



Original Research Article

COVID 19 infection during first and second wave in Madhya Pradesh, Central India

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ABSTRACT

Background and Objective: India has experienced a massive surge of COVID 19 cases and death since its appearance in January 2020.

Aims: In the present cohort study, the percent positivity of non-hospitalized COVID-19 cases among male and female of different age group were analysed, during both first and second wave.

Materials and Methods: A total of 1,75,739 patients, from non-hospitalised settings, were referred/walked-in to our diagnostic centre in the present cohort study between August 2020-June 2021. The collection and testing were approved by NABL, Government of India.

Results: Of the tested samples, 40999 (39.15%) males and 28730 (40.46%) females were positive for COVID-19. The second wave (February 2021-June 2021) detected higher number of positive cases (13,922 vs 55,807, $p < 0.001$). During the first wave (August 2020-January 2021), percent positivity was more amongst male ($31.28 \pm 10.75\%$), but second wave recorded higher percent positivity amongst female ($56.8 \pm 12.24\%$). Asymptomatic female cases were higher during both waves (2,769; $54.05 \pm 5.85\%$ vs 14,166; $59.48 \pm 9.88\%$). The highest percent positivity was amongst older >60 years of age (37.47 ± 16.69) in both waves and lowest amongst 18-30 years ($23.17 \pm 13.86\%$). Fever was the predominant symptom in both waves (95%) followed by cough (70% vs 86%). Abdominal pain, nausea and chest pain were prominent in the first wave.

Conclusion: Few reports are available from India on non-hospitalized COVID-19 patients. This study will help for developing knowledge on the role of symptomatic and asymptomatic cases in transmission of SARS-CoV-2 in Central part of India amongst non-hospitalized cases and will help for developing strategies to prepare for impending subsequent waves in disparate population groups across India.

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1. Introduction

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), a novel evolutionary divergent RNA virus, is responsible for the present devastating coronavirus disease 19 (COVID-19) pandemic.¹ The current outbreak of COVID-19 caused by SARS-CoV-2, was first reported in

December 2019 in Wuhan, China and spread globally.^{1,2} COVID-19 has already affected over 2 billion people, claiming more than 4 million lives in over 200 nations all over the world.³ Step-wise mutations have led to the gradual spill over of virus and after crossing the inter-species interface, the virus has adapted itself for a stable human-to-human transmission. The spread of SARS-CoV-2 virus has been unprecedentedly fast, and was declared a pandemic by the World Health Organization (WHO) on

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With a population of more than 1.3 billion people, India had reported second highest cases of COVID-19. In India, COVID-19 was first detected on January 30, 2020. Over 33 million cases and 4.4 lakh deaths have been reported from the country till August 31, 2020.³ The country experienced the first peak of COVID-19 in the middle of September 2020 and was followed by a progressive decrease till January 2021. Several countries have seen a two-wave pattern of reported cases, with a first wave in spring and a second in late summer and autumn.⁴⁻⁷ India experienced a huge second surge of COVID 19 cases since March 2021 and extended till May 2021.⁸⁻¹¹

COVID-19 positive cases were reported among both symptomatic and asymptomatic cases. Therefore, it is utmost important to have a proper diagnosis to combat the rapid transmission of the virus.¹² In India, very few reports have been published describing demographic and clinical attributes of the second wave. In the present study positive covid cases have been studied in the first and the second wave based on available data on age, gender, symptomatic and asymptomatic condition.

2. Materials and Methods

2.1. Study design

A total of 1,75,739 samples were tested at Molecular Biology Laboratory of our diagnostic centre for Covid 19 through qualitative Real Time Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) during August 2020-June 2021. The non-hospitalised patients were referred or walked in for testing at the centre. The sample collection, testing and reporting was approved by National Accreditation Board of Testing and Calibration Laboratories (NABL), Government of India and quality control was maintained according to their guidelines. Stratification of the two waves of pandemic was performed according to Kumar et al, 2021.⁸ Therefore, patients tested during August 2020- January 2021 were included in the study for first wave and all those tested during February 2021 to June 2021 were included as the second wave.

