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Original Article

Microbiology Section

Utility of Various Clinical Samples in the Diagnosis of Neonatal SARS-CoV-2 Infection: A Retrospective Observational Study

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ABSTRACT

Introduction: Coronavirus Disease-2019 (COVID-19) in pregnancy was thought to be associated with an increased risk of stillbirth, Intrauterine Growth Restriction (IUGR), and preterm birth. The current study was undertaken to assess the burden of Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) infection in neonates born to COVID-19-positive mothers.

Aim: To assess the role of various clinical samples in the diagnosis of perinatal transmission of SARS-CoV-2 in all three waves of the pandemic.

Materials and Methods: A retrospective observational study was carried out in a COVID-19 dedicated tertiary care hospital in Pune, Maharashtra, India from April 2020 to February 2022. Swabs from the umbilical stump and nasopharynx of neonates were collected after birth from neonates born to mothers who were COVID-19 positive at the time of admission for delivery, along with the mother's placental swab.

Results: Over a period of two years, a total of 360 neonates born to 351 COVID-19 positive mothers were included. Thirty neonates showed evidence of SARS-CoV-2 infection. The maximum rate of infection was in the first wave (10.86%). Among the three types of swabs, the umbilical cord sample showed more COVID-19 Reverse Transcription Polymerase Chain Reaction (RT-PCR) positivity (4.88%), followed by the nasopharyngeal swab (4.72%) and placental (3.64%) swab.

Conclusion: In the present study, the nasopharyngeal and umbilical swabs were found to be better clinical samples than placental swabs in the diagnosis of SARS-CoV-2 infection in neonates. The rate of perinatal transmission was 8.5%, providing strong evidence of perinatal transmission.

Keywords: Coronavirus disease-2019, Nasopharyngeal swab, Perinatal transmission, Placental swab, Umbilical swab

INTRODUCTION

The SARS-CoV-2 pandemic has entered its third year and has affected almost all age groups equally, although the severity has decreased. However, the spread or transmission of the virus remains the same. The most vulnerable age groups are neonates, pregnant females, and elderly people [1]. COVID-19 during pregnancy is associated with an increased risk of stillbirth, IUGR and preterm birth. The adverse effects are more significant in symptomatic women. Neonates born to SARS-CoV-2 positive mothers are at risk of perinatal transmission of the infection. Perinatal transmission is defined as a positive COVID-19 RT-PCR report in a neonate within the first 72 hours after birth [2,3]. However, the incidence of vertical transmission still remains unknown [4]. COVID-19 infection during pregnancy is linked to increased preterm birth, Low Birth Weight (LBW), and complications related to prematurity [5,6]. Infected newborns are mostly asymptomatic or present with mild clinical symptoms such as shortness of breath, fever, or gastrointestinal symptoms [7]. Limited information is available about the manifestation and outcomes of neonates born to SARS-CoV-2 positive mothers in developing countries. The current available data on the consequences of SARS-CoV-2 infection during pregnancy, for the foetus, and the neonate is mostly in the form of case reports, small case series, retrospective cohorts, or cross-sectional studies, compiled in a recent systematic review [8,9]. Early diagnosis of SARS-CoV-2 infection is crucial to reduce adverse events in neonates. The type of clinical specimen affects the diagnosis of SARS-CoV-2. Therefore, this study aims to determine the best clinical samples, such as swabs from the nasopharynx, placenta, and umbilical stump, with the highest sensitivity for diagnosing COVID-19 in neonates. Limited evidence exists on the perinatal transmission of SARS- CoV-2 infection especially from the developing world [10]. Additionally, the study aims to assess the rate of SARS-CoV-2 infection in neonates born to mothers who were COVID-19 positive at the time of delivery during the first, second, and third waves of the pandemic.

MATERIALS AND METHODS

A retrospective observational study was conducted in the Department of Microbiology at Byramjee Jeejeebhoy Government Medical College and Sassoon General Hospital, Pune, Maharashtra, India. The study duration was from April 2020 to February 2022, covering the first, second, and third waves of the pandemic. The time period of first wave was from 12th April 2020 to 14th January 2021, the period of second wave was from10th March 2021 to 12th July 2021 and the third wave was from 28th December 2021 to 16th February 2022 [11]. Data analysis was performed in September 2022. Ethical approval was obtained from the Institutional Ethics Committee (IEC) (0721244-244).

