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Original Research Article

A prospective study on vertical transmission of sars-cov-2 in pregnancy in a tertiary referral centre in south india

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Abstract
Purpose: The purpose of this study was to determine the possibility of vertical transmission of COVID-19 from COVID-19 positive mothers to new-borns and the associated factors.
Methods: This prospective observational study included a total of 128 antenatal COVID-19-positive women. The present study was conducted in a tertiary-care public hospital in south India. This study evaluated the SARS-CoV-2 positivity rate in neonates born to SARS-CoV-2-positive mothers who tested RT-PCR positive for this virus in their nasopharyngeal swab. During their hospital stay, these neonates' clinical outcomes were also evaluated.
Result: Out of 128 pregnancies, 45 study subjects' placenta specimens were COVID-19 positive, 3 neonates and 2 stillborn were RTPCR positive, and 1 neonate's cord blood was also positive. As among 4 stillbirth babies, 2 stillbirth babies were COVID-19 positive therefore there is a statistically significant relationship between stillbirth and vertical transmission ($p = 0.007$). In the present study, all COVID-19-positive babies were preterm, therefore there is statistically significance between preterm and vertical transmission ($p = 0.00$).
Conclusion: This study demonstrated that there is a possibility of vertical transmission of COVID-19 from COVID-19 positive mothers to new-borns.
Key Words: COVID-19, LSCS, Intensive Care Unit.

Introduction

Early in December 2019, Wuhan saw the first COVID-19 infection finding. Fever, cough, dyspnoea, malaise, weariness, and sputum secretion are the most typical symptoms. Additionally, SARS-CoV-2 infection is not just confined to the respiratory system; it can also trigger an over reactive immune system, which can result in the failure of numerous organs and death [1]. Compared to other disease-causing coronaviruses, SARS-CoV-2 has a much higher potential for transmission. [3] This raises concerns about the possibility of vertical transmission of illness from ill mothers to their infants. There are only limited studies in this area. However, initial reports from China seemed to indicate that there was no vertical transmission [2, 3].

The detection of COVID-19 in a pregnant woman is concerning because infections with other coronaviruses, like SARS and MERS, have been linked to severe maternal and neonatal morbidity and mortality, as well as unfavourable pregnancy outcomes like miscarriage, premature birth, and stillbirth. [4] Pregnant women, however, may experience a serious clinical course, according to early reports and lessons learned from SARS, MERS, and other respiratory illnesses. There is currently insufficient knowledge about pregnant COVID-19 patients upon which to base recommendations for pregnancy-specific treatment. Systems for monitoring COVID-19 cases must incorporate data on maternal and foetal outcomes as well as pregnancy status. [4].

The term vertical transmission refers to the spread of an infectious pathogen via the placenta in utero, bodily fluid contact during childbirth, or direct contact because of breastfeeding after delivery from the mother to the foetus during the antepartum and intrapartum phases of pregnancy, or from the mother to the new-born during the postpartum period. Although it has been shown that a variety of infectious vectors are capable of vertical transmission, there has recently been discussion on the likelihood of vertical transmission of SARS-CoV-2 from the infected mother to the foetus or new-born. Coronaviruses are not known to be vertically transmitted. [5]

The aim of this study was to investigate the possibility of vertical transmission of SARS-CoV-2 from mothers who tested positive for COVID-19 to their new-borns, as well as the contributing factors.

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Materials and methods

Study participants: A total of 128 antenatal women and their new born were included in this prospective single-centre observational study.

Study setting: The present study was conducted in a tertiary-level public hospital in Kerala (SAT Hospital, Govt. Medical College, Trivandrum) by the Department of Obstetrics and Gynaecology in association with the Department of Microbiology. This hospital is an important referral centre in South India. COVID-19 positive pregnant women admitted to the Department of Obstetrics and Gynaecology were included in this study.

Study duration: May 2021 to May 2022

Selection criteria

Inclusion criteria:

- Those who are SARS-COV-2 positive at the time of abortion or delivery

Exclusion criteria:

- Antenatal women who do not consent to participate in the study.
- All cases of Preterm Premature Rupture of Membranes

Sampling technique: All study subjects that meet the eligibility requirements enrolled consecutively.