2.2. Sample collection

The inclusion criterion was patient with symptomatic and asymptomatic SARS-CoV-2 infection using deep swab.¹³ Samples were collected from the upper respiratory tract (nasopharyngeal and oropharyngeal exudate) and detection of the virus and early diagnosis is possible in them.^{14,15} The samples were collected in viral transport media (Himedia) at defined collection areas or from hospitals by trained technical expert, maintaining the universal precaution and were immediately transported with three-layer packing in refrigerated conditions¹³ to Molecular Biology laboratory. Of total tested samples, 1,04,733 were males & 71,006

were females. The symptoms were noted from the patients according to Indian Council of Medical Research (ICMR) specimen referral form (SRF), guided by Govt. of India. These data are not individually identifiable.

2.3. RNA extraction

RNA was extracted from 200 μ l of collected samples using Zybion automation extraction kit on automated extraction system (Zybion, China) and eluted into a 50 μ l elution buffer. The extracted RNA was stored at -80°C in aliquots.

2.4. Reverse transcription polymerase chain reaction (RT-PCR)

RT-PCR was performed using Meril Real time PCR COVID 19 kit on Real time PCR Instrument, Quantstudio 5 (ThermoFisher, USA), targeting ORF1ab gene and nucleoprotein N gene. The PCR detection system includes an endogenous internal control in order to avoid false negative results.

The Real time PCR was programmed with an initial cycle of reverse transcription at 50°C for 15 min 1 cycle, then cDNA initial denaturation 95°C for 3 min for 1 cycle and 40 cycles for denaturation for 95°C for 15 s. Further, annealing, extension and fluorescence measurement 55°C for 40 s for 40 cycles.

2.5. Data management

Demographic and clinical data were submitted at ICMR, according to the guidelines. For the study, the data were collected in an electronic data capture portal, developed and maintained by the ICMR. The data was analysed with Microsoft Excel.

2.6. Statistical analyses

Data is given as numbers and percentages or means and standard deviations. Statistical comparisons between two groups were made using the 't' test and chi square χ^2 test. Statistical significance was set at $p < 0.05$. All calculations were made using the Graphpad Prism software (GraphPad Software, Inc.). The study was approved by the Institutional ethical committee.

3. Results

The raw data of this study are as (S1 File). During the study period from August 2020 to June 2021, a total of 1,75,739 cases of suspected SARS-COV-2 were tested in Molecular Biology Laboratory. Of the tested individuals, 104733 (59.59%) were male and 71006 (40.41%) were female. A total of 40,999 (39.15%) males and 28,730 (40.46%) females, were laboratory confirmed as SARS COV-2 cases by RT-PCR targeting the ORF1ab and N gene (Table 1). During the second wave, the greater number of

patients were infected; 32331 males and 23476 females were COVID 19 RT PCR positive whereas in the first wave 8668 male and 5254 female cases were tested positive.

Gender wise distribution of COVID 19 cases during the study period revealed a significantly higher ($p < 0.01$) number of male percent positive cases in the first wave ($31.28 \pm 10.75\%$) as compared to females ($27.89 \pm 8.07\%$) whereas during the second wave, female cases were significantly higher ($33.57 \pm 17.63\%$) ($p < 0.01$) than male ($31.80 \pm 17.28\%$) (Figure 1). The percent positivity was calculated among total cases.

Clinical history was recorded for patients at the time of sample collection. Patients' clinical history was noted as symptomatic and asymptomatic in most cases at ICMR referral form as noting of individual symptoms was not possible at the time of outbreak. Male symptomatic patients were higher (4897 cases, 35.17%) in the first wave and the second wave recorded more male asymptomatic positive cases (19636, 35.79%) (Table 1). Amongst the females, asymptomatic positive cases were higher during both the first wave (2769 cases, 19.88%) and the second (14166 cases; 25.95%, $p < 0.01$) wave. Male asymptomatic positive cases were higher (23658 cases) during the study period, though female asymptomatic cases recorded the highest percent positive cases ($59.48 \pm 9.8\%$) in the second wave (Table 2).