Inclusion criteria: Neonates born to symptomatic/asymptomatic pregnant females who tested positive for COVID-19 using Rapid Antigen Test (RAT), RT-PCR, or Cartridge Based Nucleic Acid Amplification Test (CBNAAT) at the time of admission for delivery. Neonates born to COVID-19 positive mothers referred from Pune Corporation hospitals within seven days of birth due to clinical suspicion of SARS-CoV-2 infection were included in the study.

Exclusion criteria: Neonates born to COVID-19 positive mothers who developed symptoms after seven days of birth. Neonates born to mothers who tested negative for COVID-19 at the time of delivery were excluded from the study.

Study samples: Swabs from the umbilical stump and nasopharynx of neonates born to COVID-19 positive mothers were collected after birth, along with the mother's placental swab. All three samples were collected in Viral Transport Medium (VTM)/saline and transported to

the laboratory following Indian Council of Medical Research (ICMR) guidelines [12].

Processing and testing: Ribonucleic acid (RNA) extraction was performed using an automated extractor (Thermo Fisher Scientific India Pvt., Ltd., Kingfisher flex) and the MagMax RNA extraction kit. The RT-PCR kits used in the study were supplied and validated by ICMR-National Institute of Virology (NIV) Pune, Maharashtra, India. The kits used in the first, second, and third waves were as follows: ICMR NIV RT-PCR kit, Huwell Life Sciences Quanti plus Multiplex, and Covipath COVID-19 RT-PCR kit by Thermo Fisher Scientific India Pvt., Ltd., respectively. The samples were tested accordingly [11].

In addition to the mentioned kits, Cepheid Xpert Xpress was used for urgent reporting of samples in all three waves. The ICMR-NIV RT-PCR kit used in the first wave detected the Envelope (*E*) gene and Open Reading Frame (*ORF*) gene of the SARS-CoV-2 virus, with the B actin gene as an internal control. The Huwell kit used in the second wave detected the Nucleocapsid (*N*) gene, *E* gene, and had an internal control for the SARS-CoV-2 virus. The Covi Path kit used in the third wave detected the *ORF*1ab gene and *N* gene, with *RNase* P as the internal control. The cycling conditions for RT-PCR and the interpretation of results were done according to the instructions provided by the respective kit manufacturers.

In cases of emergency, such as a neonate in respiratory distress after birth, Cepheid Xpert Xpress-CBNAAT was used for rapid detection of SARS-CoV-2. The Cepheid Xpert CBNAAT kit detected the *E* gene, *N2* gene of the SARS-CoV-2 virus, and had the SPC gene as an internal control.

A sample was reported as positive if two out of three genes were detected according to the kit manufacturer's instructions. The cut-off Cycle threshold (Ct) value <35 was used as per ICMR guidelines [13]. If Two out of three SARS-CoV-2 virus genes had a Ct value equal to 35, it was reported as inconclusive based on the ICMR's recommendation mentioned in the kit literature. In cases of an inconclusive result, the test was repeated after 24-48 hours using a repeat sample from the neonate, as recommended by the ICMR.

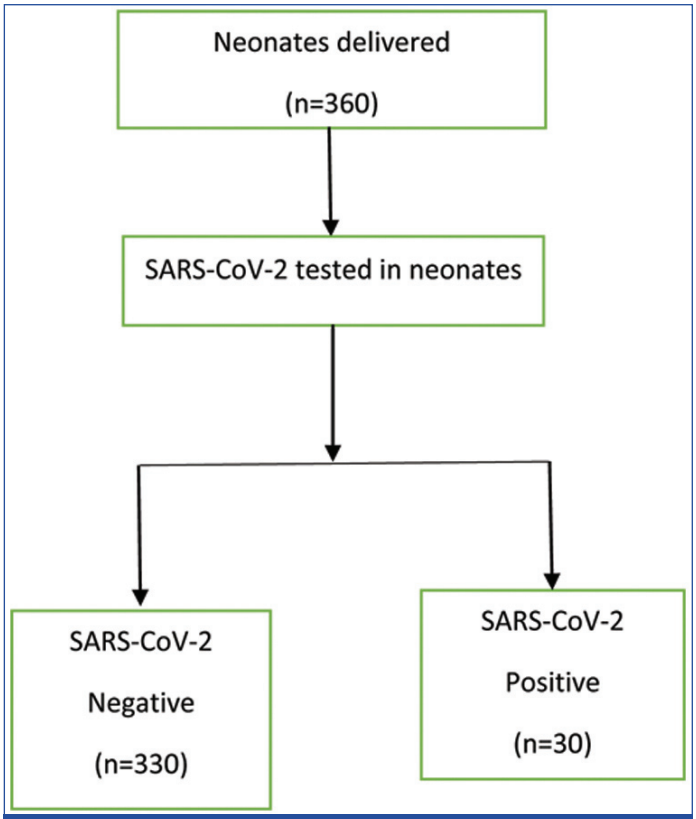
STATISTICAL ANALYSIS

The data was entered and analysed in MS Excel 2013 for descriptive statistical tests.

