

ORIGINAL ARTICLE

Prevalence of SARS-CoV-2 Infection during the COVID-19 Second Wave in Chitral, PakistanMahnoor Rizwan^{1*}, Tabasum Naz^{1*}, Muhammad Bilal Khan¹, Angray S Kang², Abbas Khan³, Taimoor Khan³, Liaqat Ali^{4*}**ABSTRACT****Objective:** To determine the prevalence of COVID-19 during the second wave in Chitral, Pakistan.**Study Design:** Cross sectional prevalence study.**Place and Duration of the Study:** The study was conducted in Health Department of Agha Khan Health Service (AKHS) Chitral from 1st September 2020 to 31th March 2021.**Material and Methods:** This data has been collected from Agha Khan Health Service (AKHS) of Chitral during the second wave. The positive cases reported were tested using PCR. A prevalence-based study was conducted to analyze the severity of COVID-19 in Chitral during second wave. A total of 2315 suspected symptomatic individuals consented for whole blood samples and nasal swabs for viral antigen detection by PCR. RNA extraction was performed using EMAG (BIOMERIUUX, France). Real-time reverse-transcription PCR was performed to detect the viral load.**Results:** The second wave of COVID-19 in Chitral district did not cause any serious harm because of the vaccination and public awareness about the infection and social distancing. Overall, 15% of the population was tested positive and the peak was in months of November and December 2020. The higher rate of positivity (69%) was shown in upper rural areas of Chitral district, KP province, Pakistan More than 70% of population got vaccinated by March 2021 that is the highest number of vaccinated people in a city in Pakistan.**Conclusion:** Our prevalence based data analysis indicate that the second wave of Corona virus hit the country but had limited effects in Chitral, possibly due to proper practice of preventive measures and awareness among the citizens including social distancing, good hygienic care and timely vaccination. Most of the cases reported during second wave were in the month of November/December 2020.**Key Words:** Immunity, Outbreak, Prevalence, SARS-CoV-2, Vaccination.**How to cite this:** Rizwan M, Naz T, Khan MB, Kang AS, Khan A, Khan T, Ali L. Prevalence of SARS-CoV-2 Infection during the COVID-19 Second Wave in Chitral, Pakistan. *Life and Science*. 2022; 3(3): 99-104. doi: <http://doi.org/10.37185/LnS.1.1.240>This work is licensed under a Creative Commons Attribution-NonCommercial 4.0 International license. (<https://creativecommons.org/licenses/by-nc/4.0/>). Non-commercial uses of the work are permitted, provided the original work is properly cited.¹Department of Life Sciences

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Funding Source: NIL; Conflict of Interest: NIL

Received: July 18, 2021; Revised: Oct 25, 2021

Accepted: Dec 13, 2021

Introduction

Coronaviruses are important human and animal pathogens. At the end of 2019, a novel coronavirus was identified as the cause of a cluster of pneumonia cases in Wuhan in Hubei Province of China. The rapid transmission of this viral infection, resulted in an epidemic throughout China, followed by the global pandemic. In February 2020, the World Health Organization (WHO) designated the disease as coronavirus disease 19 (COVID-19).¹ The virus that causes COVID-19 is designated severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2); previously, it was referred to as novel corona virus 2019 (2019-nCoV). Full-genome sequencing and phylogenetic analysis indicated that the coronavirus that causes COVID-19 is a beta-coronavirus in the

