Original Article

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Microbiology Section

Burden of Severe Acute Respiratory Syndrome Coronavirus 2 and its Seasonal Trends in Patients Attending a Tertiary Healthcare Centre in Rajasthan: A Retrospective Observational Study

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ABSTRACT

Introduction: The pandemic of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) has drastically affected the global population, leading to high rates of morbidity and mortality. This virus originated in China had quickly spread to different countries worldwide, paralysing healthcare systems. Alongside supportive therapy, isolation, and contact tracing, vaccines have also played a crucial role in rescuing the human population from the virus.

Aim: To evaluate the burden of SARS-CoV-2 and its seasonal trends.

Materials and Methods: This retrospective observational study was conducted in the Department of Microbiology at the Ananta Institute of Medical Sciences and Research Centre in Rajsamand, Rajasthan, India. Data was collected from July 2020 to September 2022 and analysed from January 2023 to March 2023. The study included a sample size of 14,050. Nasopharyngeal and throat swab samples were collected into a single tube of Viral Transport Medium (VTM). Ribonucleic Acid (RNA) was extracted from the VTM, and real-time Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) was performed using different kits approved by the Indian Council of Medical Research (ICMR). Results were interpreted according to the manufacturer's instructions. A Chi-squared test was performed using GraphPad Prism version 9.2.0.332. A p-value of <0.05 was considered significant.

Results: A total of 14,050 samples were evaluated, of which 2,861 (20.36%) tested positive for SARS-CoV-2. The highest positivity rate of 581 (4.13%) was observed in the age group of 41-50 years (p-value <0.0001). The maximum positivity for SARS-CoV-2 was found among individuals aged 21-60 years, accounting for 2,086 (14.85%) cases. Among the samples obtained from the Inpatient Department (IPD) and Outpatient Department (OPD), 913 (13.83%) (p-value=0.0014) and 1,948 (26.16%) tested positive for SARS-CoV-2, respectively. Male patients accounted for 1,869 (21%) (p-value=0.0194) positive cases, while female patients accounted for 992 (19.26%) (p-value <0.0001) positive cases. The highest positivity rate was recorded in April 2021, with 921 (6.55%) cases. Seasonal trends of SARS-CoV-2 showed two major peaks and a minor peak between July 2020 and September 2022. Symptomatic patients had a positivity rate of 987 (31.75%), while asymptomatic patients had a rate of 1,874 (17.13%) (p-value <0.0001).

Conclusion: Enhanced precautionary measures are required for individuals aged 21-60 years, as they are more vulnerable to SARS-CoV-2. Asymptomatic patients had a positivity rate of 17.13%. Given the mixed trend of seasonal transmission of SARS-CoV-2, continuous surveillance of the virus is necessary. The study results will be useful for epidemiological purposes and for planning strategies aimed at reducing the duration of the pandemic.

Keywords: Asymptomatic patients, Mixed trends, Pandemic, Respiratory virus, Seasonal variation

INTRODUCTION

The outbreak caused by SARS-CoV-2 has resulted in unpredictable morbidity and mortality across the globe [1]. Transmission of this novel virus is through direct contact and respiratory droplets from an infected person [2]. India has faced a severe impact of COVID-19 [3] and has reported the third-highest number of SARS-CoV-2 cases globally [4]. The peak of the first wave of the pandemic in India was delayed by around 8 weeks due to a national lockdown. From March 25th to April 14th, 2020, a nationwide lockdown was implemented [5]. The Indian healthcare system faced an unprecedented burden of COVID-19 during the second wave [6]. New variants of concern have increased the transmissibility of the virus, leading to higher hospitalisation and death rates. Booster doses of vaccines have been administered worldwide to maintain protection against the disease caused by this virus [1,7].

Seasonal trends of SARS-CoV-2 are of significant interest as they help us better understand the role of the virus in infection transmission during different seasons [8]. Many respiratory viruses exhibit established seasonal variations. Some studies have suggested an association between temperature, humidity, and SARS-CoV-2 incidence [9,10]. Apart from climate changes, other factors such as virus viability, stability, and host immunity may also contribute to the virus survival or suppression [11]. Data regarding the burden of SARS-CoV-2 along with seasonal trends are lacking in the study region. Knowledge of the burden and seasonal trends of SARS-CoV-2 is crucial as it provides more accurate information about the current trend and potential changes over time. The aim of this study was to analyse the burden of SARS-CoV-2 in patients attending a tertiary healthcare centre. Another objective of the study was to analyse the seasonal trends in patients attending a tertiary healthcare centre.

MATERIALS AND METHODS

This was a retrospective observational study conducted in the Department of Microbiology at the Ananta Institute of Medical

Sciences and Research Centre, Rajsamand, Rajasthan, India. The data was collected from July 2020 to September 2022, and the analysis was done from January 2023 to March 2023. As it was a time-bound study, only the samples available during the study duration were considered. The sample size of the study was 14,050.

Ethical approval for the study was obtained from the Institutional Ethics Committee (IEC) (Letter No. AIMS/IEC/2023/07). Informed consent was waived due to the retrospective nature of the study.

