

Prevalence of Asymptomatic SARS-CoV-2 Infection among Pregnant Women: A Retrospective Study from a Tertiary Care Hospital in North India

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ABSTRACT

Introduction: COVID-19, caused by the SARS-CoV-2 virus, had a widespread impact on lives worldwide. Its global impact has transcended geographical barriers, affecting people of all ages, races, and genders. Pregnancy induces critical physiological changes in women that can increase their susceptibility to infections. As a result, pregnant women may be at a higher risk of acquiring infections compared to non-pregnant individuals. This retrospective study aimed to determine the prevalence of COVID-19 among pregnant women from April 2020 to January 2022. **Methods:** Screening was performed on a total of 4929 pregnant women nearing their expected delivery date. Nasopharyngeal and/or oropharyngeal samples were collected and analyzed for SARS-CoV-2 detection using real-time RT-PCR. **Result:** Pregnant women in the study had a mean age of 30.28 years, and the overall prevalence of COVID-19 was 3.6%. Positivity rates varied between zero and 23.2% during different intervals, with increases in positivity coinciding with the peaks of the country's first, second, and third waves of COVID-19. Pregnant females exhibited a higher positivity rate for COVID-19 compared to the general population. **Conclusions:** The presence of COVID-19-positive patients in our study group, which comprised entirely of asymptomatic individuals, underscores the importance of active screening among at-risk populations, particularly during periods of increased activity in the general population. These findings can be of vital importance for the management of COVID-19 in pregnant patients, as well as policymaking at all levels.

INTRODUCTION

On March 11th, 2020, the World Health Organization (WHO) declared the outbreak of the newly discovered SARS-CoV-2 virus, which causes COVID-19, a pandemic, following much debate [1]. The newly discovered β -coronavirus rapidly spread across mainland China and beyond, facilitated by human-to-human transmission and intercontinental travel [2]. According to World Health Organization estimates as of March 2023, there have been approximately 684 million cases of SARS-CoV-2 infection worldwide, resulting in over 6.8 million deaths India has reported 44 million confirmed cases and half a million deaths [3]. The first recorded COVID-19 case in India was reported in Kerala, and the virus soon spread beyond its boundaries, affecting the entire country [4]. Most infected patients exhibit mild-to-asymptomatic illness, although a minority can rapidly progress to acute respiratory distress syndrome (ARDS), multi-organ dysfunction syndrome (MODS), and death [5]. The coronavirus family is responsible for causing the

common cold, which is prevalent during the winter season. While the benign nature of most coronaviruses is reflected in their mild symptoms, two significant epidemics caused by viruses from this family have occurred in the past few decades: severe acute respiratory syndrome (SARS), caused by SARS-CoV, and Middle East respiratory syndrome (MERS), caused by MERS-CoV. These outbreaks had respective case fatality rates (CFRs) of 10% and 37%, resulting in a total of 10,000 deaths globally [6]. At the time of writing, COVID-19 has a global infection fatality rate (IFR) of 1-3%, yet it has caused more deaths than both MERS and SARS combined [7]. It is noteworthy that both SARS and MERS have been strongly associated with maternal morbidity and mortality, as evidenced by previous studies [8, 9]. Presently, the scientific community is actively investigating the impact of SARS-CoV-2 infection on pregnant women. Pregnant women, due to their immunosuppressed state, are particularly susceptible to

viral infections that can lead to adverse maternal and perinatal outcomes. The unique physiological changes during pregnancy, including alterations in anatomy, bodily functions, and immune status, create a state of immunosuppression, rendering over 100 million pregnant women worldwide at high risk of SARS-CoV-2 infection [10]. These physiological changes, coupled with a reduction in cell-mediated immunity, heighten susceptibility to intracellular pathogens, particularly viruses [12]. During the pandemic, pregnant patients with COVID-19 should receive heightened attention and be considered a potential at-risk group [13]. Although most pregnant women are asymptomatic at admission, screening can safeguard against and prevent disease transmission among other pregnant women, infants, obstetric care providers, and the general public [14]. Unfortunately, there is limited knowledge and experience regarding COVID-19 during pregnancy. Presently, pregnant women seem to have experienced fewer COVID-19-related adverse events than those observed during SARS and MERS outbreaks [15]. As the pandemic continues, preventing and controlling infections among this population subset can become increasingly challenging. This article takes a retrospective cross-sectional approach to address the lack of research on asymptomatic pregnant women with COVID-19 infection in developing countries. The resulting data will enhance the understanding and management of COVID-19 infection in pregnant women.