same subgenus as the severe acute respiratory syndrome (SARS) virus (as well as several bat coronaviruses), but in a different clade. The Coronavirus Study Group of the International Committee on Taxonomy of Viruses has proposed that this virus be designated severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). The Middle East respiratory syndrome (MERS) virus, another beta-coronavirus (bat originated virus), appears more distantly related. It appears likely that bats are the primary source; whether COVID-19 virus is transmitted directly from bats or through some other mechanism (i.e., through an intermediate host) is unknown. Coronavirus belongs to the RNA virus's family, Coronaviridae. The virus is layered with envelope and has a positive sense RNA with single strand and it has a layer of capsid around nucleus, which is of helical symmetry. The human pathogenic coronaviruses, HCoV229E, HCoVHKU1, HCoVNL63 and HCoVOC43 persistently cause infection in the human population and cause general symptoms of the common cold.² The severe illness caused by this family of virus are acute respiratory syndrome (SARS) and the Middle East Respiratory Syndrome (MERS).³ The coronavirus genome is of approximately 30-kilo bases. Generally, it has four structural proteins, Membrane protein (M), Nucleocapsid protein (N), Envelop protein (E) and Spike protein (S) and many other proteins (non-structural).⁴

Following infection with SARS-CoV-2, the majority of patients develop detectable serum antibodies to the receptor-binding domain of the viral spike protein and associated neutralizing activity. However, the magnitude of antibody response may be associated with severity of disease, and patients with mild infection may not mount detectable neutralizing antibodies.⁵ When neutralizing antibodies are elicited, they generally decline over several months after infection, although most studies suggest that neutralizing activity is maintained for up to six to eight months.⁶ Like other viruses, SARS-CoV-2 evolves over time. Most mutations in the SARS-CoV-2 genome have no impact on viral function. Certain variants have garnered widespread attention because of their rapid emergence within populations and evidence for transmission or clinical implications; these are considered variants of

concern. The host receptor for SARS-CoV-2 cell entry is the same as for SARS-CoV, the angiotensin-converting enzyme 2 (ACE2). SARS-CoV-2 binds to ACE2 through the receptor-binding domain of its spike protein.⁷

Coronaviruses range in size from 60 nm to 140 nm in diameter.⁸ HKU1, NL63, 229E, and OC43 are four corona viruses that have been found in humans and cause moderate respiratory illness such as the common cold, whilst SARS, and the Middle East respiratory syndrome (MERS) more severe respiratory disease.⁹ Viruses circulate in a variety of hosts, including humans, birds, and animals, triggering epidemics and pandemics. SARS-CoV-2 has infected nearly 5.5 million people in 212 countries in the six months since the initial cases were recorded, with at least 350000 deaths. While the overall fatality rate of COVID-19 is believed to be around 2%, 10%–20% of people diagnosed with the virus are hospitalized, and more than 50% of COVID-19 patients who are severely ill die as a result of multiple organ dysfunction and severe complications. The disease is spread through inhalation or contact with contaminated droplets, with a 2 to 14-day incubation period.¹⁰

The immunological response of the host to SARS-CoV-2 appears to be important in disease development and clinical symptoms.¹¹ In patients with severe COVID-19, SARS-CoV-2 not only triggers antiviral immune responses, but it can also cause uncontrolled inflammatory responses characterized by significant pro-inflammatory cytokine production, resulting in lymphopenia, lymphocyte dysfunction, and granulocyte and monocyte abnormalities.^{12,13} Immune abnormalities caused by SARS-CoV-2 could lead to microbial infections, septic shock, and severe multiple organ failure. Fever, cough, sore throat, dyspnea, weariness, and malaise are common symptoms. In most people, the condition is minor; nonetheless, it can progress to pneumonia and acute respiratory distress syndrome (ARDS) in the elderly and those with comorbidities and multi organ dysfunction. Many people are asymptomatic.¹⁴ Given the unprecedented global impact of COVID-19, the administration of COVID-19 vaccination is a high global health priority. Different vaccines have been synthesized so far and administered globally. Pakistan is also importing

vaccines like Pfizer, sinoform, sinovac, Oxford/Astra-Zeneca etc. and giving vaccine shots to people above age of 18. Vaccination helps in synthesizing antibodies against SARS-CoV-2 and aids our immune system.¹⁵ In Pakistan, vaccination of COVID-19 has been a challenge due to lack of awareness and education among public especially the rural areas of Pakistan but after the second wave more awareness and knowledge has been spread through different channels among public to convince them for vaccination and get protected against this deadly virus.¹⁶