Inclusion criteria: All patients suspected of SARS-CoV-2 who presented to the OPD and IPD, and whose samples yielded either positive or negative test results during the initial testing, were included in the study.

Exclusion criteria: All samples that yielded inconclusive test results were excluded from the study.

Data collection: Data for the study was collected from the records of the Department of Microbiology, Ananta Institute of Medical Sciences and Research Centre, as well as from Excel sheets and Specimen Referral Form (SRF).

Study Procedure

Sample collection and transportation: The study procedure involved the collection and transportation of samples. To enhance the yield of the virus, two swabs were collected from each patient following the standard protocol [12]. This included a nasopharyngeal swab and a throat swab, which were placed into a single tube of VTM. The samples were properly labeled, assigned an SRF number, and transported in a cold chain to the laboratory.

Extraction of RNA from clinical samples: RNA was extracted from the VTM containing the nasopharyngeal swab and throat swab using the automated RNA extractor QIAcube connect (Qiagen USA). Manual extraction of RNA was also performed in a biosafety level-2 facility using the QIAamp viral RNA kit (Qiagen USA) as per the manufacturer's instructions [13,14].

Detection of viral genes: Real-time RT-PCR was performed using different kits approved by the ICMR on the Rotor-Gene Q 5plex RT-PCR Platform (Qiagen USA). The following kits were used to detect different viral genes of SARS-CoV-2, as described in previous studies [15,16]: STANDARD M nCoV Real-Time-Detection kit (*E* gene, *ORF1ab/RdRp* gene, IC), TRUPCR® SARS-CoV-2 Kit (*RDRP* and *N* gene, *E* gene, and Rnase p gene), SARS-COV-2 R-GENE® kit (*N* gene, *RdRp* gene, and IC), and PathoDetect COVID-19 Qualitative PCR Kit (*E* gene, *ORF1*(RDRP)/N gene, Rnase P). Results were interpreted as shown in [Table/Fig-1] [17-20].

Interpretation of results as per TRUPCR® SARS-CoV-2 Kit [17] Threshold cut-off cycle Ct is ≤35				
Case	**RNaseP**	***E* gene**	***RdRp* and *N* gene**	**Results**
1	±	+	+	SARS-CoV-2 positive
2	±	-	+	SARS-CoV-2 positive
3	±	+	-	Sarbecovirus positive
4	+	-	-	Negative
5	-	-	-	Invalid
Interpretation of results as per SARS-CoV-2 R-GENE®kit [18]. Threshold cut-off cycle- Any Ct value is considered as positive				
Case	***N* gene**	***RdRp* gene**	**IC**	**Results**
1	+	+	+	Positive for SARS-CoV-2 RNA
2	+	-	+	Positive for SARS-CoV-2 RNA
3	-	+	+	Positive for SARS-CoV-2 RNA
4	-	-	+	SARS-CoV-2 RNA not detected
5	-	-	-	Invalid
Interpretation of results as per STANDARD M nCoV Real-Time-Detection kit [19]. Threshold cut-off cycle Ct is ≤36 for *E* Gene and *ORF1ab (RdRp)* gene and for IC ≤26				
Case	***E* gene**	***ORF1ab (RdRp)* gene**	**IC**	**Results**
1	±	+	±	Positive for SARS-CoV-2 RNA
2	+	-	±	SARS-CoV-2 presumptive positive
3	-	-	+	SARS-CoV-2 Negative
4	-	-	-	Invalid
Interpretation of results as per PathoDetect COVID-19 Qualitative PCR Kit [20] Threshold cut-off Ct is ≤40 for *E* gene and *ORF1 (RdRP)/N* gene. Threshold cut-off Ct is ≤38 for RNaseP				
Case	***E* gene**	***ORF1 (RdRP)/N* gene**	**RNaseP**	**Results**
1	+	+	+	Positive for SARS-CoV-2 RNA
2	-	+	+	Positive for SARS-CoV-2 RNA
3	+	-	+	COVID-19 presumptive positive re-extraction and retest recommended
4	-	-	+	Negative
5	-	-	-	Inhibition re-extraction and retest recommended
6	Ct-value ≤40-≤45	Ct-value ≤40-≤45	+	Re-extraction and retest recommended

[Table/Fig-1]: Interpretation of results of SARS-CoV-2.

STATISTICAL ANALYSIS

Statistical analysis involved conducting a Chi-square test using GraphPad Prism version 9.2.0.332. A p-value of <0.05 was considered significant. Descriptive statistics, including percentages and frequencies, were utilised.

RESULTS

A total of 14,287 samples were collected from the Department of Microbiology at Ananta Institute of Medical Sciences and Research Center. Out of these 14,287 samples, 237 were inconclusive and were not included in the study. Therefore, a total of 14,050 samples were analysed. Among them, 2,861 samples (20.36%) tested positive for SARS-CoV-2 [Table/Fig-2].

