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# Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network



RESEARCH ARTICLE

BIOSCIENCE RESEARCH, 2021 18(2): 1608-1613.

OPEN ACCESS

## Molecular epidemiology of SARS-COV-2 in Mardan, Khyber Pakhtunkhwa Pakistan: A real world clinical experience

Faheem Anwar<sup>1</sup>, Muhammad Zubair<sup>2</sup>, Muzamil Shah<sup>\*3,2</sup>, Sohail Ahmad<sup>1</sup>, Ihteshamul haq<sup>1</sup>, Maria Mehmood<sup>4</sup>, Shumaila Bakht<sup>5</sup>, Fazli Zahir<sup>6</sup>, Zia Ud Din<sup>5</sup>, Faizan Ullah<sup>1</sup>, Shakir Ullah<sup>1</sup>, Taiba Bibi<sup>1</sup>, Zeeshan Nasar<sup>1</sup>, Inam Ullah<sup>7</sup> and Obaid Ullah Shah<sup>8</sup>

<sup>1</sup>Department of Biotechnology and Genetic Engineering, Hazara University, Mansehra 21300, KP, Pakistan

<sup>2</sup>Department of Pathology, Bacha Khan Medical College Mardan KP Pakistan

<sup>3</sup>Department of Microbiology, Abdul Wali Khan University Mardan Peshawar KP Pakistan

<sup>4</sup>Department of Pathology, Khyber Medical College Peshawar KP Pakistan

<sup>5</sup>Department of Human Nutrition the University of Agriculture Peshawar KP Pakistan

<sup>6</sup>Department of Allied Health Sciences, Iqra National University Peshawar KP, Pakistan

<sup>7</sup>Center for Biotechnology and Microbiology, University of Swat, KP, Pakistan

<sup>8</sup>Department of Biotechnology, Abdul Wali Khan University Mardan Peshawar KP Pakistan

\*Correspondence: [muzamilshah804@gmail.com](mailto:muzamilshah804@gmail.com) Received 04-03-2021, Revised: 06-05-2021, Accepted: 19-05-2021 e-Published: 20-05-2021

The coronavirus disease 2019 (Covid-19) outbreak represents a thankfully rare yet extremely salient incident that had an adverse effect on individuals, families, and societies throughout the world. COVID-19 has the highest infectivity and spreading rate as compared to other human coronaviruses. SARS-CoV-2 was recognized as the disease causative agent that initially targets humans' respiratory system that may lead to multi-organ failure and death. To date, COVID-19 spread to 220 countries/regions of the world, including Pakistan. This study aimed to examine the prevalence of COVID-19 among the general population in District Mardan of Khyber Pakhtunkhwa Province, Pakistan. A total of 4199 suspected individuals (2898 were males, and 1301 were females) were screened through RT-PCR. The age of suspected individuals was ranging from 10-90 years, and the average age was found  $42 \pm 20.8$  years. Among all suspected individuals, 753 were SARS-CoV-2 positive and diagnosed with COVID-19. Among the positive individuals, 500 (66%) were males, and 253 (34%) were females. The overall prevalence was found at 17.93%. Most of the SARS-CoV-2 positive individuals were belonging to age-group 31 to 40 (n=182), followed by age-group 21 to 30 (n=166). The prevalence of COVID-19 in the Mardan district is very high and needs WHO recommended SOPs to control the spread of COVID-19.

**Keywords:** COVID-19; SARS-CoV-2; Prevalence; District Mardan

### INTRODUCTION

The coronavirus disease 2019 (COVID-19) outbreak was first described in Wuhan city, Hubei province of China, in late December 2019 Guan et al.2020. It is believed that this disease has

originated from the local human seafood market in Hunan and is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) Shereen et al.2020.COVID-19 is a very contagious disease and spread throughout the