The virus has spread rapidly all over the world. Pakistan's first case of COVID-19 emerged in Karachi and from there the virus spread in whole country and the highlighted regions were kept under control for certain period of time to limit the spread of the infection.^{17,18} The most affected areas were Punjab, KPK and Sindh.¹⁹ Chitral comes under the province KPK with total population of 447,362, divided into two zones i.e, Upper Chitral and Lower Chitral. The cases of corona virus increased during the first wave day by day because of lack of awareness and social distancing with minimum medical facilities. Many cities got affected with greater positivity rate.

Materials and Methods

The study was conducted in Health Department of Agha Khan Health Service (AKHS) Chitral from 1st September 2020 to 31th March 2021 during the second wave. The positive cases reported were tested using PCR. The protocol followed by the laboratories were as follows:

Sample Collection and Processing

During Covid-19 infection, the total numbers of 2315 suspected symptomatic individuals were consented for whole blood samples and nasal swabs for viral antigen detection by PCR, were collected from different general and private hospitals of District Chitral. Serum was separated from whole blood by centrifugation following a standard protocol and stored at -80°C. For establishing the serological status for antibodies, the rapid device tests were performed using kit (GP Getein, Biotech & Acu-Tell Rapid In-Vitro Diagnostic kit) to detect the presence of antibodies

Against nucleocapsid, from the suspected persons. All the tests were performed according to the respective manufacture's protocols.

This method is a qualitative membrane based immunoassay (IgG and IgM) for the detection of SARS-CoV-2 antibodies against nucleocapsid from whole blood or serum.²⁰

After performing the antibody detection by rapid device tests the individuals having positive antibodies of coronavirus were further confirmed by Real time PCR using (ARGENE SARS- COV-2-GENE) detection kit.¹⁸

Viral RNA Extraction

RNA extraction was performed using EMAG (BIOMERIUUX, France), following the instructions.

Real-time reverse-transcription PCR

Specifically, the SARSCoV2 genome in a triplex reaction: N gene of SARSCoV2 at 530 nm, Size is 148 bp, RdRp gene of SARSCoV2 at 670 nm size is 136 bp, and internal control at 560 nm. Generically the Sarbecovirus (SARS-CoV, SARSCoV2, SARSlike) genomes in a triplex reaction,

Size of the amplified fragment is 148 bp E gene of Sarbecovirus at 530 nm, cellular control (HPRT1 gene) at 670 nm and the size of amplified fragment is 108 bp, The Cell control (Cc) checks for the presence of cells in the sample, which reflects the quality of the sampling and internal control at 560 nm.

A 25 µL reaction contained 5 µL of RNA, 12.5 µL of 2 × reaction buffer provided with the Superscript III one step RT-PCR system with Platinum Taq Polymerase (Invitrogen, Darmstadt, Germany; containing 0.4 mM of each deoxyribonucleotide triphosphates (dNTP) and 3.2 mM magnesium sulphate), 1 µL of reverse transcriptase/ Taq mixture from the kit, 0.4 µL of a 50 mM magnesium sulphate solution (Invitrogen), and 1 µg of bovine serum albumin (Roche). All oligonucleotides were synthesized by Tib-Molbiol (Berlin, Germany). Thermal cycling was performed at 55 °C for 10 min for reverse transcription, followed by 95 °C for 3 min and then 45 cycles of 95 °C for 15s, 58 °C for 30s and the process of RT-PCR is proceeded on (CFX96-Real Time System BIO RAD USA).

Results

According to the prevalence study in Chitral between September 2020 to March 2021, the cases positive for corona virus are low in both upper and lower regions due to the preventative measures taken by government. The total number of patients reported with symptoms were 2315 out of which only 435

cases were positive confirmed by rapid antibody testing and real time PCR as shown in figure 1.