Sample Size: complete enumeration of the study subjects during the study period

Study Procedure:

Biological samples are collected at admission, delivery, and post-partum. Maternal samples include a nasopharyngeal swab and a vaginal swab in order to test the positivity for SARS-CoV-2. At delivery, placental tissue was collected in a viral transport medium from the foetal surface, including a full-thickness cut section of the placenta. A 10 ml umbilical cord blood sample was collected at 24 weeks of gestation after cleaning the cord with sterile gauze and physiological solution. Both were obtained in a sterile way by a dedicated operator. In the case of a caesarean section, if possible, amniotic fluid was collected before the membrane ruptured. In women undergoing vaginal delivery, amniotic fluid was collected by amniocentesis where feasible after obtaining informed consent. The collected samples were transported within 24 hours to the virology lab (VRDL) in Govt. MCH Trivandrum, maintaining the cold chain, for RT-PCR testing. Upon delivery, a nasopharyngeal swab was taken from new-borns within 24 to 48 hours for COVID-19 testing. After delivery, antenatal women and babies are followed up until discharge from the hospital.

RT-PCR testing for detection of SARS-CoV RNA:

The collected samples were transported to the VRDL lab under the Department of Microbiology at the Government Medical College in Trivandrum, maintaining the cold chain. At the lab, the tissues were homogenized and RNA was extracted following the viral RNA extraction kit protocol (QiAmp viral RNA extraction kit). The presence of SARS-CoV-2 was detected by running a real-time PCR using the extracted RNA using an appropriate PCR kit (SeegenAllplexTM 2019-nCoV Assay). AllplexTM 2019-nCoV Assay is a qualitative in vitro diagnostic RT-PCR test for the qualitative detection of nucleic acid from SARS-CoV-2. It detects three target genes (the E gene, the RdRP gene, and the N gene). This assay uses the fluorophore FAM for the analysis of the E gene, HEX for the internal control (IC) gene, Cal Red 610 for the RdRP gene, and Quasar 670 for the N gene. Test results are positive for SARS-Cov-2 RNA when all 3 genes are detected OR when any two of the E, RdRP, or N genes are detected OR when either of the N or RdRp genes is detected. The test is negative when the IC is positive but none of the above genes are detected. A test is invalid when the IC is negative and none of the above genes are detected. Ct value 40: Detected (+), > 40 or N/A: Not detected (-) for all targets.

Study variables:

Maternal status of COVID-19, maternal co-morbidities, gestational age of onset of COVID-19, laboratory investigations, neonatal nasopharyngeal swab for COVID-19, maternal and foetal outcome & complications, breastfeeding, duration of hospital stay.

Data collection and analysis

For each patient, data was obtained using a pre-designed proforma. The information gathered was entered into a Microsoft Excel spreadsheet. SPSS version 26.0 was used for statistical analysis. Quantitative data was presented as the mean± standard deviation, whereas qualitative data was presented as frequency and percentage. When comparing the two means, an independent sample t-test for significance was performed. To analyse relations between two qualitative parameters, the Fisher's exact test of significance was performed. P-values less than 0.05 were considered significant.

Ethical considerations

Prior approval was obtained from the ethics committee of the institute, and written informed consent was obtained from all antenatal women in their mother tongue. A participant information sheet was given to all study subjects in their native language, informing them about the procedure involved. Study subjects were informed that they

