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Original research

Molecular epidemiology of SARS-COV-2: current scenario in Gilgit, Pakistan

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Abstract

The epidemic of SARS-CoV-2 emerged in China (Wuhan) at the end of 2019, which created a global pandemic, subsequently causing a significant health crisis. Although this disease has become prevalent worldwide, there is very limited data available on its epidemiology and its molecular characteristics in Gilgit, Pakistan. The aims of this research to share a summary of the current SARS-CoV-2 epidemiological landscape in Gilgit through epidemiological surveillance and molecular characterization. A cross-sectional study involved collecting a total of 117,986 nasopharyngeal swabs from symptomatic patients in Gilgit. The viral RNA was extracted and amplified using the Thermo-Fisher Auto Extraction Kit and TaqPath Amplification Kit on the Quant Studio™ 5 Real-Time RT-PCR Detection System, respectively. Whole-genome sequencing of selected samples was also conducted. The results showed an overall prevalence of SARS-CoV-2 of 6.89%; the percentage was higher in males (59.27%) than in females (40.72%). Year-wise prevalence showed a much higher prevalence of SARS-CoV-2 in 2020 (10.52%), followed by 2022 (10.33%) and 2021 (4.02%). Also, the mortality rate was highest in 2020 (0.17%), followed by 2021 (0.06%) and 2022 (0.02%). The results of whole-genome sequencing revealed the presence of the Delta variant (B.1.617.2) variant of concern in all 2021 samples and the Omicron variant in the majority of 2022 samples. Our study highlights that the effective control measures taken during the pandemic were the reason for the low prevalence.

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Introduction: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was first discovered in China (Wuhan) in December 2019 and it set off a worldwide pandemic [1]. SARS-CoV-2 belongs to the genus Beta coronavirus and the Coronaviridae family, which also contains two other viruses that caused earlier outbreaks: MERS and SARS-CoV [2]. Phylogenetic analysis revealed genetic similarities of SARS-CoV-2 with a bat SARS-like virus and SARS-CoV, while the intermediate host for this virus is pangolins [3].

SARS-CoV-2 symptoms can be different from patient to patient like some patient may be asymptomatic or mild to severe respiratory symptoms with various complications. Different tools like a combination of PCR and radiological examination is usually required for diagnosis [4]. Only supportive care is available currently, and there are no specific therapeutic options available. In China, on the other hand, they are testing targeted medications for this virus. Also, the virus's super-spreading nature has contributed to the rapid spread of the disease [5].

Besides its contagious nature, the virus can stay alive on surfaces like cardboard and stainless steel for up to 16-24 hours. SARS-CoV-2 spreads through respiratory droplets that can be inhaled, as these droplets can travel 1 to 2 meters through the air [6,7]. Although most coronaviruses do not cause serious illness in humans, the COVID-19 has led to significant illness and death worldwide.

Coronaviruses are classified into 4 different genera (α, β, γ, and δ) based on their genetic and antigenic traits. Mammals are the primary host of α and β coronaviruses, while birds are the primary host of γ and δ coronaviruses, though some can also infect mammals. Most coronaviruses generally do not cause severe disease in humans, except for SARS-CoV and MERS-CoV. The recent pandemic COVID-19 was caused by a novel β-coronavirus, SARS-CoV-2. It is genetically and epidemiologically not similar to SARS-CoV and MERS-CoV [8]. Up till now three highly pathogenic coronavirus types have been identified including SARS-CoV, MERS-CoV and SARS-CoV-2.

Pakistan reported its first COVID-19 case in February 2020 and has since experienced a significant number of cases and deaths, including in Gilgit. The northern region of Gilgit, Pakistan, is a mountainous area with a relatively low population density. Nonetheless, the region remains an important tourist destination and a transit hub for travelers to and from China, which increases the risk of introducing and spreading SARS-CoV-2. The epidemiology and molecular features of SARS-CoV-2 in this region are rather insufficiently studied, and further studies are necessary to clarify the processes of viral infection and genetic drift. Accordingly, the purpose of the paper is to give a recent evaluation of SARS-CoV-2 in Gilgit by way of epidemiological tracking and molecular examination. The obtained data will further contribute to the knowledge of the dynamics of transmission, genetic variation, and evolution patterns of the virus in this region. The findings are to be used to direct the application of effective public health interventions that can be used to control the spread of the virus in Gilgit and similar locations.

Methods:

Study design: This cross-sectional study was carried out in the Provincial Headquarter Hospital at Gilgit in Pakistan where the patients who presented with the symptoms were sampled at the hospital in four districts that have already been mentioned at Gilgit-Baltistan. The period of the research was January 2020 to December 2022. The sample consisted of 117,986 nasopharyngeal swabs collected on symptomatic subjects in Gilgit, mainly on the districts of Ghizer, Hunza, Nagar, and Gilgit. The use of sterile swabs and the transportation of the specimens into the laboratory in viral transport media (VTM) were used.

