DOI: 10.7860/JCDR/2024/68267.19341

Original Article

Microbiology Section

The Decline of COVID-19 Pandemic- A Journey from Fear to Freedom: A Retrospective Study from Northwest Punjab, India

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ABSTRACT

Introduction: Globally, Coronavirus Disease-2019 (COVID-19) pandemic era is on the decline, and now, after three years, much lower rates of mortality and morbidity are witnessed. The emergence of new variants and subvariants like Omicron is leading into a transition phase where one would only see sporadic surges. After the 3rd wave, Punjab also experienced such surges, prompting this retrospective study to observe the trend of COVID-19 and emerging variants in the Northwest region of Punjab, India.

Aim: To assess the prevalence of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) infection during the past year and to analyse demographic variables like age and gender distribution of positive cases of SARS-CoV-2.

Materials and Methods: The present study was a retrospective study, with study period of one year from 1st July 2022 to 30th June 2023, all samples (n=3,04,273) from suspected SARS-CoV-2 patients received at the Virology Research and Diagnostic Laboratory at Government Medical College, Amritsar, Punjab, India, were included in the study. The results of Real-Time Polymerase Chain Reaction (RT-PCR) were analysed to determine the prevalence in that region, and basic demographic variables of SARS-CoV-2 cases were compared. Additionally, 100 random positive samples were sent for whole-genome sequencing to study the prevalent variants and subvariants in this region. The results were analysed to study the prevalence of COVID-19 cases in the region. Demographic parameters like age group distribution and sex distribution were calculated. To study the dynamics of transmission during the one-year study period, an epidemiological curve was plotted over the period of 12 months.

Results: Out of the total 3,04,273 samples, 2,102 samples (0.69%) tested positive for the COVID-19 virus by RT-PCR. The study showed more prevalence of infection among young adults, with 853 (40.6%) cases in the age group of 21-40 years, followed by 614 (29.2%) cases in the 41-60 years age group. Of the total positive cases, 1153 (54.85%) were males, compared to 949 (45.15%) were females. The maximum number of positive cases was reported during the months of July 2022 to September 2022, totaling 1,273 cases. Whole-genome sequencing results showed predominance of the Omicron variant, with 44 (49.44%) strains belonging to the XBB lineage of Omicron.

Conclusion: There was a definite reduction in the prevalence of SARS-CoV-2 cases during the study period and that Omicron and its subvariants, like XBB lineages, are prevalent in this part of India as well. Despite the decrease in the number and severity of COVID-19 cases, maintaining vigilance and monitoring sporadic cases using tools like Whole Genome Sequencing (WGS) can help in tracking major pandemics in the future.

Keywords: Coronavirus disease-19, Severe acute respiratory syndrome-coronavirus-2, Subvariants of omicron, Whole genome sequencing

INTRODUCTION

The COVID-19 emerged in late 2019 in China, causing a pandemic of acute respiratory disease that became a global health problem. On 30th January 2020, the World Health Organisation (WHO) declared SARS-CoV-2 outbreak, constituted a public health emergency of international concern, with more than 80,000 confirmed cases reported worldwide as of 28th February 2020 [1]. Since then, the Coronavirus has affected millions of people around the globe. After three years of the highest alert over the COVID-19 virus, on May 5th, 2023, the WHO declared that COVID-19 no longer represents a "health Emergency" [2]. This declaration represents a major step towards ending the COVID-19 pandemic. Although WHO's declaration, nearly two million new cases and 12,000 deaths were reported globally in the month of May 2023 (1st to 28th May 2023) [3]. Among South East Asian Region, the highest number of new cases was reported from India (44,355) during this same period [3]. The region of Punjab also noticed a rise in COVID-19 cases. Such figures raise the question of whether the pandemic is really over or if we are just going into the endemic phase of SARS-CoV-2.

Since the dawn of the SARS-CoV-2 pandemic, various novel variants, lineages, and sublineages have been named according to the evolution of the virus. Internationally, variants have been named Alpha, Beta, Gamma, Delta, and Omicron. The evolution of these variants is attributed to vaccination and natural immunity in humans. Omicron (B.1.1.529), the most recent one was first reported in November 2021 from South Africa and eventually became the dominant strain because of its high transmissibility [4]. After the first and second waves, the third wave of COVID-19 in India occurred between December 2021 and March 2022. This wave was predominantly driven by the Omicron variant, which contains more than 30 mutations in the spike protein. Omicron is characterised by unique characteristics like a high replication rate, increased transmissibility, and immune evasion. Clinically, patients encountered asymptomatic to mild symptoms [5]. Even after the third wave, cases of COVID-19 continued, caused by Omicron and its lineages.