RESULTS

The following flow chart illustrates the summary of the study result [Table/Fig-1].

Over a period of two years, a total of 360 neonates (7 from twin births and 1 from triplet birth, making it 351+7+2=360) born to 351 COVID-19 positive mothers were included in this study [Table/Fig-1a,b]. The distribution of these 360 neonates across different waves is as follows: 184 from the first wave, 97 from the second wave, and 79 from the third wave. Among the 360 neonates, 30 showed evidence of SARS-CoV-2 infection. Twenty-eight neonates tested positive by RT-PCR, and two tested positive by CBNAAT. Of the 30 COVID-19 positive neonates, 20 were from the first wave, six from the second wave, and four from the third wave. The highest infection rate was observed in the first wave (10.86%), followed by the second wave (6.38%) and the third wave (5.06%) [Table/Fig-1a]. A total of 818 samples were collected from these 360 neonates for SARS-CoV-2 infection testing [Table/Fig-2]. Among these samples, 37 tested positive for COVID-19. Umbilical cord samples (4.9%) showed the highest positivity rate, followed by nasopharyngeal swabs (4.72%) and placental swabs (3.64%) [Table/Fig-3]. Most of these positive samples were detected within the first 24 hours of birth [Table/Fig-4]. Out of the 30 SARS-CoV-2 positive neonates, 15 were female and 15 were male. Detailed information on 22 of these neonates is available) [Table/Fig-5]. They were delivered either by Normal Vaginal Delivery (NVD) or by Lower Segment Caesarean



[Table/Fig-1]: Illustrates the study flow chart.

Section (LSCS). LSCS was performed mainly due to previous LSCS or foetal distress. The average weight of the neonates was 2.64 kg (ranging from 1.75 kg to 3.8 kg). The percentage of preterm delivery was 27.27%, and 31.8% of the neonates had low birth weight. Most of these 22 neonates were asymptomatic and were discharged from the hospital on day 3 (NVD) or day 7 (LSCS) after counseling the parents about breastfeeding and the signs of COVID-19 in neonates. Only one neonate exhibited typical signs and symptoms of SARS-CoV-2 (respiratory distress, gastrointestinal symptoms) and required NICU admission for more than 10 days. There were no neonatal deaths.

Wave	No. of neonates	SARS CoV-2 Positive	Percentage (%)
1st	184	20	10.86
2nd	97	6	6.18
3rd	79	4	5.06

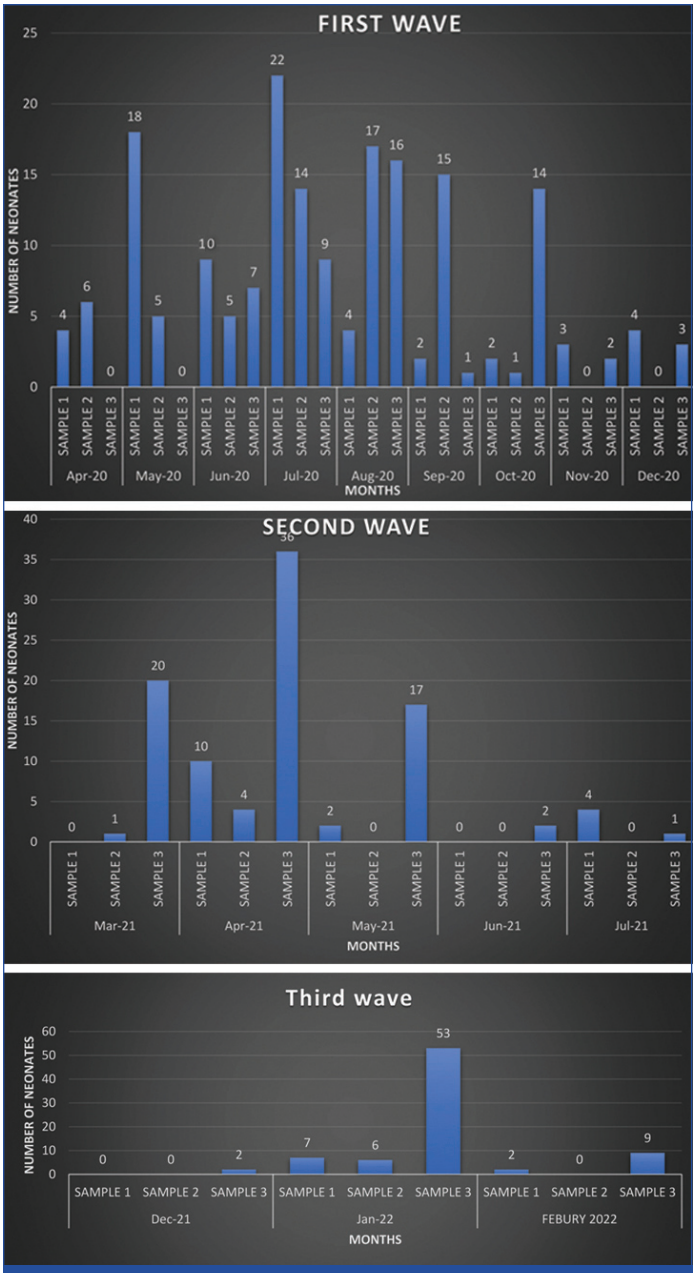
[Table/Fig-1a]: Number of neonates positive in the three different wave.