globe in a very short period Adhikari et al.2020 .After global spread, the World Health organization recognized and declared it as a pandemic on March 12, 2020 Ali et al.2020.The SARS-CoV-2 is a member of coronaviruses belonging to the genus of *beta-coronaviruses* in the *Coronaviridae* family that causes respiratory illnesses in humans [5]. The first outbreak of human coronavirus was caused by severe acute respiratory syndrome coronavirus (SARS-CoV) in China in 2002 Zhong et al.2003. In 2012, in Saudi Arabia, another outbreak was caused by the human coronavirus, and the Middle East Respiratory Syndrome coronavirus (MERS-CoV) was recognized as the causative agent of that outbreak Bleibtreu et al.2019. The SARS-CoV-2 has high infectivity rate and low fatality and mortality rate compared to SARS-CoV and MERS-CoV Shereen et al.2020 and Guan et al.2020.As of December 8, 2020, the COVID-19 infected 66.4 million people with 1.5 million mortalities worldwide World Health Organization.<https://www.who.int/emergencies/diseases/novel-coronavirus-2019>

COVID-19 is a zoonosis and can be transferred from animals to humans, humans to humans, and animals to animals and can be spread through person to person contact and aerosol air Mackenzie and Smith 2020. COVID-19 has various clinical signs and symptoms ranging from mild/ asymptomatic infection to acute respiratory distress syndrome (ARDS), multi-organ failure, and life-threatening illness Zhou and Zhou 2019.The initial symptoms of COVID-19 include fever, cough, fatigue, and shortness of breath, myalgia, and when the condition becomes worsened, then it may lead to vomiting, nausea, diarrhea, septic shock, failure of organs that may lead to death Zhou and Zhou 2019. Real-Time Polymerase Chain Reaction (RT-PCR) is the WHO recommended technique for the diagnosis of COVID-19 Ravichandran et al. 2020

RT-PCR detects different genes of SARS-CoV-2 RNA, such as ORF1a, N gene, and E gene Ravichandran et al. 2020.To date, no FDA approved drugs are available for the treatment of COVID-19 Drożdżał et al. 2020.. Numerous vaccines have been developed and are in different stages of clinical trials.

The first case of the COVID-19 outbreak was reported by the Ministry of National Health Services, Regulation & Coordination Pakistan on February 26, 2020 Waris et al. 2020. As of December 8, 2020, the ministry of health services Pakistan reported 0.42 million infected cases

along with 8487 mortalities Ministry of national health services, Govt of Pakistan. <http://covid.gov.pk/>

The Government of Pakistan is taking all the measures such as designated hospitals, isolation wards, quarantine centers, and specialized diagnosis centers to combat this deadly outbreak Waris et al. 2020. In this study, we explored the epidemiology and transmission patterns of patients with COVID-19 in Pakistan. We have also tried to acknowledge how many patients have recovered in quarantine and isolation centers by treatment and factors involved in the control COVID-19 outbreak.

## MATERIALS AND METHODS

### Study design and participants

This retrospective study was carried out from March 25 to July 25, 2020, on 4199 consecutive patients who had COVID-19 symptoms such as fever, cough, shortness of breath, and muscles pain and suspected individuals who had contact history with confirmed COVID-19 patients who were admitted in various quarantine centers of District Mardan and isolation wards of Mardan Medical Complex, District Mardan, Khyber Pakhtunkhwa, Pakistan.

### Inclusion and Exclusion Criteria

All the symptomatic patients who had symptoms like fever, shortness of breath, and cough had included in this study. All the included individuals had ages ranging from 10-90 years. Individuals who were immunocompromised, non-specific history such as no specific location and medical history and covert patients were excluded from the study.

### Sample Collection

According to WHO guidelines, nasopharyngeal swab samples were collected from all of the symptomatic and suspected individuals admitted in various isolation wards and quarantine centers of District Mardan. These samples were then sent to the Department of Pathology, Mardan Medical Complex, for further processing. All the necessary standard operating procedures were taken during sample collection and shipment.

### Sample Processing

All the collected samples were then analyzed with Real-Time PCR (Rotor-Gene Q) for the

diagnosis of COVID-19/ detection of SARS-CoV-2 RNA/genome.

### Statistical Analysis

The Chi-Square test was applied for the analysis of data using IBM SPSS 0version 250.  $P < 0.05$  was considered significant.

