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Coronavirus Pandemic

Age and sex distribution trends of SARS-CoV-2 infections: Insights from three epidemic waves in Puducherry, India

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Abstract

Introduction: This study analyzed the age and sex distribution of COVID-19 patients during the initial three COVID-19 waves in Puducherry, India, from August 2020 to March 2022, to understand the distribution of infection across different demographic groups.

Methods: The disease surveillance program conducted at ICMR-Vector Control Research Centre processed 79,705 Throat Swab/Nasal Swab (TSNS) samples received from various institutions in Puducherry through the Integrated Disease Surveillance Program (IDSP). Real-time reverse-transcriptase-polymerase chain reaction (rRT-PCR) was performed following approved protocols.

Results: Test positivity rates during the second (14.6%) and third waves (25.1%) were significantly higher than the first wave (11.4%). In the first wave, children ($p < 0.001$) and elderly individuals ($p = 0.017$) had a lower risk of testing positive than adults. However, in the second wave, elderly individuals had a 1.12 (95% CI: 1.03 – 1.23) times greater risk of contracting COVID-19 ($p = 0.013$). Children had a lower risk of testing positive across all waves ($p < 0.001$). A significant sex difference was noted only in the first wave, with males having a 1.27 (1.18-1.37) times greater chance of being COVID-19 positive. The mean age of female patients was significantly younger than male patients in the third wave ($p = 0.008$). The third wave showed an increasing trend of infection across all age groups and sexes, especially among younger individuals.

Conclusions: The study highlights an increasing trend of infections across all age groups and sexes during the third wave. Micro-epidemiological analyses are crucial for developing targeted intervention strategies that address age and sex demographics effectively.

Key words: COVID-19; rRT–PCR; disease transmission; age trend; sex distribution; Puducherry; India

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Introduction

Coronavirus disease 2019 (COVID-19) originated in Hubei Province, China, in 2019 and spread to approximately 200 countries by the end of April 2020 [1]. It has rapidly spread across the world with extensive health, social, economic, and political implications. India, a low-and middle-income country with the second largest population in the world, has put forth a long, brave fight against the pandemic by imposing early lockdowns and rapidly establishing testing centers across the nation. Since the first reported case of COVID-19 in India on 30 January 2020, the country has experienced multiple phases of the pandemic, with significant peaks and troughs contributing to the waves of infection. Distinct viral strains shaped India's three major COVID-19 waves: the first wave (mid-2020) by the original strain, the second wave (early 2021) by the Delta variant (B.1.617.2), and the third wave (late 2021 to early 2022) by the Omicron variant (B.1.1.529) [2,3]. By mid-2022, India had witnessed more than 43 million COVID-19 cases, including 526,000 deaths [4]. While the Omicron variant spread rapidly, it was linked to a lower incidence of hospitalization and death compared to the

Delta variant [5].

Although SARS-CoV-2 is known to affect all age groups, several assumptions exist on age-related susceptibility and outcome variations. Research from various parts of the world has indicated that the second wave seemed to affect younger age groups compared to the first [6–11]. In contrast, Germany reported an increase in the average age of the affected population over time, while a study from central India found no significant age-related differences in infection rates [12,13]. The sex distribution of infections has also been the focus of studies worldwide, with males generally showing higher rates of severe disease and mortality compared to females, though infection rates do not always follow the same pattern [14–17]. This was substantiated by studies in countries like Spain and Nigeria, where males were found to have higher infection rates than females [18,19].

Sociodemographic susceptibility may be influenced by a variety of factors, including pandemic control measures such as quarantine and vaccination, in addition to genetic influences. The biological determinants of age and sex susceptibility to COVID-19 in India have been studied, revealing that females in younger age groups (< 35 years) have a greater chance of infection than males. Moreover, it has been observed that the probability of infection gradually decreases with age in women and increases with age in men [20]. Environmental factors were also found to be associated with COVID-19 vulnerability [21]. COVID-19 transmission in India was reported to be worsened by inadequate access to basic amenities such as indoor latrines, drainage, electricity, and drinking water, while the presence of elderly populations slowed its spread [22]. In many low-income and rural households, shared communal spaces for essential activities made physical distancing difficult. Furthermore, poor healthcare facilities and low education levels correlated with fatality rates [22]. Given India's unique situation with its large population size, complex socioeconomic structure, geographical and cultural disparities, and interstate migration, understanding the sociodemographic patterns of a pandemic is crucial for public health interventions.

Puducherry is a Union Territory in the coastal region of southern India, with four geographically separate districts. It is a destination for tourists across the state and national borders, accounting for less than 0.5% of the COVID-19 cases reported in India. The state has an estimated population of approximately 1.6 million, with a sex ratio exceeding the national average, at 1037 females for every 1000 males [23]. The elderly population (aged 60 and above) accounts for about 10% of the total population, while around 60% falls within the 18-60 age group. In this study, we present an analysis of the COVID-19 laboratory data at the Indian Council of Medical Research (ICMR)-Vector Control Research Centre (VCRC), during the three initial epidemic waves of COVID-19 (August 2020 – March 2022) to identify trends in COVID-19 positivity with regard to age and sex distribution.

Methodology

Laboratory Setup and Sample Collection

The ICMR-Vector Control Research Centre, Puducherry, operationalized a COVID-19 testing laboratory in August 2020 to support the state government's testing efforts. The laboratory processed throat and nasal swab (TSNS) samples were collected from individuals presenting with suspected COVID-19 symptoms such as fever, cough, fatigue, headache, sore throat, rhinitis, myalgia, dyspnea, etc. The samples collected from 32 Primary Health Centers (PHCs) and Indira Gandhi Medical College and Research Institute (IGMC & RI), a tertiary care hospital in Puducherry by the Integrated Disease Surveillance Program (IDSP) and State Public Health Department of Puducherry were transported in 2 or 3mL of the viral transport medium (VTM) to COVID-19 testing laboratory at ICMR-VCRC. Each sample was accompanied by the ICMR Specimen Referral Form for COVID-19, which contained relevant details including the individual's name, age, sex, address, contact number, and type of specimen collected.