MATERIAL AND METHODS

Study design. This cross-sectional study was conducted in the virology division of a tertiary care hospital. Over the course of 22 months (April 2020-January 2022), the department collected and processed 4,929 samples. Clinical, demographic, and epidemiological information were recorded at the time of sample collection.

Inclusion criteria. Pregnant women who were asymptomatic and scheduled for surgery or near their expected delivery date were eligible.

Sample collection and transport. Oropharyngeal and nasopharyngeal swab specimens were collected in a single viral transport medium (VTM, HiMedia) tube, immediately immersed, and transported to the virology laboratory in triple-layered packaging at 4 °C. To protect patient confidentiality, personal information and hospitalization numbers were removed from the samples. All healthcare workers involved in sample collection and transport received training on appropriate standard operating procedures. The study was approved by the institutional ethics committee of the hospital (SIMS 131/IEC-SKIMS/2022-188). Confirmation of COVID-19 diagnosis was determined by real-time reverse transcription polymerase chain reaction (RT-PCR) testing of nasopharyngeal and/or oropharyngeal swab specimens.

Sample Processing. The samples were processed upon receipt in a biosafety level II (BSL II) laboratory using a biological safety cabinet (BSC-type IIb). RNA extraction and purification were performed on all specimens using the available QIAamp Viral RNA Kit (Qiagen), Imperial life sciences RNA extraction kit, and Genetix RNA extraction kits. The extracted and purified RNA was reverse transcribed to cDNA and amplified following the manufacturer's instructions using thermocyclers, including the Applied Biosystems 7500/7500 Fast Real-Time PCR Instrument System (Thermo Fisher Scientific) and CFX96 Real-Time PCR Detection System (Bio-Rad). Some samples were directly processed using COBAS® 6800 System (Roche Diagnostics). The genes targeted for detecting SARS-CoV-2 ribonucleic acid (RNA) in the NP swabs included the Envelope (*E* gene), Open Reading Frame 1b (*ORF-1b*), Spike (*S* gene), RNA-dependent RNA polymerase (*RdRP*) gene, and Nucleoprotein (*N* gene). During the early phase of the pandemic, the kits developed by the National Institute of Virology, Pune, were utilized in accordance with ICMR recommendations. The screening test kit that targeted the *E* gene was based on a two-step process. Positive samples identified by the screening test were confirmed using a second reaction that targeted the *ORF* and *RdRP* genes. Other kits, including the ABI TaqMan 2019-nCoV Control, Roche Diagnostics Light Mix Modular SARS-CoV-2 (COVID19), Seegene Allplex 2019-nCoV assay, Meril Diagnostics Meril COVID-19 One-step RT-PCR Kit, and BioSewoom Real-Q 2019-nCoV Detection Kit, were used as available. If processing delay was expected to exceed 6 hours, the samples were stored at 2-8 °C for a maximum of 72 h after collection. The extracted nucleic acid was stored at -70 °C in a freezer.

Interpretation of results. To ensure integrity and verify RT-PCR assay results, an internal control (IC) was analyzed with each patient sample. Furthermore, each batch included one replicate of the positive control and one replicate of the negative control. A positive test result was declared for a cycle threshold value (Ct value) < 35, and a negative result was declared for a Ct value of ≥ 38. Ct values ranging from 35 to less than 38 were reported as inconclusive and required repeat sampling (Fig. 1).