Wave	No. of samples	SARS CoV-2 positive	Percentage (%)
1st	351	23	6.55
2nd	254	8	3.14
3rd	213	6	2.81

[Table/Fig-1b]: Number of samples collected and its positivity in three different wave.

DISCUSSION

In the current study, a rate of SARS-CoV-2 infection in neonates born to COVID-19 positive mothers was noted at 8.33% (30/360). The highest positivity was observed during the first wave (10.86%), while the second and third waves had lower rates of 6.18% and 5.06% respectively. Comparing to other studies, More K et al., reported a 10.8% positivity rate in the first wave, Malik S et al., reported 4.2% positivity in neonates from Maharashtra, and a study from China reported 8% positivity in the first wave [14-16]. During the second wave, the present study had a 6.18% positivity rate, higher than Malik S et al., [15] (4.6%) and lower than Roohi A and Janaki V (3.1%) from Telangana. In the third wave, the present study reported 5.06% positivity, while Madhavi N et al., from Andhra Pradesh reported no cases of infection in neonates, and Farhadi R et al.,



[Table/Fig-2]: Number of samples collected in different wave from the neonates.

Wave	Placental		Umbilical		Nasopharyngeal	
	No. of samples	SARS CoV-2 positive	No. of samples	SARS CoV-2 positive	No. of samples	SARS CoV-2 positive
1st	52	3 (5.72%)	115	8 (6.9%)	184	12 (6.52%)
2nd	76	2 (2.63%)	81	3 (3.7%)	97	3 (3.19%)
3rd	64	2 (3.1%)	70	2 (2.8%)	79	2 (2.53%)
Total	192	7 (3.64%)	266	13 (4.9%)	360	17 (4.72%)

[Table/Fig-3]: Positivity of different clinical sample collected from neonates.

Parameter	Number of neonates
Type of delivery	
LSCS	10
NVD	11
Assisted NVD	1
Birth	
Term (≥37 weeks)	16
Late preterm (34-36 weeks)	6
Weight	
≥2.5 kg	15
1.5-2.5 kg	7

[Table/Fig-5]: Characteristics of COVID-19 positive neonates (n=22).
LSCS: Lower segment caesarean; section; NVD: Normal vaginal delivery

from Iran reported a positivity of 8.69% (2/23) [17-19]. Variability in study population characteristics and diagnostic methods of SARS-CoV-2 may contribute to the wide range of results.

In the present study, the usefulness of nasopharyngeal, umbilical, and placental swabs in diagnosing perinatal COVID-19 was assessed. The review of literature indicates that nasopharyngeal swabs are the most commonly used sample for perinatal transmission diagnosis [8]. In this study, an overall positivity of 4.72% was observed for nasopharyngeal swabs collected from neonates across all three waves. During the first wave, a positivity of 6.18% was observed, which was similar to the findings reported by Sharma R et al., from Noida (6.6%-2/30) [20]. However, Kumar C et al., from Jodhpur reported a higher positivity rate of 9.8% (19/193) in nasopharyngeal swabs [21]. Reports from Kuwait, UK, Italy, Turkey, and France showed a wide range (2.7-6.1%) of positivity for SARS-CoV-2 infection in neonates using nasopharyngeal swabs [22-26].