Self Reported Symptomatic and PCR Positive COVID-19

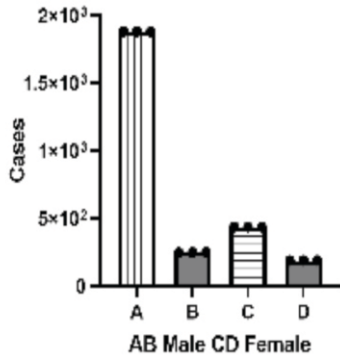


Fig 1: Symptomatic and PCR test confirmed COVID-19 Infections by gender in both upper and lower regions of Chitral

The male patients reporting with covid-19 were 1880 with complaint of minor or major symptoms for covid-19, 248 were confirmed by PCR test. Whereas out of 435 females reporting symptoms 187 were positive by the PCR test. The peak of cases was observed in month of November and December 2020 as shown in figure 2.

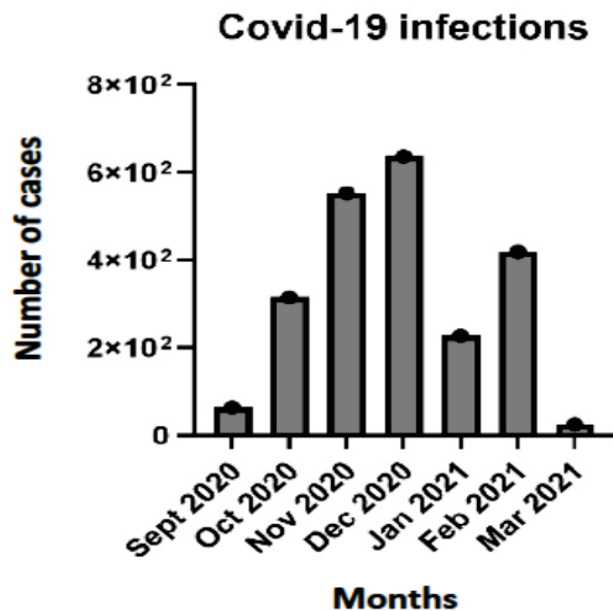


Fig 2: Symptomatic self-reporting of suspected Covid-19 infection between September 2020 and March 2021

Majority of cases were reported from the upper rural region i.e, 69% of Chitral as illustrated in figure 3.

% Covid-19 Infections in Chitral

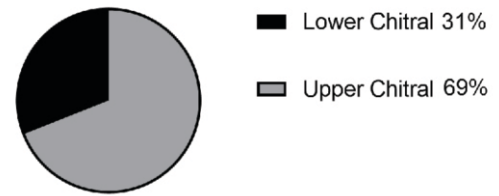


Fig 3: Positive cases of Covid 19 infection in Lower and Upper Chitral

The patients symptomatic for corona were tested by PCR test to confirm infection. Vaccination in order to counter this deadly virus was initiated by the Government of Pakistan. Vaccine was given to 18 years of age and above. So far 54765 people have been vaccinated and as awareness of the benefits of vaccine spreads the number of people vaccinated is increasing, mostly with the Chinese Sinovac vaccine as shown in figure 4.