RESULTS

The virology division of the department collected and analyzed 4,929 samples, with 179 (3.6%) positive for SARS-CoV-2 infection. Additionally, 16 results were inconclusive according to the kit manufacturer's instructions, and 25 specimens were rejected for various reasons, including incomplete or unlabeled VTM (n=17) and insufficient VTM or leaking container (n=8). Sample distribution was highest among hospitalized patients, followed by those with short-stay admission and outpatients who did not require admission. The highest positivity rate (7.9%) was observed among outpatient department (OPD) samples, followed by those needing a

short stay (4.5%), and the lowest positivity rate was observed among admitted patients (3.1%) (Table 1). The

test results were assessed after validation of the positive and negative controls.

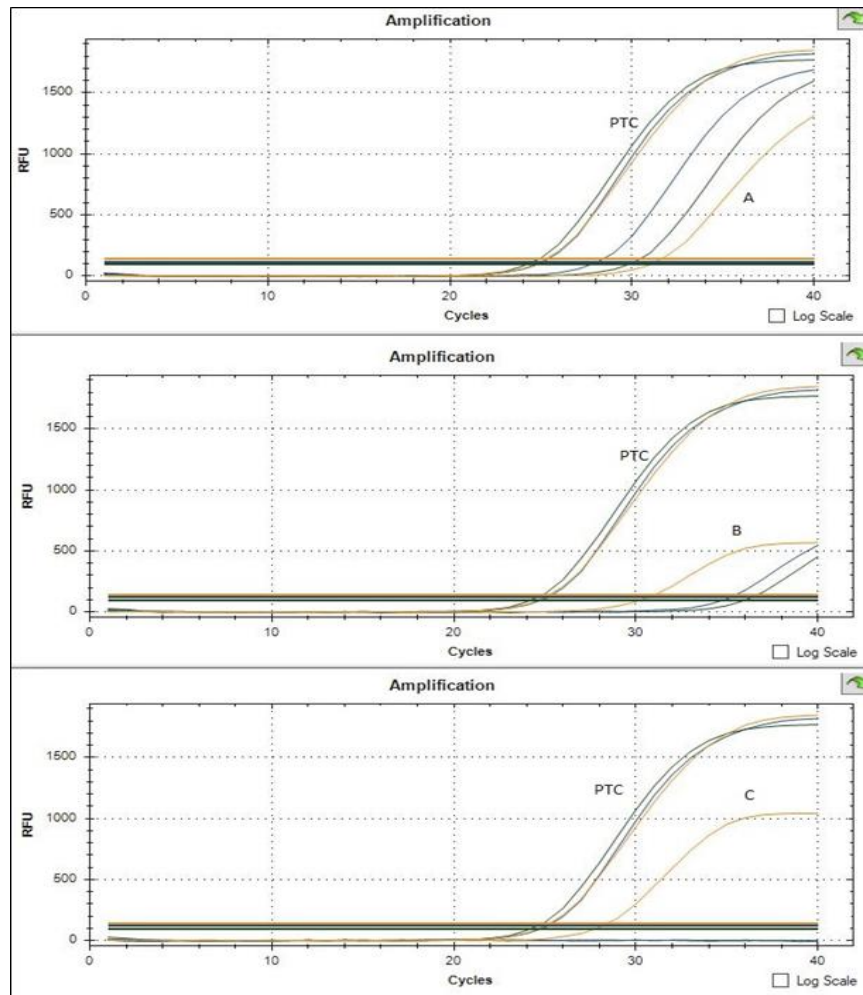


Fig 1. Amplification plots of three samples and a positive control (PTC) replicate. Sample A was positive for SARS-CoV-2, Sample B was inconclusive, and Sample C was negative

Table 1. Comparison of demographic variables with the rate of positivity in the studied population

		Negative	Positive	Positivity	Total	P-value (chi-square test)
Block location						
	In patient department	3799	120	3.1	3953	P < 0.001 *
	Outpatient department	422	36	7.9	463	
	Short stay admission	488	23	4.5	513	
Age group (years)						
	≤20	60	2	3.2	63	P = 0.033*
	21-30	2876	121	4	3017	
	31-40	1272	32	2.5	1323	
	≥40	51	5	8.9	57	
Residence						
J & K	Jammu	37	0	0	37	
	Kashmir	4618	178	3.7	4837	
	Ladakh	11	0	0	11	
Outside state	Punjab	1	0	0	1	
	Uttar Pradesh	3	0	0	3	
	West Bengal	1	0	0	1	
Not available	NA	38	1	2.6	39	

* *P*-value was calculated using the Chi-Square test.