RNA Extraction and Real-Time PCR

Viral nucleic acid was extracted from the TSNS samples using the QIAamp viral RNA mini kit (Qiagen, Hilden, Germany), RNAsure COVID-19 RNA extraction kit (Trivitron Healthcare Pvt Ltd, Chennai, India), and MagRNA-II Viral RNA Extraction kit (GENES2ME Pvt Ltd, Haryana, India). These extractions followed the manufacturer's protocols. The extracted RNA was then quantified for concentration and purity using the Thermo Scientific™ μDrop.

Extracted viral RNA isolates were subjected to real-time polymerase chain reaction (RT-PCR) using one of the 10 commercially available RT-PCR kits that had been approved by ICMR for the detection of SARS-CoV-2. The kits were sourced from the ICMR depot and the Department of Health and Family Welfare, Puducherry. Details of the RT-PCR kits, including their origin, reaction conditions, RNA volumes, target genes, and threshold cycle (Ct) values, are provided in the

Table 1. COVID-19 RT–PCR results among the samples tested.

Wave	COVID-19 test results			Total number of samples tested
	Positive (%)	Negative (%)	Others (%) *	
First	3,092 (11.4)	21,778 (80.6)	2,165 (8.0)	27,035
Second	5,964 (14.6)	33,651 (82.7)	1,084 (2.7)	40,699
Third	3,006 (25.1)	8,794 (73.5)	171 (1.4)	11,971
Total	12,062 (15.1)	64,223 (80.6)	3,420 (4.3)	79,705

*Inconclusive results/ rejected samples due to insufficient quantity.

Supplementary Table 1.

The RT-PCR tests were performed using either the Light Cycler 96 (Roche) or CFX96 real-time systems, following the respective manufacturer's instructions. The data generated during the COVID-19 pandemic were analyzed to assess trends in age and sex distribution of SARS-CoV-2 infections.

Data Analysis

The data analysis was conducted using a total of 79,705 samples, which were collected during three distinct waves of the COVID-19 pandemic in Puducherry. These included 27,035 samples from the first wave (August 2020 to November 2020), 40,699 samples from the second wave (May 2021 to December 2021), and 11,971 samples from the third wave (January 2022 to March 2022). The delineation of COVID-19 pandemic waves lacks a universally accepted definition, as different authors have proposed varying criteria [24]. These criteria can include factors such as infection rates, the emergence of new variants, changes in public health measures, etc. For this study, we defined a new wave based on a weekly test positivity rate of more than 5%, which was also used to guide the relaxation of restrictions in containment zones [25].

The collected data were entered into Microsoft Excel and analyzed using Stata 14.0 software. Descriptive statistics, including mean and standard deviation, were used to represent age in years. Participants were classified into age groups, and proportions were calculated for comparison across the waves. For adjusted analysis, three age categories were made based on the COVID-19 vaccination policy in India (≤ 18 years, 19-60 years, and > 60 years). The chi-square test was used to compare age and sex distributions across the three waves, while the mean age of male and female patients was compared using analysis of variance (ANOVA). Logistic regression analysis was used to examine the risk of contracting COVID-19 in different age and sex groups. A p-value of less than 0.05 was used to determine statistical significance.

Ethical Considerations

Approval for a waiver of participant consent, as well as for the use of data generated during the three waves of the pandemic, was obtained from the Institutional Human Ethics Committee of ICMR-VCRC, Puducherry (IHEC-0122/N/S).

Results

Test Positivity Rates Across Waves

The results provide insights from the analysis of 79,705 samples tested during the three waves of COVID-19 in Puducherry. Across the three waves, the test positivity rates increased significantly, starting at 11.4% in the first wave, rising to 14.6% in the second wave, and peaking at 25.1% in the third wave. An overview of the RT-PCR results of samples tested across the three waves of the pandemic is given in Table 1. Compared to the first wave, the odds ratios of COVID-19-positive samples were 1.33 (1.27-1.39), $p < 0.001$ for the second wave, and 2.60 (2.46-2.75) for the third wave.

Age and Sex Distribution

The mean age of COVID-19-positive patients was similar in the first two waves but decreased slightly in the third wave. Specifically, the mean age was 38.4 (± 17.8) years in the first wave, 38.5 (± 16.6) years in the second wave, and 36.5 (± 16.8) years in the third wave. The most affected age group across all waves was 41-60 years, followed by the 19-40 years category. All age groups showed a significantly greater positivity rate

Figure 1. Age distribution of COVID-19 patients stratified by sex.

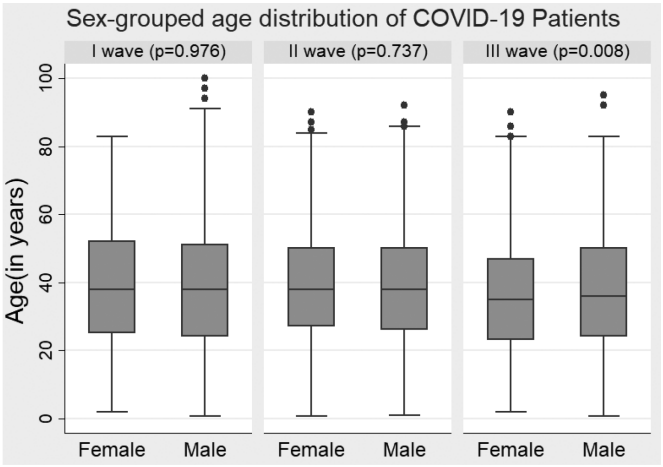

Table 2. Age and sex distribution of patients with laboratory-confirmed COVID-19.