The highest prevalence of positivity, 581 samples (4.13%), was observed in the age group of 41-50 years. This was followed by the age groups of 31-40 years with 567 samples (4.03%) and 51-60 years with 515 samples (3.66%), respectively. The maximum positivity was found between the age groups of 21-60 years, accounting for 2,086 samples (14.85%) of the total samples [Table/Fig-2].

Age group (years)	Positive samples (n%)	Negative samples (n%)	Total samples (n%)
0-10	26 (0.18)	263 (1.87)	289 (2.05)
11-20	139 (0.99)	779 (5.54)	918 (6.53)
21-30	423 (3.01)	2556 (18.19)	2979 (21.20)
31-40	567 (4.03)	2122 (15.10)	2689 (19.14)
41-50	581 (4.13)	1838 (13.08)	2419 (17.21)
51-60	515 (3.66)	1621 (11.53)	2136 (15.20)
61-70	384 (2.73)	1269 (9.03)	1653 (11.76)
71-80	166 (1.18)	599 (4.26)	765 (5.44)
81-90	55 (0.39)	131 (0.93)	186 (1.32)
91-100	5 (0.03)	11 (0.07)	16 (0.11)
Total	2861 (20.36)	11189 (79.64)	14050 (100)

[Table/Fig-2]: Burden of SARS-CoV-2 in different age groups (N=14050).
Chi-square test-p-value <0.0001 Chi-squared equals 24.075 with 1 degrees of freedom. The two-tailed p-value is less than 0.0001

Among male patients, 1,869 (21%) tested positive for SARS-CoV-2 [Table/Fig-3], with the highest prevalence observed in the age group of 31-40 years, accounting for 415 cases (4.66%). In total female

Age group (years)	Male (N=8899)			Female (N=5151)		
	Positive sample (n%)	Negative sample (n%)	Total sample (n%)	Positive sample (n%)	Negative sample (n%)	Total sample (n%)
0-10	26 (0.29)	164 (1.84)	190 (2.13)	0	99 (1.92)	99 (1.92)
11-20	75 (0.84)	416 (4.67)	491 (5.52)	64 (1.24)	363 (7.04)	427 (8.29)
21-30	333 (3.74)	1318 (14.81)	1651 (18.55)	90 (1.74)	1238 (24.03)	1328 (25.78)
31-40	415 (4.66)	1389 (15.60)	1804 (20.27)	152 (2.95)	733 (14.23)	885 (17.18)
41-50	378 (4.25)	1244 (13.98)	1622 (18.22)	203 (3.94)	594 (11.53)	797 (15.47)
51-60	314 (3.53)	1076 (12.09)	1390 (15.62)	201 (3.90)	545 (10.58)	746 (14.48)
61-70	202 (2.27)	889 (9.99)	1091 (12.26)	182 (3.53)	380 (7.37)	562 (10.91)
71-80	88 (0.98)	431 (4.84)	519 (5.83)	78 (1.51)	168 (3.26)	246 (4.77)
81-90	35 (0.39)	96 (1.07)	131 (1.47)	20 (0.38)	35 (0.68)	55 (1.06)
91-100	3 (0.03)	7 (0.07)	10 (0.11)	2 (0.03)	4 (0.07)	6 (0.11)
Total	1869 (21.00)	7030 (79.00)	8899 (100)	992 (19.26)	4159 (80.74)	5151 (100)
Chi squared equals 5.466 with 1 degrees of freedom. The two-tailed p-value equals 0.0194 p=0.0194. p-value <0.0001 Chi-squared equals 180.845 with 1 degrees of freedom. The two-tailed p-value is less than 0.0001.						

[Table/Fig-3]: Gender wise burden of SARS-CoV-2.

patients, 992 (19.26%) were found to be positive for SARS-CoV-2 [Table/Fig-3], with the highest prevalence observed in the age group of 41-50 years, accounting for 203 cases (3.94%).

When the data of the predominant positive age group of males was analysed with other age groups of males using the Chi-square test, a p-value of 0.0194 was obtained. Similarly, when the data of the predominant positive age group of females was analysed with other age groups of females using the Chi-square test, a p-value of <0.0001 was obtained.

Out of the total samples, 6,603 (47%) were from IPD patients [Table/Fig-4]. Among them, 913 samples (13.83%) tested positive for SARS-CoV-2, with the highest prevalence observed in the age group of 51-60 years, accounting for 186 cases (2.82%) among IPD patients. When the data of the predominant positive age group of IPD patients was analysed with other age groups of IPD patients using the Chi-square test, a p-value of 0.0014 was obtained.

Additionally, 7,447 samples (53%) were from OPD patients [Table/Fig-4]. Among the OPD samples, 1,948 (26.16%) tested positive for SARS-CoV-2, with the highest prevalence observed in the age group of 31-40 years, accounting for 416 cases (5.58%). When the data of the predominant positive age group of OPD patients was analysed with other age groups of OPD patients using the Chi-square test, a p-value of 0.2619 was obtained.

The monthly burden of SARS-CoV-2 from July 2020 to September 2022 is presented in [Table/Fig-5]. The highest prevalence of positivity was observed in the month of April 2021, accounting for 921 cases (6.55%).