The overall positivity of placental swabs was found to be 3.64%, with a positivity of 5.76% (3/57) observed during the first wave. A study by Kumar C et al., from Jodhpur reported a higher positivity rate of 8.1% (2/62) in placental samples [21]. As for umbilical swabs, the overall positivity rate was 4.9%, which was almost similar to that of nasopharyngeal swabs (4.72%). There is no known study that has used umbilical swabs for detecting perinatal COVID-19. Limited retrospective or prospective studies have been conducted to compare different neonatal samples for the diagnosis of SARS-CoV-2 infection [15].

Perinatal transmission is defined as a positive RT-PCR test result in a neonate within the first 72 hours of birth, encompassing both intrauterine and intrapartum transmission [2,3]. Horizontal transmission is considered when a neonate tested negative on RT-PCR within the first 72 hours but subsequently tested positive after 72 hours, regardless of the mother's SARS-CoV-2 status [2,3]. Analysis of the data showed that 73.9% (266/360) of neonatal samples were tested within 24 hours of birth [Table/Fig-3]. Out of the 360 samples, 29 neonates (8.05%) tested positive for SARS-CoV-2 infection within 72 hours of birth. Of the 30 positive neonates, 29 tested positive within 72 hours, while one neonate tested positive

Wave		Within 24 hours		24-48 hours		48-72 hours		After 72 hours	
		Sample	SARS CoV-2 Positive	Sample	SARS CoV-2 Positive	Sample	SARS CoV-2 Positive	Sample	SARS CoV-2 Positive
1st	Samples	282	17	20	3	5	2	44	1
	Neonates	(115)	(14)	(20)	(3)	(5)	(2)	(44)	(1)
2nd	Samples	238	8	4	0	2	0	10	0
	Neonates	(81)	(6)	(4)		(2)		(10)	
3rd	Samples	204	6	2	0	0	0	7	0
	Neonates	(70)	(4)	(2)				(7)	
Total	Samples	724	31	26	3	7	2	61	1
	Neonates	(266)	(24)	(26)	(3)	(7)	(2)	(61)	(1)

[Table/Fig-4]: Time at which swabs were given for SARS-CoV-2 RT-PCR test.

on the 5th day after birth. Thus, the rate of perinatal transmission in this study is 8.3%. More K et al., from Chandigarh also reported an 8% (106/1330) rate of perinatal transmission [14], and Kumar C et al., from Jodhpur reported a 9.8% rate of perinatal transmission [21]. In contrast, a study by Edlow AG et al., from Boston revealed no cases of perinatal transmission in neonates [27]. The positivity of umbilical and placental swabs collected within 24 hours of birth suggests the possibility of perinatal transmission [21]. The 6.7% (24/360) of neonates who tested positive within 24 hours may have acquired the infection intrauterinely, while those who tested positive on the second or third day after birth may have been infected through intrauterine or intrapartum transmission.

In the present study, an equal number of male and female neonates were found to be infected, indicating no specific gender affinity for SARS-CoV-2 infection. This study also provides evidence that perinatal infection of SARS-CoV-2 is typically asymptomatic or mild in neonates. The rate of prematurity in this study was 27% (6/22), and the rate of neonates with low birth weight (LBW) was 32% (7/22). A study by More K et al., reported a higher incidence of prematurity (32%) and LBW (42%) [14]. Among the COVID-19 positive neonates in this study, five were preterm in the first wave, while only one case of prematurity was observed in the third wave. There were no cases of preterm birth in neonates born to COVID-19 positive mothers during the second wave. This contrasts with a study by Malik S et al., from Maharashtra, which reported a higher incidence of prematurity during the second wave [28]. This retrospective study showed a relatively low rate of perinatal transmission (8.3%).

Nasopharyngeal and umbilical swabs showed equal potential for diagnosing perinatal transmission of SARS-CoV-2 infection. The positivity of placental samples (3.64%) raises the possibility of vertical transmission of SARS-CoV-2, but further studies with a larger number of samples are needed to confirm this.

Limitation(s)

Since this study was retrospective, authors could not obtain detailed information on eight neonates born to COVID-19 positive mothers.

The difference in positivity rates may be attributed to the use of different testing kits in different waves.

CONCLUSION(S)

This was the first study from Maharashtra to report on the performance of different clinical samples for diagnosing perinatal SARS-CoV-2 infection and the burden of COVID-19 in neonates born to SARS-CoV-2 infected mothers. The detection of SARS-CoV-2 RNA in umbilical and placental swabs provides strong evidence of perinatal transmission. However, confirmation of perinatal transmission requires genome sequencing of both the mother and neonate. This study found that the rate of preterm delivery and low birth weight was higher in the first wave compared to the second and third waves. These findings support the possibility of perinatal transmission.

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