The mean age was 30.28 years (SD: 3.737; range: 18-45 years). During different intervals, the COVID-19 positivity rate ranged from 0% to 23.2%, with peaks

coinciding with the first, second, and third waves of COVID-19 in the country (Fig. 2).

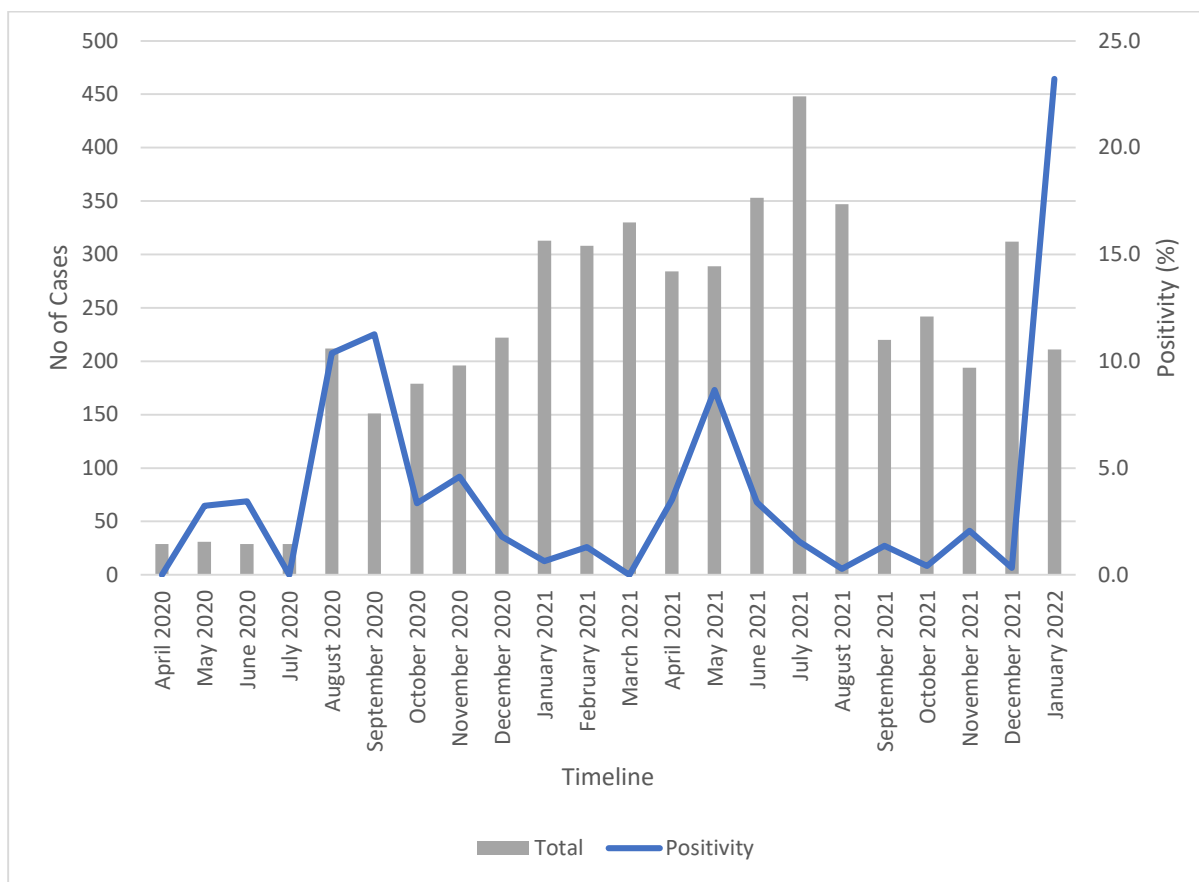


Fig 2. Trend of sample numbers and positivity rates during different intervals

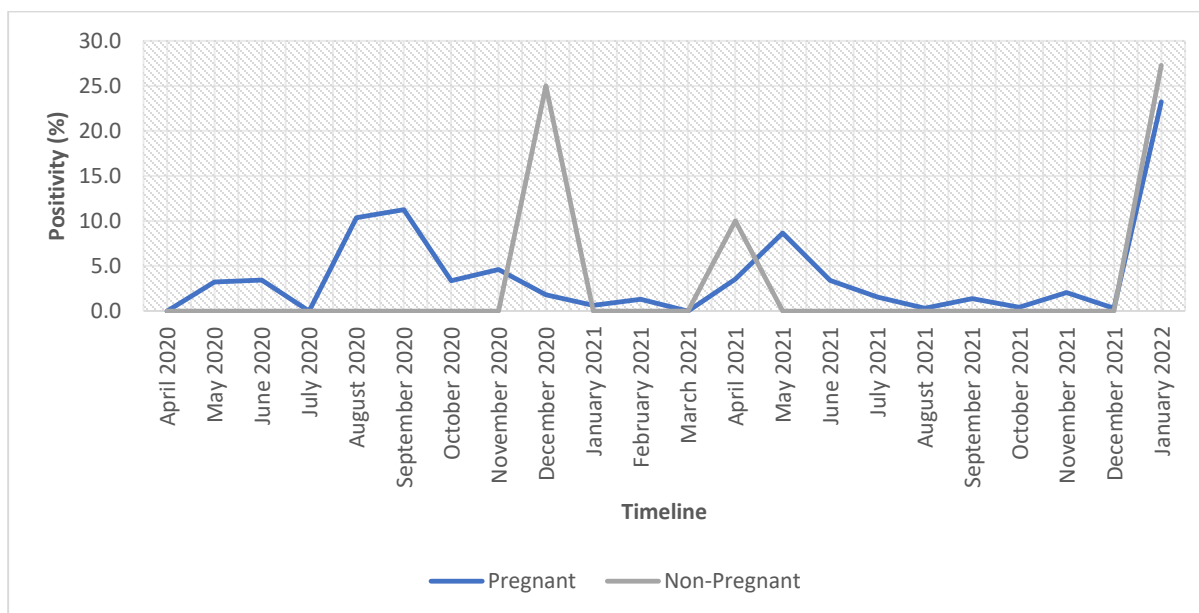


Fig 3. Comparison of COVID-19 positivity rates between pregnant patients and those admitted for gynecological reasons

DISCUSSION

Maternal morbidity and mortality related to viral pneumonia during pregnancy are a heightened concern, particularly during the ongoing COVID-19 pandemic [16]. The maternal mortality rate during the infamous Spanish flu of 1918 and the Asian flu epidemic of 1957 ranged from 30% to 50% [17]. Similarly, SARS has a significant impact, and pregnant women appear to have a worse clinical course with a case fatality rate of 25% [8]. SARS-CoV-2 is a highly infectious virus that can spread across national and international borders [17]. COVID-19 has garnered global attention from researchers. Although studies have examined COVID-19 infection in the general population, few have investigated the prevalence and specific effects of COVID-19 on pregnancy outcomes. Significant differences in demographic and clinical patterns have led to major policy shifts. The aim of this study is to provide information on the demographic profile of pregnant patients with COVID-19, which can guide future management of these women.

Most positive pregnant women were young (mean age: 30.28 years), which is similar to a study in Wuhan (age range: 26-40 years) [18]. Conversely, a study conducted at a tertiary care hospital in Northern India reported a mean age of 40.3 years, but their sample size was limited [19]. At the time of study enrollment, all patients were asymptomatic. Asymptomatic patients should be closely monitored, as some may progress to severe disease. A similar study conducted in Jaipur, India found that 33.3% of COVID-19 positive patients were asymptomatic [20]. Identifying pregnant women who are COVID-19 positive but asymptomatic is essential for monitoring their pregnancy and considering issues such as the mode of delivery. Unfortunately, limited reporting on asymptomatic COVID-19 positive pregnant women makes it difficult to determine the safest delivery method for these patients. Selecting the appropriate delivery method and approach for severe COVID-19 cases is a complex decision that necessitates a thorough evaluation of the gestational age, maternal health status, and fetal well-being. It is crucial to stabilize the mother before any emergency delivery for fetal indications, as this can

improve the fetal outcome [21]. However, further studies with larger sample sizes are necessary to confirm the appropriate delivery mode for pregnant women with COVID-19 infection.