Percent positivity was calculated amongst male and female cases (Table 2, Figure 2). Asymptomatic cases were reported higher during the second wave. Female asymptomatic cases recorded the highest percent positive cases ($56 \pm 8.02\%$) during the study period. The percent positivity of male symptomatic cases was higher ($54.51 \pm 6.9\%$) amongst the male cases in the first wave, but in contrast to that, amongst the female, asymptomatic cases were higher ($54.05 \pm 5.8\%$). Interestingly, percent positivity was higher among both asymptomatic male (58.26%) and asymptomatic females ($59.48 \pm 9.8\%$) in the second wave.

Age wise distribution of COVID 19 cases at various time points is presented in (Table 2, Figure 3). Age group was categorized according to the vaccination strategy of Govt. of India. A significantly higher number of cases amongst older age groups > 60 years (mean $37.47 \pm 16.69\%$) was reported throughout the year. The younger age groups 18-30 years (mean $23.17 \pm 13.85\%$) followed by < 18 years (mean $25.86 \pm 13.84\%$) were less affected by COVID 19 during the study period (Table 2, Figure 3). The same pattern of infection was also observed during both the first wave and the second wave (Table 2). Monthly distribution showed the highest percent positivity in April 2021 (56.19%) and lowest infection in June 2021 (3.82%).

The study was conducted to determine the percent positivity of asymptomatic individuals and those with symptoms in various age groups. In younger age groups of < 18 years and 18-30 years, asymptomatic cases

were significantly higher amongst both male and female throughout the year (Figure 3, Table 3). Amongst < 18 years, male asymptomatic (mean 68 ± 6.62) and female asymptomatic (mean $66.4 \pm 7.31\%$) cases were significantly higher. Amongst the middle age groups i.e., 31-45 years and 46-60 years, more symptomatic cases were reported during the first wave, whereas asymptomatic patients reported during the second wave for both genders. In the age group of 31-45 years, percent positivity of symptomatic males ($59 \pm 8\%$) were significantly higher during the first wave whereas female asymptomatic cases were higher during the second wave ($59 \pm 10.85\%$). Likely, in the age group of 46-60 years, percent positivity amongst male ($61 \pm 7\%$) were significantly higher in the first wave whereas in the second wave, female percent positivity was higher ($59 \pm 10\%$).

Symptomatic COVID 19 positive cases were included in the study to observe the clinical features during both the first and second the wave (Table 3). A total of 28,924 symptomatic cases were detected COVID 19 positive; of these 7382 were in the first wave and 21051 in the second wave. Symptoms were noted from 2000 cases randomly; 1000 patients from each wave (Figure 4). During both the waves, fever was the predominant symptom (62% vs 65%, ns), followed by cough (45% vs 62.5%, $p < 0.01$), sore throat (27.2% vs 22.9%, $p < 0.01$), loss of taste (3% vs 9%, $p < 0.01$), loss of smell (3% vs 8.1%, $p < 0.01$), breathlessness (5.9% vs 2.6%, $p < 0.01$). Interestingly, clinical symptoms including abdominal pain, chest pain and nausea were reported in the first wave, but lesser/not reported by the patients in the second wave.