Demographics of COVID-19 patients	Wave I		Wave II		Wave III		*p*
	Number of samples tested	Positive (%)	Number of samples tested	Positive (%)	Number of samples tested	Positive (%)	
Age (years)							
≤ 5	731	47 (6.4)	1,089	100 (9.2)	271	45 (16.6)	< 0.001
6 – 18	4,916	387 (7.9)	6,067	598 (9.9)	1,819	403 (22.2)	< 0.001
19 – 40	10,852	1,277 (11.8)	18,732	2,693 (14.4)	5,739	1,372 (23.9)	< 0.001
41 – 60	7,421	1,035 (14.0)	11,289	1,969 (17.4)	3,099	938 (30.3)	< 0.001
> 60	3,115	346 (11.1)	3,522	604 (17.2)	1,043	248 (23.8)	< 0.001
Gender							
Male	14,077	1,765 (12.5)	22,813	3,305 (14.5)	6,664	1,693 (25.4)	< 0.001
Female	12,958	1,327 (10.2)	17,886	2,659 (14.9)	5,307	1,313 (24.7)	< 0.001

* Chi-square test.

when moving from the first wave to the third wave ($p < 0.001$ for all age categories). Although males constituted a greater number of patients in all the waves, the test positivity rate was comparable between males and females in the second and third waves. The age and sex distribution of the patients with laboratory-confirmed COVID-19 cases over the study period are shown in Table 2.

The mean age of the male COVID-19 patients in the first three waves was 38.56 (± 17.58), 38.38 (± 16.70), and 37.20 (± 17.11) years, respectively, which is a statistically significant difference ($p = 0.001$). Among the female patients, the mean age decreased to 35.55 (± 16.47) years in the third wave, compared to 38.54 (± 18.09) years and 38.52 (± 16.59) years in the first and second waves, respectively ($p < 0.001$). Figure 1 shows the sex distribution of the COVID-19 patients by age. The mean age of the female patients was significantly younger than that of the male patients in the third wave of the pandemic ($p = 0.008$).

Wave-stratified Adjusted Risk analysis

Patients were classified as children (≤ 18 years), adults (19-60 years), or elderly (> 60 years). The adjusted analysis showed that in the first wave, both children (OR = 0.57, 95% CI: 0.51-0.63, $p < 0.001$) and elderly (OR = 0.86, 95% CI: 0.77-0.97, $p = 0.017$) had a lower risk of testing positive for COVID-19 compared

Table 3. The risk associated with COVID-19 in terms of age and gender.

Period	Demographics of COVID-19 patients	Number of samples tested	Positive (%)	Adjusted OR (95% CI)	*p*
	Age (years)				
	19 – 60	18,273	2,312 (12.6)	1	-
	≤ 18	5,647	434 (7.7)	0.57 (0.51 – 0.63)	< 0.001
Wave 1	> 60	3,115	346 (11.1)	0.86 (0.77 – 0.97)	0.017
	Gender				
	Male	14,077	1,765 (12.5)	1.27 (1.18 – 1.37)	< 0.001
	Female	12,958	1,327 (10.2)	1	-
	Age (years)				
	19 – 60	30,021	4,662 (15.5)	1	-
	≤ 18	7,156	698 (9.7)	0.59 (0.54 – 0.64)	< 0.001
Wave 2	> 60	3,522	604 (17.2)	1.12 (1.03 – 1.23)	0.013
	Gender				
	Male	22,813	3,305 (14.5)	0.97 (0.92 – 1.03)	0.352
	Female	17,886	2,659 (14.9)	1	-
	Age (years)				
	19 – 60	8,838	2,310 (26.1)	1	-
	≤ 18	2,090	448 (21.4)	0.77 (0.69 – 0.86)	< 0.001
(Wave 3)	> 60	1,043	248 (23.8)	0.88 (0.76 – 1.02)	0.102
	Gender				
	Male	6,664	1,693 (25.4)	1.04 (0.95 – 1.13)	0.411
	Female	5,307	1,313 (24.7)	1	-
	Age (years)				
	19 – 60	57,132	9,284 (16.2)	1	-
	≤ 18	14,893	1,580 (10.6)	0.62 (0.59 – 0.66)	< 0.001
	> 60	7,680	1,198 (15.6)	0.99 (0.92 – 1.06)	0.727
	Gender				
Combined	Male	43,554	6,763 (15.5)	1.06 (1.02 – 1.10)	0.003
	Female	36,151	5,299 (14.7)	1	-
	Wave				
	First	27,035	3,092 (11.4)	1	-
	Second	40,699	5,964 (14.6)	1.31 (1.25 – 1.37)	< 0.001
	Third	11,971	3,006 (25.1)	2.56 (2.42 – 2.71)	< 0.001

to the adult population. However, in the second wave, older individuals had a 1.12 (95% CI: 1.03-1.23, p = 0.013) times greater risk of testing positive for COVID-19. Children had a significantly lower risk of testing positive than adults in all the waves (OR = 0.62, 95% CI: 0.59-0.66, $p < 0.001$).

The sex difference was significant in the first wave, where males had a 1.27 (95% CI: 1.18-1.37, $p < 0.001$) times greater chance of being COVID-19 positive. The sex difference in COVID-19 positivity was not significant in the second or third waves. The wave-stratified adjusted risks for age and sex are shown in Table 3.