Vaccination in Chitral

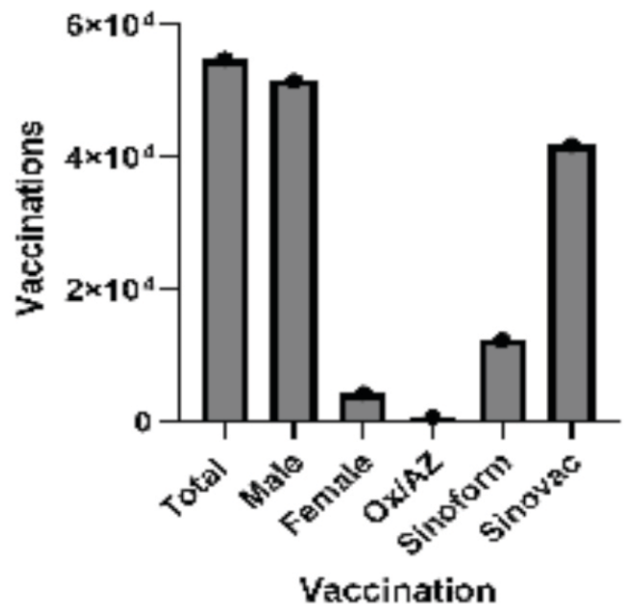


Fig 4: Vaccination in Chitral: By gender and brand of vaccine (Oxford/Astra-Zeneca, Sinoform and Sinovac)

Discussion

Our prevalence based data analysis indicate that the second wave of Corona virus hit the country but had limited effects in Chitral, possibly due to practice of preventative measures and awareness among the citizens that caused social distancing, good hygienic care and timely vaccination.

The patients testing positive for coronavirus were cross checked and declared positive after PCR tests. Since the antibody testing is not reliable of current infection, PCR testing is mandatory for reporting positive cases. PCR test can also give false positive results if the controls are inappropriate or the sample is not properly taken or due to error.²¹ The antibody testing is good enough to get an estimate of the antibody levels which can give a good representation about the immune response and prior exposure, but cannot be relied upon alone and PCR testing is mandatory.²² From the self-reporting of symptomatic cases and subsequent PCR test confirmation, it would appear that the number of positive cases is low which would imply public engagement, awareness and concern about this fatal infection (figure 1).

At the end of first wave the government announced vaccination for people age 40 and above, during the second wave the vaccine was available for all citizens of Pakistan above 18 years of age free of cost, which increased the vaccine uptake. The most commonly used vaccine Sinovac designed and manufactured by the People Republic of China.²³ It comprises of two doses, the second is injected 40 days after the first dose. However as single dose vaccines become available the uptake in Covid-19 vaccination programs is increasing. The efficacies of different vaccines among different age groups are still unknown in Pakistan. However, the third wave with the delta variant is more serious and the vaccine is not as effective in preventing infection.²⁴ However, it may prevent serious disease, hospitalization and death. A number of questions remain about the vaccine efficacy in the clinic, the ability to induce neutralizing capacity of the antibodies will be of utmost relevance. On a population level, the protective effect for re-infections needs to be determined. The vaccine has shown few side effects such as swelling and redness at site of injection and generally considered safe.²⁵ Currently, Chitral is only city of KP province, Pakistan in which vaccinated population is greater than non-vaccinated.

The second wave of COVID-19 in Chitral district did not cause any serious harm because of the vaccination and public awareness about the infection and social distancing. Overall, 15% of the population was tested positive and the peak was in

months of November and December 2020 (Figure 2). The higher rate of positivity was shown in upper rural areas of Chitral district, KP province, Pakistan i.e., 69%. More than 70% of population got vaccinated by March 2021 that is the highest number of vaccinated people in a city in Pakistan.

Conclusion

At the end of 2019, a novel coronavirus was identified as the cause of a cluster of pneumonia cases in Wuhan, a city in the Hubei Province of China. It rapidly spread, resulting in an epidemic throughout China, followed by a global pandemic. The virus constantly mutates itself in every wave bringing more severity in the infection. Our prevalence based data analysis indicate that the second wave of Corona virus hit the country but had limited effects in Chitral, possibly due to high preventative measures and awareness among the citizens that caused social distancing, good hygienic care and timely vaccination. Most of the cases reported during second wave were in the month of November/December 2020. The data have shown that the infection was controlled by following government guidelines. According to a latest survey Chitral is the only major city of KP, Pakistan in which vaccinated population is greater than non-vaccinated. With advancement in molecular biology and virology, researchers will be able to discover more therapeutic strategies to overcome this pandemic.

Acknowledgment

We thank Mr. Rahmat Khan, the marketing officer of health-care department Chitral, to cooperate and help with us by providing and managing the data.

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