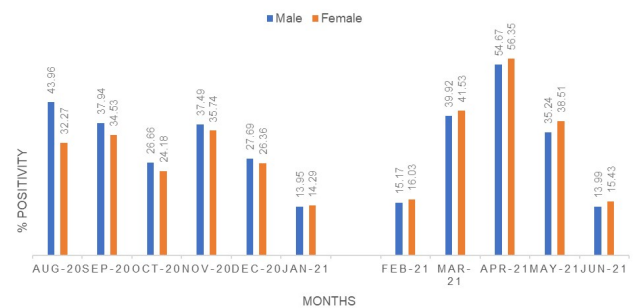


Fig. 1: Month wise percent positivity of COVID-19 cases among males and females

4. Discussion

On January 30, 2020, the World Health Organization declared the COVID 19 outbreak as Public Health Emergency of International Concern, and proclaimed as a pandemic on 11 March 2020. The first wave of the disease was reported in Europe (Spain, Italy, UK, Neitherland) in February-March 2020 and lasted till May-June 2020, whereas the second wave commenced in mid-September 2020 and extended until December 2020 and coincided with

Table 1: Gender wise distribution of COVID 19 cases. Percent positivity was calculated out of total cases. Percentage is written in bracket.

	Male	Female	p value	Male Symptomatic	Male Asymptomatic	p value	Female Symptomatic	Female Asymptomatic	p value
Total cases	40999 (37.38%)	28730 (38.62%)		17341 (24.87%)	23658 (33.93%)	p<0.001	11583 (11.61%)	17147 (24.59%)	p<0.001
First wave (August 2020- January 2021)									
Total	8668 (31.28%)	5254 (27.89%)		4897 (35.17%)	3771 (27.08%)	p<0.001	2485 (17.84%)	2769 (19.88%)	p<0.001
Second wave (August 2020- January 2021)									
Total	32331 (31.8%)	23476 (33.57%)		12149 (22.15%)	19636 (35.79%)	p<0.001	8902 (16.31%)	14166 (25.95%)	p<0.001

Table 2: Percent positivity of COVID 19 cases among various age group

Month	Age group					MEAN	
	<18 years	18-30 years	31-45 years	46-60 years	>60 years		
Aug-20	44	32	49	51	39	38.83	First wave
Sep-20	38	35	62	38	54	35.35	
Oct-20	27	24	61	39	51	25.73	
Nov-20	37	36	58	42	47	36.58	
Dec-20	28	26	50	50	43	27.15	
Jan-21	14	14	46	54	41	14.93	
Feb-21	15	16	54	46	48	15.76	Second wave
Mar-21	40	42	52	48	48	41.09	
Apr-21	55	56	42	58	43	56.19	
May-21	35	39	23	77	24	37.98	
Jun-21	14	15	37	63	38	3.82	
First wave	31	28	55	45	46	54	
SD	11	8	7	7	6	6	
Second wave	32	34	42	58	41	59	
SD	17	18	12	12	10	10	

Table 3: Clinical characteristics of patients during first and second wave of the pandemic in SSDC, Indore. Percentage is written in bracket

Features	First wave	Second wave	p value
Fever	620 (95)	622 (95)	ns
Cough	450 (70)	562 (86)	ns
Sore throat	272 (41.8)	229 (35.2)	p<0.01
Loss of smell	30 (4.5)	81 (12.5)	p<0.01
Loss of taste	30 (4.5)	90 (13.8)	p<0.01
Diarrhoea	4 (0.6)	13 (2)	p<0.01
Breathlessness	59 (9.07)	26 (2)	p<0.01
Body ache	20	20 (3.07)	ns
Nasal discharge	14	0	N.A.
Abdominal pain	12	0	N.A.
chest pain	16	0	N.A.
Nausea	3 (0.4)	1 (0.15)	p<0.01