Discussion

Several countries worldwide have experienced a three-wave pattern of the COVID-19 pandemic with varying degrees of severity. Many factors, including government initiatives, perceived danger and susceptibility among the population, and vaccine availability/acceptability, may have contributed to the difference in wave characteristics across the world [26]. A comparison of the initial three waves of COVID-19 showed a higher positivity rate in the third wave, where more than one-fourth of the samples tested were positive. In April-May 2021, new SARS-CoV-2 variants, namely, the B.1.617.1 and B.1.617.2 lineages, were detected in India. These variants are believed to have played a significant role in the surge of cases and deaths during the second wave [27]. The Omicron variant of the SARS-CoV-2 virus, which emerged later, was primarily responsible for the third wave. It was known to exhibit even greater transmissibility than earlier variants, including the Delta variant [28,29]. In addition, the results showed a clear increase in the positivity rate across all age and sex groups during the three waves of the pandemic. The working-age group population (19-60 years) was the most affected in all three waves, and children younger than 10 years were the least affected, irrespective of sex and ethnicity [27]. Global evidence suggests that the second wave of COVID-19 affected the younger adult population (18-40 years) more than the first wave [7]. France [8], Spain [9], Italy [10], and the USA [11] report a decrease in the median age of COVID-19 patients over time, unlike in Germany [12]. Patients were notably younger during the second wave, indicating the spread of the illness among the active working population. In contrast, Shukla *et al.* described a comparable age distribution of COVID-19 cases across all age groups in the first and second waves in the Gwalior district of Central India [13]. Furthermore, an 11-year shift in the mean age of positivity from the first to the second wave was reported in Uttar Pradesh, India [30]. Very few studies have reported findings from the third wave of COVID-19. Our data show that children were less affected than adults in all waves in Puducherry. A review conducted during the first wave of COVID-19 highlights that a notable proportion of asymptomatic infections in children can have significant epidemiological consequences, as these children may play a role in the transmission chain within the community [31]. The findings from a retrospective study conducted in the same setting suggest that the impact of COVID-19 on children during both the first and second waves was similar, with most cases being mild or asymptomatic and a low mortality rate [32]. The elderly individuals were protected in the initial wave, whereas they showed a significantly greater risk of infection in the second wave. The mean age of COVID-19 positivity decreased in the third wave, with younger age groups showing an increase in positivity rates. The great fear associated with the novel virus and the austere restrictive measures might have protected elderly people during the initial wave of the pandemic. Later, there was ease in the lockdown measures, and the working-age population would have taken more of the infection into their homes, transmitting it to the elderly people. With the progression of the third wave, younger people and children (who may have been mostly unvaccinated) became more prone to infection as the community started resuming its long-missed social life. Singh *et al*. made a similar observation from a tertiary care setting in Rajasthan, India, where the proportion of elderly among COVID-19 patients showed a significant increase in the third wave compared to the other two waves [33].

Reports on sex differences in COVID-19 infection vary over time and space. A systematic review of the initial phase of the pandemic revealed higher infection rates among men and highlighted smoking and alcohol consumption as major factors associated with the high prevalence [34]. A comparative study conducted in India analyzing the initial two waves of COVID-19 revealed that the positivity rate among males was consistently greater than that among females in both waves [30]. A study from Intensive Care Units (ICUs) in nine hospitals in Maharashtra, India, also reported a significant difference in admissions by sex across the first and second waves. Men accounted for 73.5% of ICU admissions in the first wave and 68.4% in the second wave [35]. A demographic comparison from a tertiary hospital in Rajasthan reported that over two-thirds across all three waves of the patients were men

[33]. A study from Central India reported that even though males were more affected in both waves, the incidence of infection among females increased by 9% during the second wave [13]. Biological factors, including immune responses, inflammatory markers, and hormonal differences, along with socio-behavioral factors, have been postulated to be involved in the sex-specific associations between COVID-19 infection and patient outcomes [14,36,37]. A study on COVID-19 vaccination highlighted a better immune response among females and a negative correlation between age and the immune response [38]. On the contrary, a nationwide retrospective study from Taiwan shows a higher proportion of women patients across all three waves of COVID-19 [39]. An ecological study using data from 177 countries reported a greater age-related correlation between the incidence of COVID-19 and female sex, while there was a stronger correlation between age and mortality rates in males [40].

Our results indicated that although males were more affected in the initial wave, the sex difference decreased with time, showing comparable positivity rates across both sexes in the second and third waves. The first wave showed greater positivity in the 19 to 60-year-old age group among the men, whereas the difference in sex-based age distribution was less marked in the female population. The positivity rate was greater among the adult and elderly males in the third wave, whereas elderly females were comparatively less affected. Doerre *et al.* reported a greater infection risk among women than men of working age and a greater risk among elderly men than among women. A greater number of contacts among women in the young and middle-aged groups was proposed to be one of the reasons for this difference [41]. Moreover, in a majority of households, women were the primary caregivers of COVID-19-infected people under home isolation [42]. Despite disparities in sex-based infection rates, most of the literature agrees that men have a greater risk of mortality than women [14–17].

This study has certain limitations that warrant discussion. Firstly, the analysis was performed with the available data, which restricts the ability to make further attributions regarding other sociodemographic and clinical factors, such as vaccination status, comorbidities, and the impact of prior infections. The lack of these data points may introduce bias, as individuals with varying access to healthcare or differing health behaviors may not be accurately represented. Secondly, defining the cutoff for a pandemic wave is inherently complex, especially for a disease like COVID-19. In this study, we have demarcated the waves based on the test positivity rate of 5% in the Puducherry setting, which may differ from the national data. Additionally, the data collection methods relied on laboratory-confirmed cases, which may not capture the full spectrum of infections, including asymptomatic cases or those untested due to limited access to healthcare facilities. External factors such as changes in public health policies and the emergence of new variants could also affect the results and interpretation of the data. Despite these limitations, the study provides valuable insights into age and sex distributions among COVID-19-positive samples in Puducherry, India, over a significant period during the pandemic.