The seasonal trends of SARS-CoV-2 revealed two major peaks and a minor peak between July 2020 and September 2022 [Table/Fig-6].

Burden of SARS-CoV-2 in IPD samples (Chi-squared equals 10.244 with 1 degrees of freedom. The two-tailed p-value equals 0.0014)			
Age group (years)	IPD positive samples (n%)	IPD negative samples (n%)	IPD Total samples (n%)
0-10	2 (0.03)	144 (2.18)	146 (2.21)
11-20	26 (0.39)	387 (5.86)	413 (6.25)
21-30	92 (1.39)	1185 (17.95)	1277 (19.34)
31-40	151 (2.28)	880 (13.33)	1031 (15.61)
41-50	176 (2.66)	898 (13.60)	1074 (16.26)
51-60	186 (2.82)	917 (13.89)	1103 (16.70)
61-70	168 (2.54)	783 (11.85)	951 (14.40)
71-80	81 (1.22)	401 (6.07)	482 (7.29)
81-90	30 (0.45)	88 (1.33)	118 (1.79)
91-100	1 (0.01)	7 (0.10)	8 (0.12)
Total	913 (13.83)	5690 (86.17)	6603 (100)

Burden of SARS-CoV-2 in OPD samples (Chi squared equals 1.259 with 1 degrees of freedom. The two-tailed p-value equals 0.2619)			
Age group (years)	OPD positive samples (n%)	OPD negative samples (n%)	OPD total samples (n%)
0-10	24 (0.32)	119 (1.59)	143 (1.92)
11-20	113 (1.52)	392 (5.26)	505 (6.78)
21-30	331 (4.44)	1371 (18.41)	1702 (22.85)
31-40	416 (5.58)	1242 (16.68)	1658 (22.26)
41-50	405 (5.44)	940 (12.62)	1345 (18.06)
51-60	329 (4.42)	704 (9.45)	1033 (13.87)
61-70	216 (2.90)	486 (6.52)	702 (9.42)
71-80	85 (1.14)	198 (2.66)	283 (3.80)
81-90	25 (0.33)	43 (0.57)	68 (0.91)
91-100	4 (0.05)	4 (0.05)	8 (0.10)
Total	1948 (26.16)	5499 (73.84)	7447 (100)

[Table/Fig-4]: Burden of SARS-CoV-2 in IPD and OPD samples.

Month and year	Positive samples n (%) N=14050
July-20	1 (0.007)
August-20	113 (0.80)
September-20	660 (4.70)
October-20	142 (1.01)
November-20	181 (1.29)
December-20	159 (1.13)
January-21	57 (0.40)
February-21	10 (0.07)
March-21	120 (0.85)
April-21	921 (6.55)
May-21	281 (2.00)
June-21	13 (0.09)
July-21	1 (0.007)
August-21	1 (0.007)
September-21	3 (0.02)
October-21	1 (0.007)
November-21	1 (0.007)
December-21	1 (0.007)
January-22	130 (0.92)
February-22	57 (0.40)
March-22	2 (0.01)
April-22	0
May-22	0

June-22	2 (0.01)
July-22	0
August-22	4 (0.03)
September-22	0
Total	2861 (20.36)

[Table/Fig-5]: Seasonal trends of SARS-CoV-2 from July 2020 to September 2022.

The first major peak was observed in September 2020, corresponding to the rainy season of the year. In that month, a total of 2,089 samples (14.87%) were tested, of which 660 samples (4.70%) were found to be positive [Table/Fig-6]. The second major peak occurred in April 2021, corresponding to the summer season. In April 2021, a total of 2,187 samples (15.56%) were tested, and 921 samples (6.55%) were found to be positive [Table/Fig-6]. Additionally, a minor peak was observed in January 2022, corresponding to the winter season. In that month, 451 samples (3.21%) were tested, and 130 samples (0.92%) were found to be positive.

Month and year	Total positive samples (n%)	Total negative samples (n%)	Total samples (n%)
July-20	1 (0.007)	98 (0.69)	99 (0.70)
August-20	113 (0.80)	915 (6.51)	1028 (7.31)
September-20	660 (4.70)	1429 (10.17)	2089 (14.87)
October-20	142 (1.01)	674 (4.80)	816 (5.80)
November-20	181 (1.29)	528 (3.76)	709 (5.04)
December-20	159 (1.13)	580 (4.13)	739 (5.26)
January-21	57 (0.40)	610 (4.34)	667 (4.75)
February-21	10 (0.07)	686 (4.88)	696 (4.95)
March-21	120 (0.85)	977 (6.95)	1097 (7.81)
April-21	921 (6.55)	1266 (9.01)	2187 (15.56)
May-21	281 (2.00)	222 (1.58)	503 (3.58)
June-21	13 (0.09)	226 (1.61)	239 (1.70)
July-21	1 (0.007)	533 (3.79)	534 (3.80)
August-21	1 (0.007)	358 (2.55)	359 (2.55)
September-21	3 (0.02)	345 (2.45)	348 (2.48)
October-21	1 (0.007)	270 (1.92)	271 (1.93)
November-21	1 (0.007)	260 (1.85)	261 (1.86)
December-21	1 (0.007)	358 (2.55)	359 (2.55)
January-22	130 (0.92)	321 (2.28)	451 (3.21)
February-22	57 (0.40)	293 (2.08)	350 (2.49)
March-22	2 (0.01)	80 (0.57)	82 (0.58)
April-22	0	43 (0.30)	43 (0.30)
May-22	0	39 (0.27)	39 (0.28)
June-22	2 (0.01)	24 (0.17)	26 (0.18)
July-22	0	17 (0.12)	17 (0.12)
August-22	4 (0.03)	21 (0.15)	25 (0.18)
September-22	0	16 (0.11)	16 (0.11)
Total	2861 (20.36)	11189 (79.64)	14050 (100)