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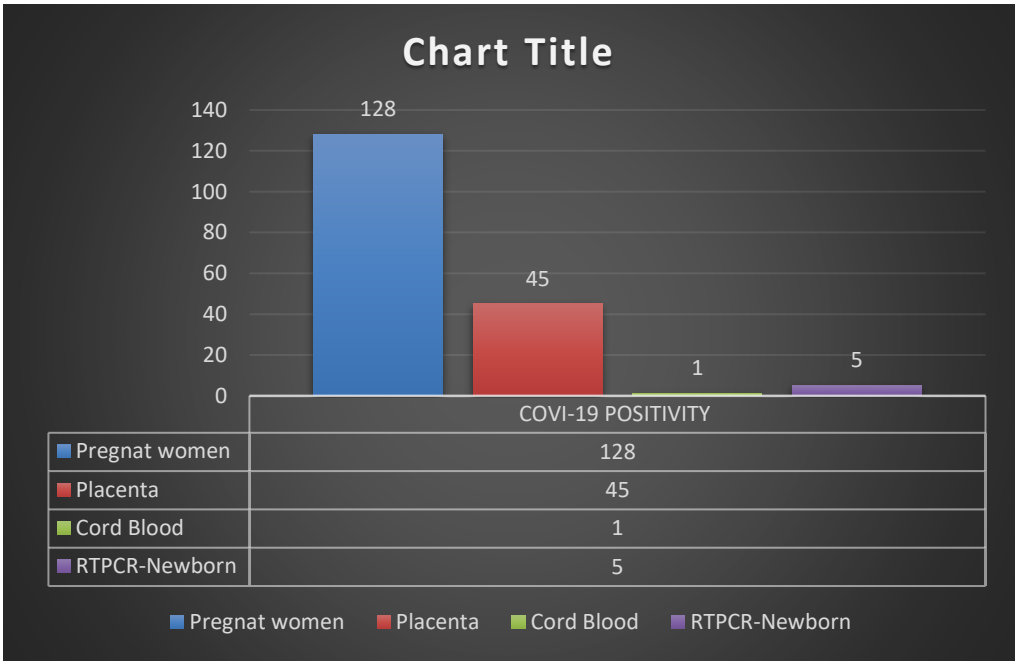
can withdraw at any time from this study and that this will not affect the treatment given to them in the hospital in any way.

Results

A total of 128, COVID-19 positive mothers were included in this study. Out of 128 pregnancies, 45 study subjects' placenta specimens were COVID-19 positive, 3 neonates and 2 stillborn were RTPCR positive, and 1 neonate's cord blood was also positive. (fig.1)



Out of 128 pregnancies, there were 124 live births and 4 still births. 24 neonates were pre-term. Out of 124 live births, 3 live births were COVID positive and out of 4 still birth babies, 2 still birth babies were COVID positive. In the present study, there were 5 vertical transmissions. (Table 1)

Table 1: Baseline characteristics and Vertical transmission

Variable	**Characteristics**	N (%)
Birth Status	Live birth Still birth	124(96.9) 4(3.1)
Gender	Male Female	63(49.2) 65(50.8)
Gestational age at delivery	Pre term term	24(18.8) 104(81.3)
Mean ±SD Gestation at the time of delivery	37.39±2.3 weeks	
Vertical Transmission	Positive Negative	5(3.9) 123(96.1)
Total		**128(100)**

Out of 5 vertical transmissions, there were 3 live births and 2 still births. Majority of the new-borns were females (50.8%). All COVID-19-positive neonates were preterm. Out of 4 still birth babies, 2 still birth babies were COVID positive which means there is a statistically significant relationship between still birth and vertical transmission (p = 0.007), and the result also showed that all COVID-9-positive babies were preterm, therefore there is a statistical significance between preterm and vertical transmission (p = 0.00). (Table 2)

Table 2: Association between Neonatal factors with Vertical Transmission

Variables s	**Characteristics**	**Vertical Transmission**		**Total**	**X2 P**
		Positive	**Negative**		
Birth Status	Live Birth	3	121	124	X2=23.371 P=.007
	Still birth	2	2	4	
Term delivery	Preterm	5	19	24	X2=22.547 P=.000
	Term	0	104	104	
Total		**5**	**123**	**128**	

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Ferritin levels in COVID-19-positive neonates' mothers were much higher than those in COVID-19 negative neonates' mothers. (The mean ±SD ferritin level is 218.33±356.5, and the p value is 0.00.) (Table 3)