Conclusions

The COVID-19 pandemic exhibits spatiotemporal dynamics characterized by continuous viral evolution, shifting control strategies, fluctuating population immunity (through prior infection and vaccination), and variations in community responses. Monitoring these factors, particularly across successive waves, is crucial for understanding transmission patterns. Our findings indicated a concerning trend in the third wave: increasing infection rates across all demographics, with a notable increase among younger age groups. While adults remain the primary drivers of transmission, increased social interactions and caregiving roles may expose younger populations and females to greater risk. We put forth some key recommendations for future pandemic preparedness. Firstly, it is important to study the demographic evolution throughout a pandemic and adjust strategies accordingly. Understanding these trends will enable the development of tailored public health interventions to prevent the spread of infection across various sociodemographic structures. Risk and behavioral change communications promoting personal protective measures should be tailored for all age groups, with particular attention to vulnerable communities. This approach requires implementing targeted informational campaigns through various media channels, taking into account literacy constraints to ensure the messages are accessible and comprehensible for all individuals. Additionally, the ongoing emergence of new variants necessitates continued surveillance across all population segments, even with substantial vaccination coverage. High mutation rates within the virus necessitate a microepidemiological approach at the local and regional levels. This can provide valuable data for developing tailored interventions that effectively mitigate transmission based on specific age and sex

demographics. Finally, gender-sensitive approaches are needed, recognizing that women's exposure risks increased due to caregiving roles during the pandemic. Future pandemic plans should provide targeted support for women in these roles while ensuring access to healthcare services for all genders. This approach will help minimize disparities in health outcomes, protect vulnerable communities, and enhance overall pandemic preparedness.

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Ethics approval and consent to participate

The proposal for the study was approved by the Institute Human Ethics Committee of ICMR Vector Control Research Centre, Puducherry (IHEC-0122/N/S).

Availability of data and materials

The datasets analyzed during the current study are available from the corresponding author upon reasonable request.

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Authors' contributions

RLJDB, VSK, and AK conceptualized the study. RLJDB, VSK, and AK supervised the study, managed the resources, and validated the findings. DS, TS, SM, DRJ, PRA, PD, YN, AE, RB, KAM, ST, RN, RP, MM, NPK, and IG were involved in data generation. ANS and AS were involved in planning and resource management. BV managed and analyzed the data. AR prepared the analysis plan and drafted the primary manuscript. VSK, AK, RLJDB, and AR were involved in revising the draft along with other authors. All the authors have reviewed and approved the final manuscript.

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Conflict of interests

No conflict of interests is declared.

References

1. Chen S, Yang J, Yang W, Wang C, Bärnighausen T (2020) COVID-19 control in China during mass population movements at New Year. Lancet 395: 764–766. doi: 10.1016/S0140-6736(20)30421-9.
2. Dhawan M, Saied AA, Mitra S, Alhumaydhi FA, Emran TB, Wilairatana P (2022) Omicron variant (B.1.1.529) and its sublineages: what do we know so far amid the emergence of recombinant variants of SARS-CoV-2? Biomed Pharmacother 154: 113522. doi: 10.1016/j.biopha.2022.113522.
3. Choudhary OP, Priyanka, Singh I, Rodriguez-Morales AJ (2021) Second wave of COVID-19 in India: dissection of the causes and lessons learnt. Travel Med Infect Dis 43: 102126. doi: 10.1016/j.tmaid.2021.102126.
4. COVID-19 INDIA (Data Source MoHFW) (n.d.) Open Government Data (OGD) Platform India. Available: https: //data.gov.in/major-indicator/covid-19-india-data-source-mohfw. Accessed: 1 May 2020.
5. Relan P, Motaze NV, Kothari K, Askie L, de Waroux OLP, van Kerkhove MD, Diaz J, Vijayaraghavan BKT (2023) Severity and outcomes of Omicron variant of SARS-CoV-2 compared to Delta variant and severity of Omicron sublineages: a systematic review and meta-analysis. BMJ Glob Health 8: e012328. doi: 10.1136/bmjgh-2023-012328.
6. Kumar G, Mukherjee A, Sharma RK, Menon GR, Sahu D, Wig N, Panda S, Rao VV, Singh S, Guleria R, Bhargava B (2021) Clinical profile of hospitalized COVID-19 patients in first and second wave of the pandemic: insights from an Indian registry based observational study. Indian J Med Res 153: 619–628. doi: 10.4103/ijmr.IJMR_1628_21
7. Boehmer TK, DeVies J, Caruso E, van Santen KL, Tang S, Black CL, Hartnett KP, Kite-Powell A, Dietz S, Lozier M, Gundlapalli AV (2020) Changing age distribution of the COVID-19 pandemic - United States, May–August 2020. MMWR Morb Mortal Wkly Rep 69: 1404–1409. doi: 10.15585/mmwr.mm6939e1.
8. Martinot M, Eyriey M, Gravier S, Kayser D, Ion C, Mohseni-Zadeh M, Ongagna JC, Schieber A, Kempf C, Group CAS