[Table/Fig-6]: Monthly burden of SARS-CoV-2 (N=14050).
p-value <0.0001 Chi-squared equals 741.755 with 1 degrees of freedom. The two-tailed p-value is less than 0.0001

When the data of the predominant positive samples month was analysed with the positive samples of other months during the study period using the Chi-square test, a p-value <0.0001 was obtained [Table/Fig-6].

A total of 3,109 samples were from symptomatic patients, and 10,941 samples were from asymptomatic patients, which showed a positivity of 987 (31.75%) and 1,874 (17.13%) (p<0.0001) for SARS-CoV-2, respectively.

The predominant symptoms associated with symptomatic patients who tested positive for SARS-CoV-2 were fever (826 cases, 83.69%), cough (667 cases, 67.57%), and shortness of breath (437 cases, 44.27%) [Table/Fig-7].

Symptoms	N=987
Fever	826 (83.69%)
Cough	667 (67.57%)
Shortness of breath	437 (44.27%)
Diarrhoea	28 (2.84%)
Vomiting	9 (0.91%)
Haemoptysis	0
Abdominal pain	18 (1.82%)
Sore throat	385 (39%)
Chest pain	8 (0.81%)
Body ache	114 (11.55%)
Sputum	8 (0.81%)

[Table/Fig-7]: Symptomatic patients tested positive for SARS-CoV-2.

DISCUSSION

This study from the state of Rajasthan provides the first assessment of the burden of SARS-CoV-2 and its seasonal trends over a period of 26 months. The world has faced the SARS-CoV-2 pandemic, which has impacted the global population's immune system, resulting in disruptions to the healthcare system [21].

The overall positivity rate of SARS-CoV-2 in present study was 20.36%. A descriptive study by Patil P et al., from Pune reported a total positivity rate of 18.33% [22]. Another study from Pune, Maharashtra [23], reported a total positivity rate of 19% when considering data from both the first and second waves. In contrast, a study from Delhi [16] reported a total positivity rate of 22.8% among patients attending OPD, IPD, and the emergency department. Present study findings were consistent with these studies. However, a previous study from Manipur, India [24], reported a positivity rate of 5% among individuals who came for SARS-CoV-2 testing, with a total of 1,528 samples tested. The variability in the positivity rates of SARS-CoV-2 across different studies may depend on factors such as the method of sample collection, sample size, duration of the study, and the types of patients included in the study.

In the current study, a higher positivity rate was observed in males (21.00%) compared to females (19.26%) among the total positive samples. A previous study from Rajasthan [25] indicated a positivity rate of 67.9% in males and 32.09% in females. Wattal C et al., from Delhi reported a positivity rate of 25.6% in males and 19.2% in females [16]. Another study by Bhandari S et al., from Jaipur, Rajasthan, reported a positivity rate of 62.7% in males and 37.3% in females [26]. A previous study from Bhilwara, Rajasthan, reported a predominance of males (70.8%) compared to females (29.2%) [27]. Although there are variations in the percentage of positivity between the present study and the above studies, the present study findings correlate with the above studies in the predominance of positivity among males compared to females. The high positivity rate among males in this study may be attributed to the higher number of samples collected from the male population. It may also be due to the enhanced expression of Angiotensin Converting Enzyme 2 (ACE2) receptors in the male population compared to the female population [28].

In the present study, IPD patients accounted for a positivity rate of 13.83%. A previous study from Delhi [16] reported a positivity rate of 35.50% for IPD patients among the total positive samples. Mo Y et al., from the Netherlands reported a positivity rate of 1.4% in IPD patients [29]. The present study findings differ from the above studies.

In the current study, the most predominantly affected age groups for SARS-CoV-2 are 21-60 years (14.85%), with a predominance of the 41-50 years (4.13%) age group. This finding was consistent with a previous study on predominantly affected age groups, although there are differences in the percentages between the age groups [24]. However, in the previous study, the predominant age group affected was 51-60 years, which differs from the present study [24]. The increased burden of the virus in the age groups between 21-60 years may be attributed to their outdoor and work environment exposure. The major symptoms observed in the present study are fever, cough, and shortness of breath. Findings with a predominance of fever and cough were reported by a study from Delhi [16]. Patil P et al., reported fever, cough, and shortness of breath as the most common symptoms [22]. A previous study from Kerala [30] reported fever as one of the common symptoms in SARS-CoV-2 patients. A study by Bongomin F et al., from Uganda reported difficulty in breathing and cough as the most common symptoms [31]. The present study findings were consistent with the above studies.