Viral RNA extraction: The procedure of extracting RNA was conducted according to given kits and protocols. Both the Thermo-Fisher Automated extractor and the Tanbead automated extractor were extracted. This was performed in a Biosafety Level 2 (BSL2) lab in order to comply with biosafety requirements.

Real-time PCR: The viral RNA samples were extracted and amplified with TaqPath amplification kit and then identified by the QuantStudio 5 Real-Time RT-PCR platforms. The assay was performed on three SARS -CoV-2 genes (ORF1ab, S, and N) to ascertain the presence of the virus.

Whole genome sequencing: Specimens that had effective positive results with CT value lower than 30 were chosen and were submitted to the National Institute of Health in Islamabad, Pakistan, to have their entire genomes sequenced. Illumina platform sequencing was done. The data produced was subjected to bioinformatics tools and software to determine the viral variants and lineages.

Statistical analysis: The IBM SPSS Version 25 and R-studio were used to conduct statistical and phylogenetic analyses, respectively. Descriptive statistics summarized the data, and chi-square tests evaluated associations between the variants and patient characteristics.

Ethical considerations: The ethical review board of the Provincial Headquarter Hospital, Gilgit, approved the study. Informed consent was obtained from all patients, and confidentiality was maintained throughout the study. Recent research was conducted in compliance with the guidelines established by the WHO and the National Institute of Health (NIH) of Pakistan.

Results: The data analysis revealed that more males tested positive for the SARS-CoV-2 virus over all three years. In 2020, there were 2,053 positive cases among males and 1139 among females. Similarly, in 2021 there were 1,443 positive cases among males and 1022 among females and 2022 there were 1,013 positive cases among males and 937 among females, the number of positive cases was higher for males than for females as shown in Table No.1. The total of PCR tests conducted in 2020, 2021, and 2022 were 33510, 63667, and 20809, respectively.

During this period of study (2020 to 2022), a total of 117,986 samples were analyzed, of which 7,607 (6.89%) were positive. The positivity rate in 2020 was 10.52% (3,192/33,510) with a rate of 0.17%. This rate decreased significantly in 2021 to 4.02% (2,465/63,667), with a rate of 0.06%. In 2022, the positivity rate increased to 10.33% (1,950/20,809), with a maximum rate of 0.02%. Chi-square analysis revealed a significant difference in the rate of change in the positive data of the study ($p < 0.01$), which is due to temporal variation in the disease. A steep fall in the rate of change of the positive rate with time (p 0.05) was observed, as shown in Table No.2 and Figure No.01.

We have discovered that the majority of patients were men (75 parts out of 100), and their average age was 44 years. They had such symptoms as cough, fever, and shortness of breath, which are typical of COVID-19 infections. However, some patients also experienced atypical symptoms like diarrhea and vomiting.

Genomic analysis of the Omicron variant in Gilgit district showed that all patients carried the BA.5.2 lineage, except for two who had the BA.2 and BA.4 lineages, respectively. Our findings indicate that BA.5.2 is the main Omicron lineage in Gilgit district. Additionally, our study identified two sub-lineages, BA.5.2.1 and BA.5.2.2, within the BA.5.2 lineage. The BA.5.2.1 sub-lineage was identified in three patients, while the BA.5.2.2 sub-lineage was found in one patient. The detection of these sub-lineages indicates that the Omicron variant is experiencing genetic diversification in Gilgit district ass shown in Table No. 03.

Discussion: This research aimed to evaluate the current status and molecular epidemiology of SARS-CoV-2 in Gilgit, Pakistan, an area with limited data on the virus. The number of nasopharyngeal swabs collected was 117,986.

The general prevalence of SARS-CoV-2 in our results was 6.89%, and it was significantly higher in males (59.27%). The prevalence in 2020 (10.52%), 2022 (10.33%), and 2021 (4.02%), was the highest. In the years 2020, 2021 and 2022, the mortality rate was the highest 0.17, 0.06 and 0.02 respectively.

The findings of this research paper suggest that gender-related differences are present regarding the occurrence of the SARS-CoV-2 infection. All three years recorded higher positive cases among males indicating that males are vulnerable to being infected by the virus. This can be explained by behavioural and social factors, i.e., more exposure to the virus, more underlying illnesses, and variations in immune reactions in males and females. These findings ought to be further confirmed through further research.

The following years of decrease in prevalence and mortality could be due to the improved management practices and the better knowledge of the disease. In all samples since 2021, the Delta variant (B.1.617.2), a variant of concern, was identified by whole-genome sequencing, and in most samples since 2022, the Omicron variant. New variants, including Omicron, demonstrate the need to continue surveillance and genomic sequencing to monitor the evolution of viruses and make informed decisions related to the population and health.