(2021) Evolution of baseline characteristics and severe outcomes in COVID-19 inpatients during the first and second waves in Northeastern France. Infect Dis Now 52: 35. doi: 10.1016/j.idnow.2021.10.002.
9. Iftimie S, López-Azcona AF, Vallverdú I, Hernández-Flix S, de Febrer G, Parra S, Hernández-Aguilera A, Riu F, Joven J, Andreychuk N, Baiges-Gaya G, Ballester F, Benavent M, Burdeos J, Català A, Castañé È, Castañé H, Colom J, Feliu M, Gabaldó X, Garrido D, Garrido P, Gil J, Guelbenzu P, Lozano C, Marimon F, Pardo P, Pujol I, Rabassa A, Revuelta L, Ríos M, Rius-Gordillo N, Rodríguez-Tomàs E, Rojewski W, Roquer-Fanlo E, Sabaté N, Teixidó A, Vasco C, Camps J, Castro A (2021) First and second waves of coronavirus disease-19: a comparative study in hospitalized patients in Reus, Spain. PLoS One 16: e0248029. doi: 10.1371/journal.pone.0248029.
10. Caramello V, Catalano A, Macciotta A, Dansero L, Sacerdote C, Costa G, Aprà F, Tua A, Boccuzzi A, Ricceri F (2022) Improvements throughout the three waves of COVID-19 pandemic: results from 4 million inhabitants of North-West Italy. J Clin Med 11: 4304. doi: 10.3390/jcm11154304.
11. Hoogenboom WS, Pham A, Anand H, Fleysher R, Buczek A, Soby S, Mirhaji P, Yee J, Duong TQ (2021) Clinical characteristics of the first and second COVID-19 waves in the Bronx, New York: A retrospective cohort study. Lancet Reg Health Am 3: 100041. doi: 10.1016/j.lana.2021.100041.
12. Budweiser S, Baş Ş, Jörres RA, Engelhardt S, Thilo C, von Delius S, Eckel F, Biller U, Lenherr K, Deerberg-Wittram J, Bauer A (2021) Comparison of the first and second waves of hospitalized patients with SARS-CoV-2. Dtsch Arztebl Int 118: 326. doi: 10.3238/arztebl.m2021.0215.
13. Shukla D, Bhadoria SS, Bansal M, Changulani R (2022) Evolution of the pandemic: analysis of demographic characteristics of COVID-19-infected patients during its two waves in Gwalior district of central India. J Family Med Prim Care 11: 1314–1321. doi: 10.4103/jfmpc.JFMPC_1189_21.
14. Ya'qoub L, Elgendy IY, Pepine CJ (2021) Sex and gender differences in COVID-19: more to be learned! Am Heart J Plus 3: 100011. doi: 10.1016/j.ahjo.2021.100011.
15. Jin J-M, Bai P, He W, Wu F, Liu X-F, Han D-M, Liu S, Yang J-K (2020) Gender differences in patients with COVID-19: focus on severity and mortality. medRxiv 2020.02.23.20026864. doi: 10.1101/2020.02.23.20026864.
16. Qian J, Zhao L, Ye R-Z, Li X-J, Liu Y-L (2020) Age-dependent gender differences in COVID-19 in mainland China: comparative study. Clin Infect Dis 71: 2488–2494. doi: 10.1093/cid/ciaa683.
17. Torres C, García J, Meslé F, Barbieri M, Bonnet F, Camarda CG, Cambois E, Caporali A, Couppié É, Poniakina S, Robine J-M (2022) Identifying age- and sex-specific COVID-19 mortality trends over time in six countries. Int J Infect Dis 128: 32. doi: 10.1016/j.ijid.2022.12.004.
18. Abulude FO, Abulude IA (2020) SARS-CoV-2 pandemic in Nigeria: the impact on age and sex distributions. Aurum J Health Sci 2: 173–189.
19. Josa-Laorden C, Crestelo-Vieitez A, García Andreu MDM, Rubio-Rivas M, Sánchez M, Toledo Samaniego N, Arnalich Fernández F, Iguaran Bermudez R, Fonseca Aizpuru EM, Vargas Núñez JA, Pesqueira Fontan PM, Serrano Ballesteros J, Freire Castro SJ, Pestaña Fernández M, Viana García A, Nuñez Rodriguez V, Giner-Galvañ V, Carrasco Sánchez FJ, Hernández Milián A, Cobos-Siles M, Napal Lecumberri JJ, Herrero García V, Pascual Pérez M de LR, Millán Núñez-Cortés J, Casas Rojo JM, On Behalf Of The Semi-Covid-Network (2021) Gender-based differences by age range in patients hospitalized with COVID-19: a Spanish observational cohort study. J Clin Med 10: 899. doi: 10.3390/jcm10050899.
20. Kushwaha S, Khanna P, Rajagopal V, Kiran T (2021) Biological attributes of age and gender variations in Indian COVID-19 cases: a retrospective data analysis. Clin Epidemiol Glob Health 11: 100788. doi: 10.1016/j.cegh.2021.100788.
21. Sarkar A, Chouhan P (2021) COVID-19: District level vulnerability assessment in India. Clin Epidemiol Glob Health 9: 204–215. doi: 10.1016/j.cegh.2020.08.017.
22. Dibyachintan S, Nandy P, Das K, Vinjanampathy S, Mitra MK (2022) Unequal lives: a sociodemographic analysis of COVID-19 transmission and mortality in India. Public Health 214: 133. doi: 10.1016/j.puhe.2022.11.009.
23. Government of India, Census (2011) Puducherry population 2022: Sex ratio and literacy rate 2024. Available: https: //www.census2011.co.in/census/state/puducherry.html. Accessed: 24 Oct 2024.
24. Ayala A, Villalobos Dintrans P, Elorrieta F, Castillo C, Vargas C, Maddaleno M (2021) Identification of COVID-19 waves: considerations for research and policy. Int J Environ Res Public Health 18: 11058. doi: 10.3390/ijerph182111058.
25. Gupta N, Rana S, Panda S, Bhargava B (2022) Use of COVID-19 test positivity rate, epidemiological, and clinical tools for guiding targeted public health interventions. Front Public Health 10: 821611. doi: 10.3389/fpubh.2022.821611.
26. El-Shabasy RM, Nayel MA, Taher MM, Abdelmonem R, Shoueir KR, Kenawy ER (2022) Three waves changes, new variant strains, and vaccination effect against COVID-19 pandemic. Int J Biol Macromol 204: 161–168. doi: 10.1016/j.ijbiomac.2022.01.118.
27. Sarkar A, Chakrabarti AK, Dutta S (2021) COVID-19 infection in India: a comparative analysis of the second wave with the first wave. Pathogens 10: 1222. doi: 10.3390/pathogens10091222.
28. CDC (2022) Omicron variant: what you need to know. Available: https: //www.cdc.gov/coronavirus/2019-ncov/variants/omicron-variant.html. Accessed: 28 Jul 2022.
29. Meo SA, Meo AS, Al-Jassir FF, Klonoff DC (2021) Omicron SARS-CoV-2 new variant: global prevalence and biological and clinical characteristics. Eur Rev Med Pharmacol Sci 25: 8012–8018. doi: 10.26355/eurrev_202112_27652.
30. Reddy MM, Zaman K, Mishra SK, Yadav P, Kant R (2021) Differences in age distribution in first and second waves of COVID-19 in eastern Uttar Pradesh, India. Diabetes Metab Syndr 15: 102327. doi: 10.1016/j.dsx.2021.102327.
31. Kuttiatt VS, Abraham PR, Menon RP, Vaidya PC, Rahi M (2020) Coronavirus disease 2019 in children: clinical and epidemiological implications. Indian J Med Res 152: 21. doi: 10.4103/ijmr.IJMR_977_20.
32. Krishnamurthy S, Kar SS, Dhodapkar R, Parameswaran N (2022) Comparison of COVID-19 infection in children during the first and second wave. Indian J Pediatr 89: 1016–1018. doi: 10.1007/s12098-022-04127-x.
33. Singh S, Sharma A, Gupta A, Joshi M, Aggarwal A, Soni N, Sana, Jain DK, Verma P, Khandelwal D, Singh V (2022) Demographic comparison of the first, second and third waves of COVID-19 in a tertiary care hospital at Jaipur, India. Lung India 39: 525. doi: 10.4103/lungindia.lungindia_265_22.
34. Abate BB, Kassie AM, Kassaw MW, Aragie TG, Masresha SA (2020) Sex difference in coronavirus disease (COVID-19): a