During the OPD visit, 7.8% of patients tested positive for COVID-19, compared to 3.1% of inpatient department (IPD) patients ($P < 0.001$). The lower positivity rate in admitted cases indicates more effective infection control practices during hospital stays and improved screening to prevent the spread of COVID-19 between patients. A total of 179 females tested positive for COVID-19, resulting in a prevalence rate of 3.6%. A study conducted on pregnant women reported a COVID-19 positivity rate of 3.4% [22]. A survey of the general population reported an 11% COVID-19 positivity rate [23]. A comparable study conducted on pregnant women in a tertiary care hospital in India reported a 14.4% prevalence of COVID-19 positive cases [24]. Differences in study periods, regions, and subgroups can account for the variability in prevalence. Epidemiological and population factors, as well as variations in prevalent health practices across locations and over time, may contribute to the differences in reported COVID-19 cases and changes in incidence trends. Furthermore, the population density in the area plays a significant role in accelerating transmission, while differences in policy implementation and timing of mitigation strategies across health centers may also account for the variability in prevalence observed in other studies.

Positivity rates varied from 0% to 23.2% across different intervals, with increases coinciding with the peaks of the first, second, and third waves of COVID-19 in the country. India's first wave of COVID-19 began in March 2020, followed by a second wave in March 2021, and a third wave that started in December 2021 [25]. The highest positivity rate of 23.2% was observed in January 2022, corresponding with the peak of the third wave in this study. The second peak in positivity was recorded in August 2020, once again coinciding with the peak of the first wave. By comparison, the positivity rate for patients admitted for gynecological surgeries was 4.4% (Table 2).

Table 2. Comparison of COVID-19 positivity rates between two study groups

		Result		Total	
Group		Positive	Negative		
Pregnancy	n	179	4709	4888	$P=0.745$
	%	3.70%	96.30%	100.00%	
Non-pregnant	n	5	109	114	
	%	4.40%	95.60%	100.00%	
Total	n	198	5258	5456	
	%	3.60%	96.40%	100.00%	

* P -value was calculated using the Chi-Square test.

Although the positivity rate was higher than that of pregnant females, a notable difference was the limited number of positive cases reported during intervening

periods, with the majority of cases occurring during the peak of COVID-19 waves in the country (Fig. 3).

Research on COVID-19 is continually evolving, representing an ever-expanding body of knowledge. The

aim of this study is to describe the demographic profile and COVID-19 prevalence among pregnant women during the first and second years of the pandemic. In this single-center study, 3.6% of female participants tested positive. Characteristic findings of this study include younger age, higher positivity rate in outpatients (non-admitted), and a correlation between peak waves of the COVID-19 pandemic in the country and increased positivity rates. The detection of COVID-19-positive patients within a study group consisting entirely of asymptomatic individuals underscores the importance of frequent testing in this subset, particularly during periods of heightened activity within the general population. These findings may have critical implications for the management of COVID-19 in pregnant patients and for policymaking at all levels.

A detailed follow-up of the patients was not conducted to track the mode and timing of delivery. In addition, perinatal outcomes were not recorded, highlighting the need for future studies to address these issues. Due to shortages in the supply chain, it was only possible to test some samples using the same RT-PCR testing kit. During the initial phases of the pandemic, several testing kits were used; however, as the supply chain stabilized, the majority of testing was conducted with a single kit (BioSewoom, Real-Q 2019-nCoV Detection Kit). Given that the kits used in this study had a similar limit of detection, the testing protocol did not have a significant impact on the study's outcome.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this manuscript.

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