The WGS is an important tool that can help study the effects of mutations or variants on disease dynamics or predict future pandemic trends [6]. This information can further help us to study the virus variants, properties transmissibility, virulence, evasion of RT-PCR detection if target genes are affected, evasion of natural/vaccine-induced immunity, and decreased susceptibility to medical

treatments [6]. The causative agent of COVID-19, SARS-CoV-2, was also done, while studying its genome through Next-Generation Sequencing (NGS) [7]. The present study was conducted to know the trend of COVID-19 in Punjab as cases were still being reported during this waning phase of the epidemic. Previous studies have been conducted during the first, second, and third waves in this region, so this work aimed to determine the prevalence during the ongoing post-Omicron wave.

The aim of the study was to assess the prevalence of SARS-CoV-2 infection during the past year and analyse demographic variables like age and gender distribution of positive cases of SARS-CoV-2. The objectives of the study were to identify the genetic diversity of the strain prevalent in the present phase and compare it with the WGS results obtained from previous studies conducted in the same region. The study also assessed the prevalence district-wise and studied the seasonal distribution of positive cases over 12 months to observe the transmission dynamics of COVID-19 in this region.

MATERIALS AND METHODS

The present study was a retrospective study conducted for the period of one-year, from 1st July 2022 to 30th June 2023, the Virology Research and Diagnostic Laboratory (VRDL) in Amritsar, Punjab, India, received 3,04,273 samples from suspected patients of SARS-CoV-2.

Inclusion criteria: All samples, from both indoor and outdoor patients and from all age groups, received within the study timeline were included. These samples belonged to six different districts (Amritsar, Pathankot, Tarn Taran, Gurdaspur, Hoshiarpur, and Kapurthala) in Punjab, India.

Exclusion criteria: Samples with incomplete request forms were once rejected but included in the study when a properly filled form was received. Samples from other districts that did not maintain proper cold chain maintenance were also excluded from the study.

Study Procedure

The swabs already placed in Viral Transport Media (VTM) were received and then sent to the processing section. RNA was extracted using the Applied Biosystems MagMAX™Viral/Pathogen II nucleic acid isolation kit as per standard protocol. RT-PCR was done on the Quantstudio 5 RT-PCR instrument using the Indian Council of Medical Research (ICMR)-approved RT-PCR kit CoviPath™ COVID-19 RT-PCR Kit (Thermofisher Scientific). The samples were analysed for different genes: *ORF1ab* gene (open reading frame 1a and b), *N*gene (nucleocapsid protein), and *RNase P* gene (Ribonuclease P). All tests were interpreted along with one positive and one negative control by observing Cycle threshold (Ct) values on the amplification curve. The analysis of tests was done by observing the presence of an exponential rise in the amplification curve with a Ct value below a given threshold.

During this one-year period, 100 random SARS-CoV-2 positive samples (Ct value <25 as per ICMR guidelines) [8] were also tested for WGS through Nanopore technology. The selected samples were packed with triple-layer packaging and sent to National genome sequencing centres authorised by ICMR to detect mutagenic strains prevalent in our region.

The data obtained was used to analyse the prevalence in this Northwest region of Punjab, and district-wise positivity was also calculated. Demographic parameters like age group distribution, sex distribution, percentages, and sex ratio were calculated.

STATISTICAL ANALYSIS

All the study data was presented in terms of descriptive statistics, and to study the dynamics of transmission during the one-year study period, an epidemiological curve was plotted over the 12-month period.