ns= not significant

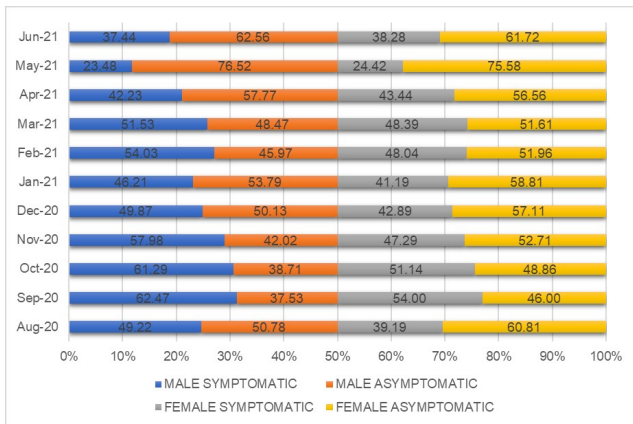


Fig. 2: Month wise percent positivity of COVID-19 cases among various age group

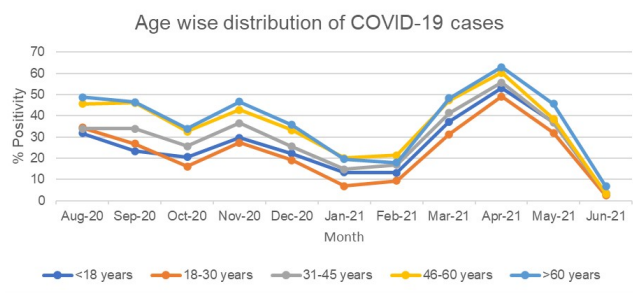


Fig. 3: Percent positivity of COVID-19 symptomatic and asymptomatic cases among various age group

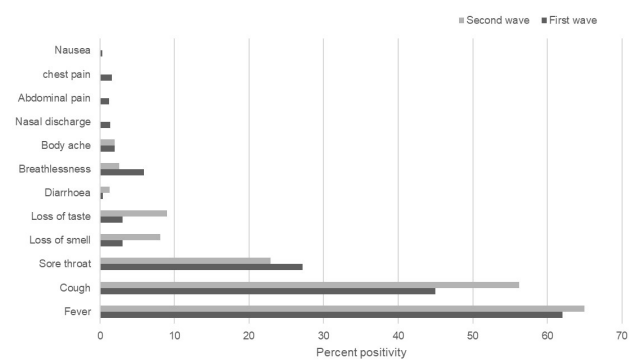


Fig. 4: Frequency percentage of symptoms among COVID-19 cases in the first and second wave

the first wave of COVID 19 pandemic in India. In India, the first case of COVID-19 was detected in India on January 30, 2020. The Government of India had imposed a nationwide lockdown on March 25, 2020 to control the pandemic. The strict lockdown drastically reduced the infection rate, till the restricted “unlock” since June 01, 2020. The cases rose in July 2020 as more than 20,000 cases were reported each day from almost every state of India. The peak of the first wave with more than 93,000 cases was recorded in the mid of

September 2020 and was followed by a progressive decrease till January 2021. The massive surge of the second COVID-19 wave began in February 2021 followed by the steep rise of cases from April 2021, where the average daily cases were much higher than the peak of the first wave. The cases gradually declined to low levels towards the end of June 2021. Till August 31, 2021, India recorded more than 32 million cases and over 4 lakh deaths (WHO). This study describes the two waves of COVID-19 epidemic in India. In India, unlike the first wave, the second wave had even spread and affected the rural parts also.¹⁶

The present study pointed out significantly higher percent ($p < 0.01$) of female cases (38.62%) compared to male (37.38%), despite more cases of male positive patients than female (40999 vs.28730) (Table 1). Unlikely, a study from Spain reported no significant difference of COVID 19 cases amongst males and females in both the waves.¹⁷ A total of 13992 samples were tested positive by RTPCR during August 2020 to January 2021 (first wave) whereas 55805 patients were tested positive from February 2021 to June 2021, i.e. during the second wave and were significantly higher ($p < 0.00001$) as compared to the first wave (Table 1). A retrospective web-based portal study over 112,860 COVID-19 patients describe the distribution of age and gender among the Indian population during the first wave.¹⁸ The male COVID-19 cases (65.39%) were more than females (34.61%) although prevalent of infection was significantly higher among females of lower age groups.¹⁸