### RESULTS

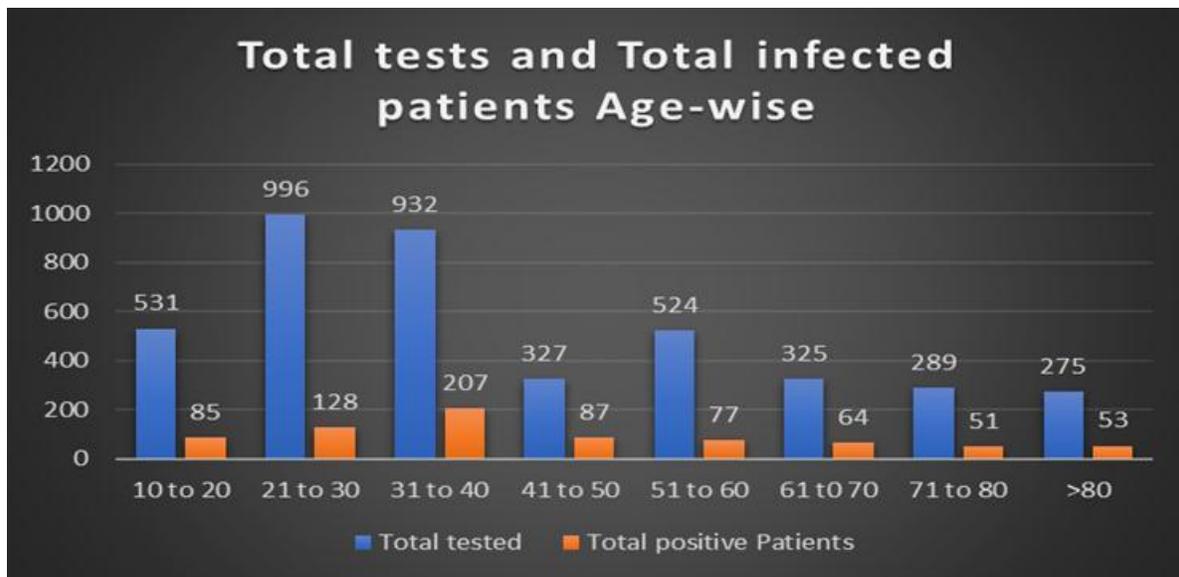
A total of 4199 suspected patients were recruited in this study. Among all individuals, 2898 were male, and 1301 were females. The age of all individuals was ranging from 10 to 90 years, and the average age was found  $42 \pm 20.8$  years. Male individuals were highly affected as compared to female individuals. The overall prevalence in Mardan was found 17.93 % (753 out of 4199), in

which 500 (66%) were males and 253 (34%) were females. No significant difference was found among males and females' individuals, as shown in table 1 and figure 1. However, a significant difference was found among different age groups 10 to 20 ( $p < 0.05$ ), 41 to 50 ( $p = 0.009$ ), 71 to 80 ( $p < 0.001$ ), and group having ages  $> 80$  ( $p < 0.05$ ). In contrast, no significant difference was found among all other groups shown in table 1 and Figure 1. Most of the affected patients belonging to age-group 31 to 40, 113 were males, and 69 were female, followed by age-group 21 to 30 (75 were males, and 12 were females). The gender and age-wise distribution of all the tested and positive cases are shown in Table 1 and Figure 2.