The least affected age groups in this study are the extremities, i.e., 0-10 years (0.18%) and 81-100 years (0.42%). Yengkhom BS et al., reported the lowest positivity in the age groups of 0-10 and 81-90 years [24]. This may be due to the fact that they have less exposure compared to young adults. Furthermore, these age groups are more cautious during SARS-CoV-2 times [24].

Among the 3,109 samples of symptomatic patients for SARS-CoV-2, 987 (31.75%) were positive, and among the 10,941 asymptomatic patients, 1,874 (17.13%) were positive. The predominance of positivity in symptomatic patients compared to asymptomatic patients in the present study was similar to an earlier study [24]. However, a study from Pune, Maharashtra [22], reported that although the predominance in samples is from asymptomatic patients, there is no difference in the positivity between asymptomatic and symptomatic patients. The higher number of samples tested in asymptomatic patients may be due to mandatory RT-PCR testing for SARS-CoV-2 for travel and individuals' self-testing to know their status. Asymptomatic individuals act as an important source of infection for transmission to susceptible populations [22].

Following January 2022, the number of samples as well as the rate of positivity for SARS-CoV-2 in this study had declined. This may be due to enhanced immunity of the population against the virus, widespread vaccination, and improved therapies. The mortality rate of hospitalised patients decreased with the increase in the number of waves of SARS-CoV-2 [32].

Respiratory viruses are predominant in winter months. Environmental factors such as sunlight, temperature, UV radiation, and humidity may be responsible for the seasonal transmission of coronaviruses and the spread of new variants of concern [11,33,34]. The present study showed two major peaks and a minor peak when analysing seasonal trends. The first major peak was observed between August 2020 to September 2020, corresponding to the first wave of the pandemic [23]. The second major peak was observed between March 2021 to April 2021, corresponding to the second wave of the pandemic [23]. A minor peak was noticed in the month of January 2022. Areas with warmer, more humid, and tropical climates may have one or two peaks but may also have a higher number of cases throughout the year [35]. Interestingly, the second peak in the present study was observed in the summer months.

A study from New York [36] reported that new cases of SARS-CoV-2 were associated with temperature and humidity. An earlier study from the Netherlands [37] reported a more severe second wave corresponding to the flu season. The seasonal trends of SARS-CoV-2 may vary in different geographical areas [8]. The activity of seasonal coronaviruses in temperate sites of China was found to be less seasonal, with high activity observed in winter, autumn, and summer [34].

In the present study, the number of cases was higher in the second wave compared to the first wave, and mixed seasonal trends were observed. These findings were consistent with a previous study from Maharashtra [23]. A study by Wiemken TL et al., reported that the rate of hospitalisations varied with seasons. However, in the present study, the burden of the disease was high in the second wave, but the hospitalisation status in different seasons was not investigated [7]. The circulation of SARS-CoV-2 was observed with a maintenance phase between the peaks. The transmissibility of SARS-CoV-2 is higher than influenza and many other seasonal respiratory viruses, which may account for its enhanced activity throughout the year compared to other pathogens. This poses substantial morbidity and mortality throughout the year [7].

The results of this study highlight the need to understand the burden of the virus along with seasonal trends, which helps the healthcare system and population better protect against serious complications. It also aids in developing necessary interventions to reduce the burden and impact on the healthcare system. Since data on the burden along with seasonal trends are lacking from the study region, further studies are required to provide more insights into the existing data, which may help in developing better protective measures. There is also a need to assess the role of vaccines in reducing the severity of the disease.

Limitation(s)

The vaccination status of the patients was not studied in this study. The mortality rate caused by SARS-CoV-2 was not determined. Co-infections associated with SARS-CoV-2, which may contribute to the severity of the infection, were not studied. Inconclusive samples were not included in the study.

CONCLUSION(S)

Enhanced precautionary measures are required for the age group of 21-60 years, as they are more vulnerable to SARS-CoV-2. Asymptomatic patients showed a positivity rate of 17.13%. Due to the mixed trend of seasonal transmission of SARS-CoV-2, continuous surveillance of the virus is necessary. The study results will be useful for epidemiological purposes and for planning strategies that may help reduce the duration of the pandemic.