Table 3: Relationship between Vertical Transmission and Maternal Biomarkers

Variable	Vertical Transmission	Mean± SD	t	p
CRP	Yes 5 No 123	4.9±1.8 3±6.3	.631 .914	.79
Ferritin	Yes 5 No 123	218.3±356.5 55.5±69.5	3.823 1.0	0.00
D Dimmer	Yes 5 No 123	3±1.3 2±1.4	1.42 1.56	.80

According to the study result, there is a relationship between placental positivity and maternal comorbidities. 91% of the placental positivity cases had maternal comorbidities, which is statistically significant (p = 0.03). Also, 71.1% of the placental positive cases were in the high-risk category (p = 0.03). (Table 4)

Table 4: Relationship between maternal factors and Placental positivity

Variables s	Characteristics	Placental positivity		Total	X2 P=
		Positive	Negative		
Maternal co morbidities	Yes	41(91.1)	64(77.1)	105(82)	X2=3.882 P=.038
	No	4(8.9)	19(22.9)	23(18)	
Maternal Risk group	High Risk	32(71.1)	44(53)	76(59.4)	**X2**=3.963 **P**=.035
	Low Risk	13(28.9)	39(47)	52(40.6)	
Maternal symptoms	Symptomatic	6(13.3)	20(24.1)	26(20.3)	X2=2.088 P= .111
	Asymptomatic	39(86.7)	63(75.9)	102(79.7)	
Severity of covid	Mild	34(75.6)	52(62.7)	86(62.7)	X2=4.652 P=.161
	Moderate	10(22.2)	31(37.3)	41(32)	
	Severe	1(2.2)	0	1(.8)	
Mode of delivery	Vaginal	19(42.2)	36(43.4)	55(43.4)	X2=.071 P=.49
	Assisted Vaginal	2(4.4)	4(4.8)	6(4.7)	
	Emergency CS	14(31.1)	24(28.9)	38(29.7)	
	Elective CS	10(22.2)	19(22.9)	29(22.7)	
NICU	Yes	6(14)	7(8.8)	13(10.6)	X2=.081 P=.24
	No	37(86)	73(91.3)	110(89.4)	
Total		**45**	**83**	**128**	

Ferritin and D-Dimmer levels were elevated in placental positive cases (p = 0.001 and 0.005, respectively). Biomarkers and placental positivity have a significant association. (Table5)

Table 5: Relationship between Placental positivity and Maternal Biomarkers

Variables	Placental positivity	N	Mean	Std. Deviation	t	P
Duration of positivity from delivery	Positive	45	9.20	4.994	1.212	.264
	Negative	83	8.05	5.205	1.227	
CRP	Positive	44	3.2909	3.93351	.173	.952
	Negative	81	3.0869	7.27357	.204	
Ferritin	Positive	45	86.255	159.81307	2.094	.001
	Negative	83	48.665	27.32515	1.565	
D dimer	Positive	45	2.4240	1.91790	1.859	.005
	Negative	82	1.9185	1.14769	1.616	
GA at diagnosis	Positive	45	36.591	2.8150	-.907	.256
	Negative	83	36.975	1.9413	-.815	

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Birth Weight	Positive	44	2.5875	.52269	-.732	.151
	Negative	83	13.848	101.78840	-1.008	

Discussion

The possibility of SARS-CoV-2 vertical transmission has started major conversation and concern. Initially, limited case series and isolated case reports contributed the majority of the knowledge on this topic. [6, 7] Furthermore, the vast majority of these data came from China, where the epidemic initially seen and North America. Large population-based data from different regions such as South East Asia are still lacking [8].

The purpose of this study was to determine the possibility of vertical transmission of SARS-CoV-2 from COVID-19-positive mothers to their new-borns and the associated factors. A total of 128 COVID-19 positive mothers were included in this study. Out of 128 pregnancies, 45 study subjects' placenta specimens were COVID-19 positive, 3 neonates and 2 stillborn were RTPCR positive, and 1 neonate's cord blood was also positive. As there is 5 vertical transmission cases, this study demonstrated that there is a possibility of vertical transmission of COVID-19 from COVID-19 mother to new-borns. Though the placental tissue tested positive in 35% of cases, only 3.9% of the neonates tested positive.