Various studies across the globe till December 2020 showed that the older males were more susceptible ($>50\%$) in getting infected by SARS-CoV-2.^{18,19} Till December 2020, aged men with medical comorbid conditions (obesity, cardiovascular disease, diabetes, cancer, chronic lung disease, chronic kidney disease) succumb to significantly higher cumulative morbidity ratio and higher case fatality rate.²⁰ In the present study, percent positive cases were higher amongst older age groups >60 years throughout the year (mean $37.47\% + 16.69$) (Table 3). The month of April 2021 recorded the highest percent of COVID 19 positive cases amongst all the age groups, maximum amongst age groups of >60 years (62.97%). Interestingly, only these age group (>60 years) was vaccinated during the second wave. Information regarding post diagnosis case fatality rate and hospitalization rate of these patient’s group was not available. Thus, correlation between vaccination status and severity rate of these patients’ group between two waves could not be stated. Cases were the lowest amongst 18-30 years of age group during the first wave (21.78%) and the second wave (24.84%). The cases dropped to the lowest level in June 2021 (mean 3.82%). Study by Kumar et al,⁸ reported more death in the younger age group amongst the hospitalised cases in the second wave. In India, vaccination programmes have been prioritized for high-risk groups such as frontline workers since January 16, 2021. The geriatric

population of >60 years of age was vaccinated since March 1, 2021. The vaccination programme for the younger population was started from May 1, 2021 resulting in largely unvaccinated younger age-groups during the second wave. Additionally, inadequacy of hospital beds, lifesaving medicine, ventilators and oxygen supply increased the changes in the age pattern for hospitalization and deaths particularly in the young population of above >20 years.^{8,10}

The first wave recorded more symptomatic positive cases amongst the male (54.51%±6.9), compared to asymptomatic cases (45.49%±6.9; $p < 0.01$) (Table 2). Interestingly, during the second wave, asymptomatic cases were significantly higher amongst both males (58.26%±12.24) and females (59.48%±9.88) which might be due to the development of sub-clinical or mild symptoms amongst non-hospitalised cases.

In the present study, fever was the most common symptom during both the waves (62% vs 65%), followed by cough (45% vs 56.2%) and sore throat (27.2% vs 22.9%). Unlikely, Kumar et al⁸ reported more breathlessness/shortness of breath amongst the hospitalized patients (42.8% vs 48.6%), whereas in our study it was significantly lower $p < 0.001$ (2.6% vs 5.9%) as patients tested at that time point were non-hospitalized. According to guideline of Clinical management of COVID-19, Ministry of Health & Family Welfare, Government of India, patients were referred to manage COVID-19 in Dedicated Covid Hospital (DCH) if the clinical condition is severe like in case of severe Pneumonia, Acute Respiratory Distress Syndrome (ARDS), sepsis or septic shock, as requirement of oxygen supplementation and need for mechanical ventilation. Genomic surveillance from various parts of India reported alpha variant (B.1.1.7) as variant of concern in the first wave whereas highly infectious Delta variant (B.1.617.2) was mainly responsible for the severe second wave of COVID-19 infections.^{5,10,21,22} The explosive second wave put India's health system under pressure and thus made hospitalization possible for severe cases only. The mild and moderately severe patients were treated at home (as per home isolation guidelines) or managed in Dedicated Covid Health Centre (DCHC).

5. Limitation

There are some limitations in the present study. The rapid surge of COVID-19 in the second wave due to more infectious SARS-CoV-2 strain made the health care system overloaded with rapid increase of large number of cases being daily reported.^{8,10} This did not make it possible to enrol the detail symptoms recording of the patients in our registry, though symptomatic category was recorded. The details of coinfection were also not available for records.

6. Conclusion

Till now, very few reports are available from India on non-hospitalized patients. This report describes the first data

on COVID -19 situation in Central India during both the first and second the wave. This study will be helpful as a reference for policy making and for developing strategies to prepare for the impending third wave in disparate population groups across India.

7. Source of Funding

None.

8. Conflicts of Interest


None.

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