REFERENCES

[1] World Health Organization (WHO). Weekly epidemiological update on COVID-19 - 1 February 2023. https://www.who.int/publications/m/item/weekly-epidemiological-update-on-covid-19---1-february-2023. [Accessed on 21 February 2023].
[2] World Health Organization (WHO). Rational use of personal protective equipment for Coronavirus Disease (COVID-19) and considerations during severe shortages: Interim guidance, 6 April 2020. https:// apps.who.int/iris/handle/10665/3316952. [Accessed on 02 February 2023].
[3] Ghosh A, Nundy S, Mallick TK. How India is dealing with COVID-19 pandemic. Sens Int. 2020;1:100021. Doi: 10.1016/j.sintl.2020.100021.
[4] World Health Organisation. Coronavirus Disease (COVID-19). https://covid19.who.int/. (Accessed 22/09/2023).
[5] Mave V, Shaikh A, Monteiro JM, Bogam P, Pujari BS, Gupte N. Impact of National and Regional lockdowns on growth of COVID-19 cases in COVID-hotspot city of Pune in Western India: A real-world data analysis. MedRxiv. 2021. Doi: 10.1101/2021.05.05.21254694.
[6] Malik MA. Fragility and challenges of health systems in pandemic: Lessons from India's second wave of Coronavirus Disease 2019 (COVID-19). Glob Health J. 2022;6(1):44-49. Doi: 10.1016/j.glohj.2022.01.006.
[7] Wiemken TL, Khan F, Puzniak L, Yang W, Simmering J, Polgreen P, et al. Seasonal trends in COVID-19 cases, hospitalizations, and mortality in the United States and Europe. Sci Rep. 2023;13(1):3886. Doi: 10.1038/s41598-023-31057-1.
[8] Kissler SM, Tedijanto C, Goldstein E, Grad YH, Lipsitch M. Projecting the transmission dynamics of SARS-CoV-2 through the post pandemic period. Science. 2020;368(6493):860-68. Doi: 10.1126/science.abb5793.
[9] Landier J, Paireau J, Rebaudet S, Legendre E, Lehot L, Fontanet A, et al. Cold and dry winter conditions are associated with greater SARS-CoV-2 transmission at regional level in western countries during the first epidemic wave. Sci Rep. 2021;11(1):12756. Doi: 10.1038/s41598-021-91798-9.
[10] Chen S, Prettner K, Kuhn M, Geldsetzer P, Wang C, Barnighausen T, et al. Climate and the spread of COVID-19. Sci Rep. 2021;11(1):9042. Doi: 10.1038/s41598-021-87692-z.
[11] Gavenciak T, Monrad JT, Leech G, Sharma M, Mindermann S, Bhatt S, et al. Seasonal variation in SARS-CoV-2 transmission intemperate climates: A Bayesian modelling study in143 European regions. PLoS Comput Biol. 2022;18(8):e1010435. Doi: 10.1371/journal.pcbi.1010435.