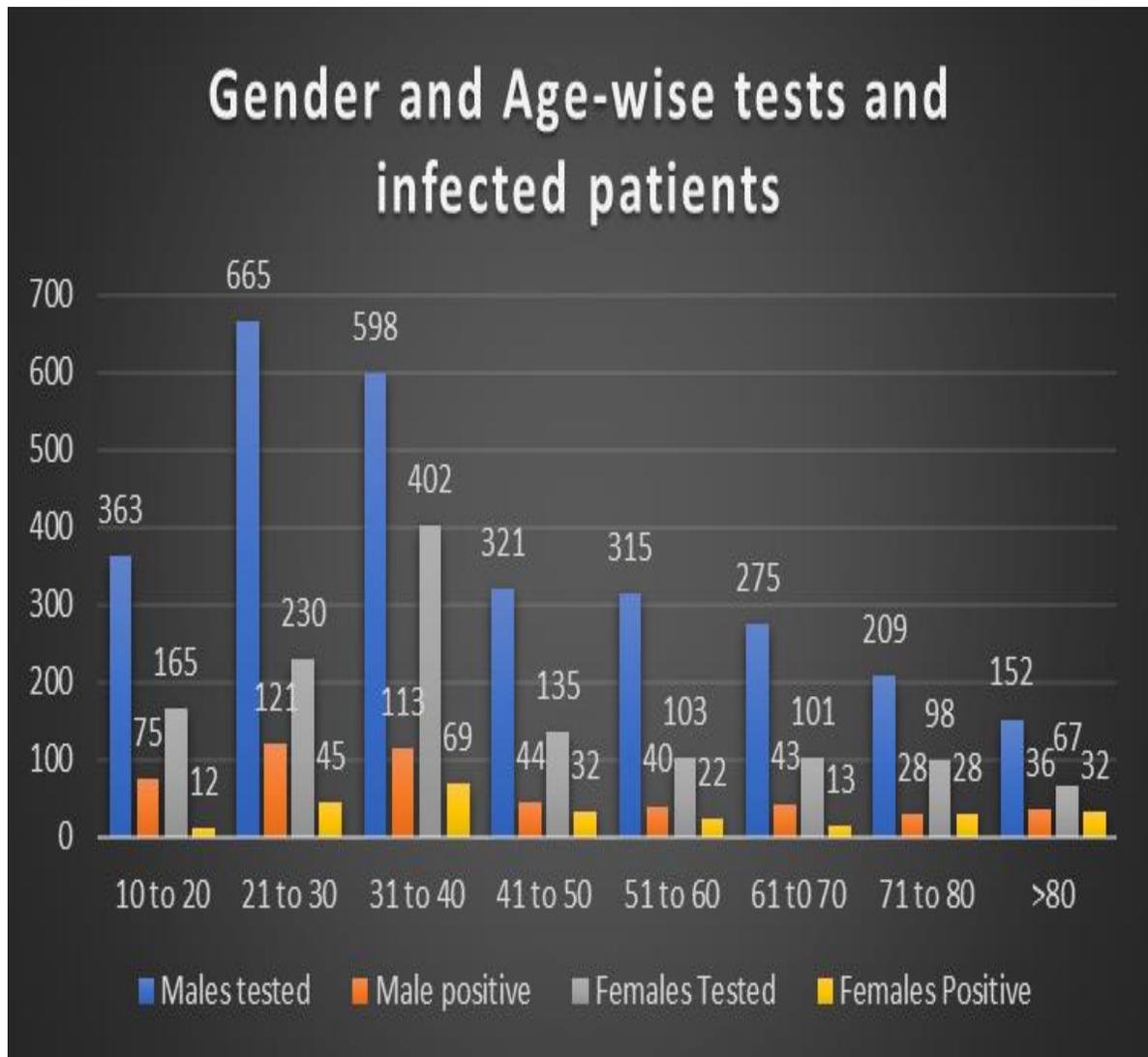
**Table 1: Gender wise distribution of COVID-19 in District Mardan**

Age	Total tested	Males tested	Male positive	Females Tested	Females Positive	Total positive Patients	P-value
10-20	528	363	75	165	12	87	< 0.05
21-30	895	665	121	230	45	166	0.64
31-40	1000	598	113	402	69	182	0.48
41-50	456	321	44	135	32	76	0.009
51-60	418	315	40	103	22	62	0.32
61-70	376	275	43	101	13	56	0.504
71-80	307	209	28	98	28	56	0.001
>80	219	152	36	67	32	68	< 0.05
Total	4199	2898	500	1301	253	753	0.087

\*P-value < 0.05 was considered significant and P-value > 0.05 (Non- Significant).