RESULTS

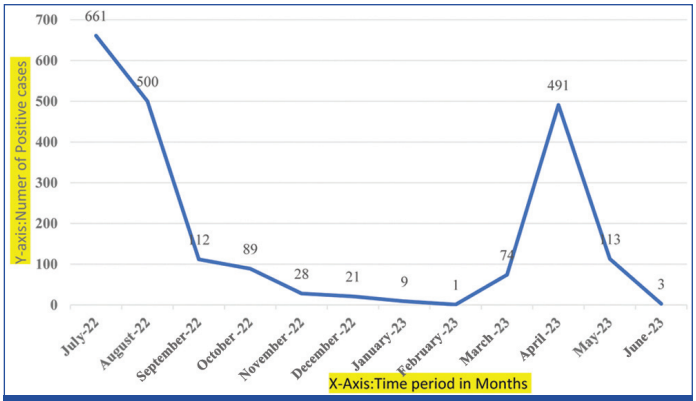
A total of 3,04,273 samples were received during the one-year study period in our lab from the Majha region of Punjab, out of which 2,102 samples were positive for the SARS-CoV-2 virus. The study showed a total positivity rate of 0.69% out of the 3,04,273 samples tested at VRDL, Government Medical College (GMC) Amritsar, which was far below the positivity rate found in the study conducted during 2021 (4.80%) from the same region [9]. The present study also showed more prevalence of COVID-19 among the population of young adults in the age group of 21-40, followed by the middle age group of 41-60, with 853 (40.6%) and 614 cases (29.2%), respectively [Table/Fig-1]. Males outnumbered females by 204 cases, with 1153 (54.85%) being males and 949 (45.15%) being females among those positive for COVID-19. The male-to-female ratio was calculated as 1.2:1 [Table/Fig-1].

Age group (years)	n (%)
0-20	326 (15.5)
21-40	853 (40.6)
41-60	614 (29.2)
>60	309 (14.7)
Total	2102 (100)
Gender	
Male	1153 (54.85)
Female	949 (45.15)

[Table/Fig-1]: Age and gender distribution of SARS-COV2 positive cases (n=2102).

District-wise, all samples were analysed, and the positivity rate was calculated separately, i.e., the total positive cases in a district divided by the total cases received from that particular district, multiplied by 100. The positivity rate of COVID-19 positive cases was found to be 0.90% in Amritsar, 0.65% in Gurdaspur, 0.72% in Pathankot, 0.24% in Tarn Taran, and 0.45% in Hoshiarpur.

Month-wise data of COVID-19 cases showed that 1273 cases were reported from July to September 2022, which declined gradually from October 2022 to February 2023, during which only 148 cases were seen but again rise in cases was observed from March 2023 to May 2023, with a maximum of 678 cases documented [Table/Fig-2].



[Table/Fig-2]: Epidemic curve of SARS-CoV-2 cases from July 2022 to June 2023.

While analysing the results of WGS (Nanopore DNA Technology), it was seen that the Omicron variant was the most prevalent variant. Out of 100 samples sent, 89 samples had clear results, while 11 samples were inconclusive. Among rest of 89, Omicron sub-lineages predominated the data, out of which 44 (49.44%) belonging to the XBB lineage (XBB1.16, XBB1.5, XBB1.92, XBB1) [Table/Fig-3].

S. No.	Variants/Subvariants on WGS	n (%)
1.	B1.1529	1 (1.12)
2.	XBB variants (XBB.1.16, XBB.1.92,XBB.1.5.1,XB B1.9,XBB.1,XBB.2)	44 (49.44)
3.	BQ+BA.5.2	7 (7.87)

4.	B1.1	12 (13.48)
5.	BA2	9 (10.11)
6.	CHH	3 (3.37)
7.	XBF	3 (3.37)
8.	Others (BR2.1,B.N.1.1,DF1,CH.1.1)	10 (11.24)
Total		89 (100)

[Table/Fig-3]: Results of whole genome sequencing-variants/subvariants distribution.

DISCUSSION

As India transitions into the endemic phase of COVID-19 since the start of January 2023, with an average of 200 cases per day compared to July 2022 when India was reporting over 20,000 cases per day [10], new sporadic surges will continue, like in other parts of the world.

Present study showed that the incidence of COVID-19 infection was higher among young adults aged 21-40 years. Being the most productive age group, they come into contact with many people daily. Similar results were seen in a study by Das AK et al., where more positivity rate was noted among the young age group [11]. Also, recent studies conducted by Gautam S et al., and Majumder S et al., have shown similar findings when discussing the clinico-demographic profile of positive patients [12,13]. In present study, a slight male predominance was noted. Many authors in their studies on coronaviruses have noted such sex bias and have outlined reasons like adaptive immune system of females with a higher number of CD4+ cells, more cytotoxic cell activity, and an increased capacity for humoural response, giving female patients advantages over male patients in COVID-19 [14]. Jaillon S et al., in their review, have also quoted innate immunity in support of this finding [15]. Similar reasons have been stated by Nasker SS et al., in their epidemiological study from the state of Odisha, India [16]. The effect of male sex and immune-related genes has also been noted by Niemi MEK et al., in their review [17].