This may be due to the placenta acting as a barrier in the transmission of virus to the foetus in the intrapartum period. In a study conducted by Kumar p (2021) et al [9] four were new-borns were SARS-CoV-2 positive by RT-PCR, out of 47 neonates born to SARS-CoV-2-positive mothers. Kumar p et al's study showed that neonates born to COVID-19-positive women can experience vertical transmission, but the risk was minimal. However in the present study showed that the risk was high as among 4 stillbirth babies, 2 stillbirth babies were COVID positive. A study conducted by Hu et al [10] demonstrated that the vertical transmission of SARS-CoV-2 from mothers infected with COVID-19 during the final days of pregnancy is possible but uncommon. [10]. This study result is similar to the present study. A study by Khan et al included 17 pregnant mothers and their 17 neonates. Two neonates with suspected SARS-CoV-2 infection and five with neonatal pneumonia were identified in the study, suggesting that adverse pregnancy outcomes may be associated with COVID-19 infection. [11]

In the present study, out of 128 pregnancies there were 124 live birth and 4 still birth. This study also showed that 24 neonates were pre-term. Out of 124 livebirths, 3 livebirths were COVID-19 positive and among 4 stillbirth babies, 2 stillbirth babies were COVID-19 positive. The study demonstrated that there were 5 vertical transmissions. Out of 5 vertical transmissions, there were 3 live births and 2 stillbirths. As among 4 stillbirth babies, 2 stillbirth babies were COVID-19 positive therefore there is a statistically significant relationship between stillbirth and vertical transmission ($p = 0.007$). In a study by Zeng et al [12] also showed that there is a possibility of vertical transmission of COVID-19 from mothers to new-borns. 33 pregnant mothers and their neonates were studied by Zeng et al. The study identified 33 neonates born to mothers with COVID-19, including 3 neonates with COVID-19. The study showed that there is a possibility of vertical transmission [12]. A study by Ferrazzi et al [13] included 42 pregnant mothers and their neonates. As per the study result 3 out of 42 neonates tested positive. This study indicates there is a possibility of vertical transmission of COVID-19and vaginal birth may be associated with a low risk of intrapartum SARS-Cov-2 transmission to the neonate. [13]

An analysis by De Sisto CL [16] showed that pregnant patients with COVID-19 had a higher risk of stillbirth than pregnant patients without COVID-19. (1.26 Versus 0.64 percent of deliveries; adjusted relative risk 1.90, 95% CI 1.69–2.15) [14] An examination of data from pregnant women with confirmed or suspected SARS-CoV-2 infection in 12 countries revealed all-cause early new-born death rates of 0.2 to 0.3 percent [15]. A systematic review by Huntley BJF et al also discovered that the rate of infant death was comparable between those who tested positive for SARS-CoV-2 and those who tested negative when admitted to labour and delivery [16].

In the present study, all COVID-19-positive babies were preterm, therefore there is statistically significance between preterm and vertical transmission ($p = 0.00$). A study by Berghella V et al [17] have similar result. In this 2023 individual participant data meta-analysis, neonates born to mothers with symptomatic COVID-19 were more likely to be born preterm (<37 weeks: RR 1.41, 95% CI 1.15-1.73) and by caesarean birth (RR 1.16, 95% CI 1.04-1.29) than neonates of COVID-19 negative mothers. [17]

Conclusion

In this study, 50% of the stillbirth showed signs of being infected with SARS-CoV-2. Even though studies have shown that the incidence of vertical transmission is low or nil, [18, 19] the present study shows that vertical transmission from mother to child can occur .An association was found between ferritin, D-dimer levels and vertical transmission. Still births had higher incidence of COVID positivity but further studies are needed to establish a relation between infection and risk of still birth. To conclude, it is essential to test pregnant women for

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SARS-CoV-2 and take strict steps to avoid spread of infections, put sick mothers in quarantine, and screen new-borns for COVID-19.

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Conflict of interest: Nil

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