**Figure 1: The age wise ratio between total tested and positive patients.**



**Figure 2: Gender and age-wise distribution of all the tested and positive individuals among various groups.**

## DISCUSSION

The COVID-19 pandemic has presented a devastating effect throughout the world. According to WHO, the COVID-19 currently spread to 220 countries/regions of the world. Pakistan reported the first case of COVID-19 on February 26, 2020, and now, approximately 0.42 million peoples have been infected with more than eight thousand mortalities. After the unexpected rise in the Covid-19 cases in Pakistan, the Government of Pakistan took various measures such as suspension of internal flights and local transport, complete lockdown, the establishment of quarantine centers, isolation wards, special hospitals for COVID-19 patients, and campaigns on electronic, print, and social media to control the spread of COVID-19.

In this study, 4199 OVID-19 suspected individuals were examined in the Mardan District of Khyber Pakhtunkhwa. All the collected samples were screened for SARS-CoV-2 detection using Rotor-Gene Q real-time PCR. Infected persons showed remarkable results and provide sufficient evidence of SARS-CoV-2 infection. SARS-CoV-2 Sreveals an 80% match of genome sequencing with SARS-CoV-1, which remains a worldwide outbreak in 2002-2003, having 8096 cases [9]. Presumed person-to-person transmission of SARS-CoV-2 was suggested based on epidemiologic and clinical evidence Ravichandran et al. 2020, Zhou and Zhou 2019. Although SARS-CoV-2 shares similar sequencing characteristics with SARS-CoV and Middle East respiratory syndrome coronavirus (MERS-CoV). Viral shedding patterns of the SARS-CoV-2 are dissimilar from SARS-CoV. Viral loads or progenies are in less quantity very after infection and become enormous in 10 days Drożdżal et al. 2020

Our observation was based on RT-PCR, which occurred positive within three weeks after the appearance of symptoms of SARS-CoV-2. We observed that individuals with many diseases and aged are susceptible and possess more symptoms of SARS-CoV-2. Previous studies also suggested that coronavirus is more likely to infect older individuals, in whom the immuno pathogenesis and induction of a prion flammatory cytokine secretion might be responsible Waris et al. 2020. Aged patients with waken defense systems take a long time for viral exclusion.

False-negative results on RT-PCR for SARS-CoV-2 are very common in clinical situations, and errors in sampling and testing. Furthermore, it is suggested from the current diagnosis and

treatment strategies for SARS-CoV-2 from the Chinese National Health Committee that the criteria for discharging a patient must have relief from the symptoms, improvement in radiography, and two successive negative RT-PCR results for SARS-CoV-2 [Ministry of national health services, Govt of Pakistan. <http://covid.gov.pk/>

Proof proposed that the epidemic of COVID-19 might have corresponded to its quick individual-to-individual transmission capacity [15]. Since a complete and well-furnished diagnosis of SARS-CoV-2 has not yet been researched. However, traditional, and possible health methods like isolation, quarantine, and community control are physical attempts to avoid its spread. The current study found evidence of the outbreak of SARS-CoV-2 in non-ICU patients in epidemic season. Based on our observation, we propose further observation and continuous test of RT-PCR from the respiratory tract to eliminate patients safely from isolation centers.

## CONCLUSION

From the current study, it could be concluded that the COVID-19 prevalence rate is too high in District Mardan, Khyber Pakhtunkhwa. The prevalence rate is 17 %. The overall male to female ratio is 2:1. The age group, 31-40, is the most affected one. The fatality and infectivity rate of COVID-19 is very high, and the infected individuals should be isolated to control the spread of the disease.

## CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

## ACKNOWLEDGEMENT

I am extremely thankful to Misbah ud din and Abdul Waris Department of Biotechnology Quaid i azam university Islamabad for his valuable suggestions and correction and positive criticism.

## AUTHOR CONTRIBUTIONS

Authors FA, MZ, MS, FA, SA, and IH conceived and designed research. FA, IH, Fu, MM, MHJ, ZN, IU and FK conducted experiments. FA, TB, SU and MZ, OUS analyzed data and wrote the manuscript. All authors read and approved the final version.

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