[12] Interim Guidelines for Collecting and Handling of Clinical Specimens for COVID-19 Testing. https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinicalspecimens.html#:~:text=Firmly%20sample%20the%20nasal%20wall,nostril%20using%20the%20same%20swab. [Accessed on 26/06/2023].
[13] QIAcube® Connect User Manual. https://portalimages.blob.core.windows.net/products/pdfs/s3evwadj_HB-2594-002_UM_QIAcubeConnect_0919_WW.pdf. [Accessed on 26/06/2023].
[14] QIAamp Viral RNA Mini Kit. https://ehslegacy.unr.edu/msdsfiles/36126.pdf. [Accessed on 26/06/2023].
[15] Rangaiah A, Shankar SM, Basawarajappa SG, Shah PA, Chandrashekar A, Munegowda A, et al. Detection of SARS-CoV-2 in clinical samples: Target-specific analysis of qualitative reverse Transcription-Polymerase Chain Reaction (RT-PCR) diagnostic kits. IJID Reg. 2021;1:163-69. Doi: 10.1016/j.ijregi.2021.11.004.
[16] Wattal C, Raveendran R, Oberoi JK, Goel N, Datta S, Rao BK, et al. Clinical presentation & laboratory diagnosis of SARS-CoV-2: An observational study from a tertiary care centre in New Delhi, India. Indian J Med Microbiol. 2021;39(3):295-99. Doi: 10.1016/j.ijmmb.2021.04.007.
[17] The Lancet Infectious Diseases. Supplementary appendix 3 (2021). https://www.thelancet.com/cms/10.1016/S1473-3099(21)00070-0/attachment/eff12e31-3319-4519-b371-03b4985a3dd2/mmc3.pdf. [Accessed on 18 August 2023].
[18] Argene SARS-CoV-2 R-GENE. Biomerieux, Ref 423735;2020:01-20. (Attached as soft copy of kit literature).
[19] M nCoV Real-Time Detection kit. https://www.fda.gov/media/137302/download. [Accessed on 22/09/2023].
[20] PathoDetect Coronavirus (COVID-19) - Qualitative PCR Kit in Humans. Alliance Transfusion- My lab discovery solutions, Catlog No: PCOVS100; Manual|VA, Pp. 1-13. (Attached as soft copy of kit literature).
[21] Peters A, Vetter P, Guitart C, Lotfinejad N, Pittet D. Understanding the emerging coronavirus: What it means for health security and infection prevention. J Hosp Infect. 2020;104(4):440-48. Doi: 10.1016/j.jhin.2020.02.023.
[22] Patil P, Thosani P, Karade S, Anand KB, Shergill SPS, Sen S, et al. Laboratory diagnosis of SARS-CoV-2 infection: Single centre experience of first 12,000 samples. J Clin Diagn Res. 2021;15(4):DC09-DC12. Doi: 10.7860/JCDR/2021/48127.14795.
[23] Bogam P, Joshi A, Nagarkar S, Jain D, Gupte N, Shashidhara LS, et al. Burden of COVID-19 and case fatality rate in Pune, India: An analysis of the first and second wave of the pandemic. IJID Reg. 2022;2:74-81. Doi: 10.1016/j.ijregi.2021.12.006.
[24] Yengkhom BS, Laifangbam S, Mahajan K, Roy A, Samom P. SARS-CoV-2 positivity using closed system RT-PCR at a tertiary care medical institute in Manipur, India. J Clin Diagn Res. 2022;16(5):DC42-DC45. Doi: 10.7860/JCDR/2022/55009.16400.
[25] Sharma RP, Gupta J, Gaur KL, Meena D, Aswal P, Sharma KK, et al. COVID-19 in Rajasthan: Status and effects of containment measures. Int J Med Public Health. 2020;10(4):202-06. Doi: 10.5530/ijmedph.2020.4.43.
[26] Bhandari S, Shaktawata AS, Sharma R, Mehta S, Pateld B, Guptab K, et al. COVID-19– A descriptive study of demographic trends in Rajasthan, listed in top five affected states of India. Menoufia Med J. 2021;34:328-32. Doi: 10.4103/mmj.mmj_183_20.
[27] Choudary MK, Jain S, Meena S, Meena D, Gour A, Sharma S. Co-morbidities and complications in covid-19 recovered patients in Bhilwara District, Rajasthan, India: A descriptive study. J Clin Diagn Res. 2022;16(3):LC33-LC36. Doi: 10.7860/JCDR/2022/52205.16159.
[28] Kopel J, Perisetti A, Roghani A, Aziz M, Gajendran M, Goyal H. Racial and gender-based differences in COVID-19. Front Public Health. 2020;28(8):418. Doi: 10.3389/fpubh.2020.00418.
[29] Mo Y, Eyre DW, Lumley SF, Walker TM, Shaw RH, O'Donnell D, et al. Transmission of community- and hospital-acquired SARS-CoV-2 in hospital settings in the UK: A cohort study. PLoS Med. 2021;18(10):e1003816. Doi: 10.1371/journal.pmed.10038.
[30] Manjiyil IJ, Areekal B, Jose R, Andrews AM, Rajagopalawarrier BK, Veetil SP, et al. Clinico-epidemiological profile and outcome of COVID-19 positive healthcare workers in a tertiary care centre in south India. J Clin Diagn Res. 2021;15(7):DC01-DC05. Doi: 10.7860/JCDR/2021/50209.15021.
[31] Bongomin F, Fleischer B, Olum R, Natukunda B, Kiguli S, Byakika-Kibwika P, et al. High Mortality during the second wave of the Coronavirus Disease 2019 (COVID-19) pandemic in Uganda: Experience from a National Referral COVID-19 treatment unit. Open Forum Infect Dis. 2021;8(11):ofab530. Doi: 10.1093/ofid/ofab530.
[32] Tandon P, Leibner ES, Hackett A, Maguire K, Mashriqi N, Kohli-Seth R. The third wave: Comparing seasonal trends in COVID-19 patient data at a large hospital system in New York City. Crit Care Explor. 2022;4(3):e0653. Doi: 10.1097/CCE.0000000000000653.
[33] Swamy MA, Malhotra B, Janardhan Reddy PV, Tiwari J. Profile of respiratory pathogens causing acute respiratory infections in hospitalised children at Rajasthan a 4 year's study. Indian J Med Microbiol. 2018;36(2):163-71. Doi: 10.4103/ijmm.IJMM_18_84.
[34] Li Y, Wang X, Nair H. Global seasonality of human seasonal coronaviruses: A clue for postpandemic circulating season of severe acute respiratory syndrome coronavirus 2? J Infect Dis. 2020;222(7):1090-97. Doi: 10.1093/infdis/jiaa436.
[35] Nichols GL, Gillingham EL, Macintyre HL, Vardoulakis S, Hajat S, Sarran CE, et al. Coronavirus seasonality, respiratory infections and weather. BMC Infect Dis. 2021;21:1101. Doi: 10.1186/s12879-021-06785-2.
[36] Adhikari A, Yin J. Short-term effects of ambient ozone, PM2.5, and meteorological factors on COVID-19 confirmed cases and deaths in Queens, New York. Int J Environ Res Public Health. 2020;17(11):4047. Doi: 10.3390/ijerph17114047.
[37] Hoogeveen MJ, Hoogeveen EK. Comparable seasonal pattern for COVID-19 and flu-like illnesses. One Health. 2021;13:100277. Doi: 10.1016/j.onehlt.2021.100277.

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