systematic review and meta-analysis. BMJ Open 10: e040129. doi: 10.1136/bmjopen-2020-040129.

35. Zirpe KG, Dixit S, Kulkarni AP, Pandit RA, Ranganathan P, Prasad S, Amanulla ZK, Kothari V, Ambapkar S, Gurav SK, Shastrabuddhe S, Gosavi V, Joshi M, Mulakavalupil B, Saldhanah C, Ambapkar S, Bapte M, Singh S, Deshmukh A, Khatib K, Zirpe A, Sayiprasad G, Joshi A (2021) The second- vs first-wave COVID-19: more of the same or a lot worse? a comparison of mortality between the two waves in patients admitted to intensive care units in nine hospitals in Western Maharashtra. Indian J Crit Care Med 25: 1343. doi: 10.5005/jp-journals-10071-24042.
36. De Francia S, Ferretti A, Chiara F, Allegra S, Mancardi D, Allice TG, Milia MG, Gregori G, Burdino E, Avanzini C, Ghisetti V, Durio A (2022) The influence of sex, gender, and age on COVID-19 data in the Piedmont region (Northwest Italy): the virus prefers men. Life 12: 643. doi: 10.3390/life12050643.
37. Haischer MH, Beilfuss R, Hart MR, Opielinski L, Wrucke D, Zirgaitis G, Uhrich TD, Hunter SK (2020) Who is wearing a mask? Gender-, age-, and location-related differences during the COVID-19 pandemic. PLoS One 15: e0240785. doi: 10.1371/journal.pone.0240785.
38. Fernandes M da CR, Vasconcelos GS, de Melo ACL, Matsui TC, Caetano LF, de Carvalho Araújo FM, Fonseca MHG (2023) Influence of age, gender, previous SARS-CoV-2 infection, and pre-existing diseases in antibody response after COVID-19 vaccination: a review. Mol Immunol 156: 148-155. doi: 10.1016/j.molimm.2023.03.007
39. Chen YH, Cheuh YN, Chen CM, Kuo HW (2023) Epidemiological characteristics of the three waves of COVID-19 epidemic in Taiwan during April 2022 to March 2023. J Formos Med Assoc 122: 1174-1182. doi: 10.1016/j.jfma.2023.05.027
40. Hu D, Lou X, Meng N, Li Z, Teng Y, Zou Y, Wang F (2021) Influence of age and gender on the epidemic of COVID-19. Wien Klin Wochenschr 133: 321-330. doi: 10.1007/s00508-021-01816-z
41. Doerre A, Doblhammer G (2022) The influence of gender on COVID-19 infections and mortality in Germany: insights from age- and gender-specific modeling of contact rates, infections, and deaths in the early phase of the pandemic. PLoS One 17: e0268119. doi: 10.1371/journal.pone.0268119
42. Wenham C, Smith J, Morgan R, Gender and COVID-19 Working Group (2020) COVID-19: the gendered impacts of the outbreak. Lancet 395: 846-848. doi: 10.1016/S0140-6736(20)30526-2

Annex – Supplementary Items

Supplementary Table 1. Details of 10 commercial RT-PCR kits used in the study.