While studying the effect of climatic variations on virus transmission, it was seen to be more transmissible in the monsoon months when the intensity and frequency of infections is more. Winters in Northwest Punjab are pretty harsh, and as temperatures starts to drop, infections also dropped significantly. But again a rise was observed in cases again in March and April when temperatures started to increase. A similar pattern was noted in a study conducted in the same region in earlier year, suggesting that the monsoon season brings about more transmission of the SARS-CoV-2 virus compared to winters [9]. Many authors have studied the correlation of transmission dynamics with temperature and relative humidity. Mehta SK et al., stated that most COVID-19 cases were reported at warmer temperatures of 24-30°C and intermediate relative humidity of 50-80% [18]. The study also stated that overall in India, cases of COVID-19 increase with an increase in relative humidity and a decrease in surface temperatures. This may be the reason in our region also, as maximum cases were seen during the monsoon season when relative humidity varies in the range of 60-70% in Punjab and temperatures are around 28-30°C. However, present study findings contrast with the study by Mane V et al., who suggested that increased humidity leads to decreased cases as the viability of the virus decreases with increased relative humidity [19]. Apart from this, a small surge of cases in March-April 2023 that occurred in the Punjab region was in parallel with nationwide data, with about 12,591 cases reported as of April 20th, 2023 [20].

Since the evolution of SARS-CoV-2, many variants have been recognised, but among them, Omicron has been considered the most evolving variant, which has been expanding its lineages. Its parent lineages are BA1, BA2, and BA4/BA5 [21]. Further BQ1 sublineages have been reported from Africa, America, and Europe, while XBB sublineages are more seen in Southeast Asia. The XBB variant resulted from recombination between second-generation BA.2 variants, and the X in XBB signifies that it has been formed by recombination between two parent lineages. The XBB variant emerged in late 2022. Gupta E et al., clearly showed that Omicron and its subvariants like XBB have been circulating in India since January 2022 [22]. Studies by Tamura T et al., on the virological characteristics of this variant showed profound resistance to humoural immunity induced by vaccination, leading to common breakthrough infections [23]. Globally, XBB variants are also on the rise. According to a recent study by Samal J et al., 90% of COVID-19 infections worldwide are due to XBB1.5 [21]. XBB1.5 has the maximum growth advantage and is believed to be the reason behind the sharp surge in cases. In India, the XBB1.6 subvariant is replacing earlier variants. In present study, genome sequencing data showed that Omicron and its subvariants like XBB1.5, XBB1.6 predominated in this part of Punjab, with some strains belonging to the BQ.1 subvariant also. Very few strains related to the original B1.1 were seen in present study. Earlier data from WGS in Punjab region showed Alpha strain predominance in 2020-2021 (95/120). However, the study conducted in the next year, i.e., 2021 to April 2022, showed Omicron and Delta in 40% and 36% of cases, with Alpha being reduced to only 24% [9,24]. Earlier knowledge with different waves has shown that viruses keep on evolve, with new strains and subvariants appearing, but those with a growth advantage over other variants become the predominant strain in circulation, replacing previous ones.

In 2023, a new Omicron strain called E.G.5 (Eris) became the reason for a spurt in cases across multiple countries. Although only a few serious cases were reported, nations like India were on alert. The SARS-CoV-2 variant E.G.5.1 is a sublineage of the Omicron variant XBB.1.9.2, and it has a growth advantage contributing to the increase in the proportion of SARS-CoV-2 cases in most regions of the world [25,26].

Limitation(s)

In present study, due to its retrospective nature, more clinical data were required to establish any correlation with the type of strain in circulation and the severity of symptoms. For WGS,a larger sample size could have been studied to ensure representative of circulating strains in the region.

CONCLUSION(S)

The present study concludes that there has been a significant reduction in the positivity of COVID-19 cases compared to previous waves in this region. The WGS data clearly shows that Omicron subvariants are leading the dashboard, replacing all other previous variants. But still COVID-19 is evolving and lurking around us, potentially threatening us at any time with a surprising new variant. After witnessing the havoc created by variants like Delta, India has learned that before overburdening our health system, we should strengthen our real-time surveillance systems. Therefore, tools like WGS are imperative in today's healthcare landscape.

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PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Oct 25, 2023
- Manual Googling: Dec 21, 2023
- iThenticate Software: Mar 05, 2024 (4%)

ETYMOLOGY: Author Origin

EMENDATIONS: 8

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? No
- Was informed consent obtained from the subjects involved in the study? No
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Oct 25, 2023**
Date of Peer Review: **Dec 07, 2023**
Date of Acceptance: **Mar 07, 2024**
Date of Publishing: **May 01, 2024**