The research paper has a number of limitations that include incomplete information about the asymptomatic cases and the possibility of sample selection bias. It also failed to determine the effect of vaccination on the prevalence and mortality of SARS -CoV 2 in Gilgit.

Conclusion: This paper presents the epidemiological situation and the molecular data of SARS -CoV-2 in Gilgit, Pakistan. The results show that SARS-CoV-2 prevalence and mortality rates decrease over the time, but new variants cause one to understand the need to monitor and conduct genomic sequencing. These findings can be used to develop policy and intervention measure to control and prevent outbreaks of SARS -CoV -2 in Gilgit and other under-researched regions. Further studies are needed on the impact of vaccination on the epidemiology of SARS-CoV-2 in Gilgit and adjacent areas.

Conflict of interest statement: The authors declare that there are no conflicts of interest.

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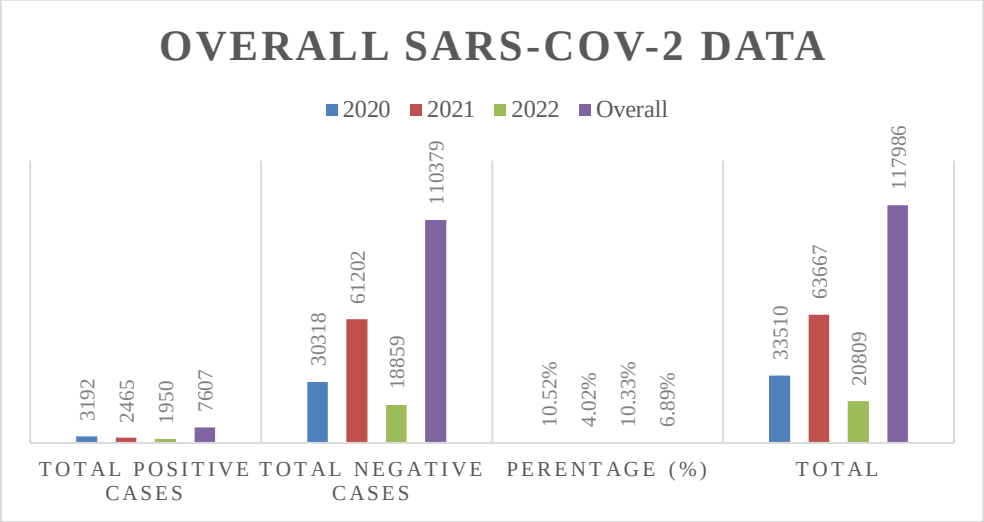

Fig 01. Overall prevalence of SARS-CoV-2

Table 01. Total Positive and Negative cases of SARS-CoV-2 in male and female

Year	Female (n)	Male (n)	Total Positive Cases	Female (%)	Male (%)
2020	1139	2053	3192	35.68%	64.31%
2021	1022	1443	2465	41.46%	58.53%
2022	937	1013	1950	48%	51.94%
Overall	3098	4509	7607	40.72%	59.27%

Table 02. Overall prevalence of SARS-CoV-2 and Mortality rate.

Year	Total Positive Cases	Total Negative Cases	Parentage (%)	Total	Mortality rate
2020	3192	30318	10.52%	33510	0.17%
2021	2465	61202	4.02%	63667	0.06%
2022	1950	18859	10.33%	20809	0.02%
Overall	7607	110379	6.89%	117986	0.08%

Table 03. Genomic distribution of SARS CoV-2 in Gilgit

Sr.No	Lab ID	Age (Years)	Gender	District	SARS-CoV-2 Variant	Lineage
1	123896	80	Male	Gilgit	Omicron	BA.2
2	124619	27	Male	Gilgit	Omicron	BA.5.2
3	124523	65	Female	Gilgit	Omicron	BA.5.2.1
4	124838	55	Male	Gilgit	Omicron	BA.5.2
5	123621	51	Male	Gilgit	Omicron	BA.2
6	123656	13	Male	Gilgit	Omicron	BA.5.2
7	124622	32	Male	Gilgit	Omicron	BA.5.2
8	124643	28	Male	Gilgit	Omicron	BA.5.2
9	124778	21	Male	Gilgit	Omicron	BA.5.2
10	124246	24	Male	Gilgit	Omicron	BA.5.2.1
11	124823	22	Male	Gilgit	Omicron	BA.5.2
12	124419	44	Male	Gilgit	Omicron	BA.5.2
13	124837	103	Female	Gilgit	Omicron	BA.5.2
14	124249	42	Female	Gilgit	Omicron	BA.4
15	124023	23	Male	Gilgit	Omicron	BA.5.2

16	114420	36	Female	Gilgit	Omicron	BA.5.2
17	124054	60	Female	Gilgit	Omicron	BA.5.2
18	124645	48	Male	Gilgit	Omicron	BA.4
19	124832	103	Female	Gilgit	Omicron	BA.5.2
20	124633	55	Female	Gilgit	Omicron	BA.5.2