Name of the Kit and kit manufacturer	Regulatory authority	Reaction mixture	Reaction conditions	Thermocycler	Target genes	Internal Control	*Ct Cut off for Positive
BGI Genomics Real-Time Fluorescent RT-PCR Kit for SARS-CoV-2.BGI Genomics Co. Ltd, China.	ICMR	Total 30 µL per reaction. 18.5 µL of SARS-CoV-2 Reaction Mix, 1.5 µL of SARS-CoV-2 Enzyme Mix and 10 µL of RNA template	50 °C 20 mins – 1 cycle, 95 °C 10 mins – 1 cycle, 40 cylces of 95 °C 15 sec and 60 °C 30 sec	LightCycler 96 (Roche) and CFX96 Real-time system (BioRad)	ORF1 ab	Beta actin	< 38
Genes2me VIRALDTECT II MµLtiplex Real-Time PCR. Genes2me Pvt Ltd, India	ICMR	Total 20 µL per reaction. 10 µL of 2X master mix, 1 µL of primer & probe mix and 9 µL of RNA template	55 °C 10 mins – 1 cycle, 95 °C 3 mins – 1 cycle, 40 cycles of 95 °C 15 sec and 60 °C 60 sec	LightCycler 96 (Roche) and CFX96 Real-time system (BioRad)	E, RdRp and N gene	Human RNaseP	< 37
Huwel Lifesciences. HLSS Manufacturing Pvt Ltd, India.	ICMR	Total 25 µL per reaction. 13 µL of master mix, 2 µL of primer, probe mix and 10 µL of RNA template.	53 °C 5 mins – 1 cycle, 95 °C 1 mins – 1 cycle, 5 cycles of 95° C 05 sec and 60 C 10 sec, 35 cycles of, 95 °C 01 sec and 60 °C 01 sec	LightCycler 96 (Roche) and CFX96 Real-time system (BioRad)	ORF1ab and N gene	Beta2-micro globµLin	≤ 38
ICMR NIV, Pune.	ICMR	**Two tube reaction mix. Tube 1** The reaction volume of 25 µL contained 12.5 µL of 2x reaction mix, 3 µL of E gene and RNaseP Primer and probe mix, 0.5 µL of SSIII or Taq enzyme mix, 5 µL of RNA template and 4 µL RNAse free water. **Tube 2** The Reaction mixture is the same as above except 3 µL of primer probe mix specific to RdRp and ORF gene	55 °C 15 mins – 1 cycle, 95 °C 3 mins – 1 cycle, 45 cycles of 95 °C 15 sec, 58 °C 30 sec and 40 °C 30 sec – 1 cycle	LightCycler 96 (Roche) and CFX96 Real-time system (BioRad)	E, RdRp and ORF gene	Human RNaseP	< 35
Meril RTPCR. Meril Diagnostics Pvt Ltd India.	ICMR	Total 20 µL per reaction. 9 µL of master mix, 1 µL of primer & probe mix and 10 µL of RNA template	50 °C 15 mins – 1 cycle, 95 °C 3 mins – 1 cycle, 40 cycles of 95 °C 15 sec and 55 °C 40 sec, 25 °C 10 sec – 1 cycle	LightCycler 96 (Roche) and CFX96 Real-time system (BioRad)	ORF1 ab, and N gene	Human RNaseP	≤ 35
MyLab PathoDetectTM COVID-19 Qualitative PCR kit. Mylab Discovery Solutions Pvt. Ltd, India	ICMR	Total 25 µL per reaction. 3 µL of detection mix, 6.25 µL of PCR mix-1, 1 µL of PCR mix-2, 8 µL of RNA template and 6.75 µL of Nuclease free water	50 °C 10 mins - 1 cycle, 95 °C 3 mins - 1 cycle, 45 cycles of 95 °C 15 sec and 60 °C 30 sec	LightCycler 96 (Roche) and CFX96 Real-time system (BioRad)	ORF1 (RdRp) and E gene	Human RNaseP	≤ 40
NeoDx CoviDx mPlex-4R SARS-CoV-2. NeoDx Biotech Labs Private Limited, DSS Imagetech, India	ICMR	Total 25 µL per reaction. 12.5 µL of 2x master mix, 1.25 µL of 20x primer and probe mix, 8 µL of RNA template and 3.25 µL of Nuclease free water.	50 °C 15 mins - 1 cycle, 95 °C 2 mins - 1 cycle, 45 cycles of 95 °C -15 sec, 58 °C - 30 sec.	LightCycler 96 (Roche) and CFX96 Real-time system (BioRad)	E, RdRP and N gene	Human RNaseP	
POCT Q-line RTPCR ki. POCT service pvt ltd, India	ICMR	Total 20 µL per reaction. 9 µL of master mix, 1 µL primer & probe mix, 5 µL of RNA template and 5 µL RNAse free water	50 °C 15 min-1 cycle, 95 °C 3 min - 1 cycle, 45 to 50 cycles of 95 °C -15 sec, 55 °C - 40 sec, 25 °C - 10 sec- 1 cycle	LightCycler 96 (Roche) and CFX96 Real-time system (BioRad)	ORF1ab and N gene	*UNG enzyme* and dUTP	< 40
Trivitron COVIDSURE multiplex RT-PCR kit. Trivitron Health Care India	ICMR	Total 25 µL per reaction. 13 µL of Enzyme mix, 4 µL primer & probe mix, 8 µL of RNA template.	45 °C 10 mins – 1 cycle, 95 °C 3 mins – 1 cycle, 40 cycles of 95 °C 15 sec and 59 °C 30 seconds	LightCycler 96 (Roche) and CFX96 Real-time system (BioRad)	ORF1ab, N and S gene	Beta-globµLin human gene	≤ 36
TRUPCR® SARS-CoV-2 Kit, Kilpest India Ltd, India	ICMR	**Two tube reaction mix. Tube 1** The reaction volume of 25 µL contained 10 µL of 2x reaction mix, 4.65 µL of E gene and RNaseP Primer and probe mix, 0.35 µL of enzyme mix, 5 µL of RNA template and 5 µL RNAse free water. **Tube 2** The Reaction mixture is the same as above except 4.65 µL of primer and probe mix specific to RdRp + N and RNaseP gene	50 °C 15 min-1 cycle, 95 °C 5 min - 1 cycle, 40 cycles of 95 °C -5 sec, 60 °C - 40 sec, 72 °C - 15 sec- 1 cycle	LightCycler 96 (Roche) and CFX96 Real-time system (BioRad)	E and RdRp + N,	Human RNaseP	RdRp, E and N - < 33; RNAse p - ≤ 35

*- Ct cut off, as per the kit manufacturer's instructions and approved by ICMR.