studies (Muralidharan *et al.,* 2020). The patient who was administered oseltamivir received negative results of viral load after 18 days on average from the hospital admission and had the shortest stay at the hospital.

Regarding eight patients (p=0.202) who did not survive presented to the hospital with high-grade fever, severe myalgia, severe cough, expectoration, headache, stuffy nose, vomiting and dyspnea. About 50% of them were hypertensive. The treatment provided to 70% of patients was hydroxychloroquine and azithromycin. As the combination prolongs the QT interval, therefore, it may be suggested to use this combination with caution in the future (Fatini, Chahinian and Yahi, 2020)

This study has certain implications. Most of the studies published in the literature are from China and the western world. This is the first study from Punjab, Pakistan to report the pattern of illness, demographics, clinical symptoms, transmission, and treatments of the SARS-CoV-2 patients. From the current study it could be hypothesized that due to the differences in the outcome of the SARS-CoV-2 illness around the globe, demographics and clinical outcomes should be reported as early as possible to increase clinical awareness among health care professionals more efficiently to combat the disease.

The current study had several limitations. Some of the limitations such as the use of convenience sampling may lead to selection bias. Small sample size was a result of the restriction imposed by the ethical committees of the hospital that allowed access to limited data due to strict preventative measures. The medical records of all the suspected cases admitted to seven hospitals from 25th March till 14th June were not allowed to access.

CONCLUSION

The number of suspected patients was higher as compared to the confirmed cases of SARS-CoV-2. Different genomes of SARS CoV 2 may exist in Punjab thereby showing differences in the clinical picture of the confirmed cases. A combination of hydroxychloroquine and azithromycin should be avoided in critically ill patients. Oseltamivir, corticosteroids, and ivermectin have shown promising results.

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