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Original Article

Pathology Section

Histopathological Changes and Clinical Outcomes in Placentas of COVID-19 Positive Mothers: A Cohort Study

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ABSTRACT

Introduction: Infection by Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) has been shown to affect multiple organs in the human body. Research shows that Coronavirus Disease (COVID-19)-positive pregnant women experience poorer perinatal outcomes related to placental infection, including higher risks of miscarriage, preeclampsia, preterm birth, and stillbirth.

Aim: To evaluate the histopathological changes in the placentas of COVID-19-positive mothers and the associated foetal outcomes.

Materials and Methods: This cohort study was conducted at Kalinga Institute of Medical Sciences (KIMS) Bhubaneswar, Odisha, India, over a period of one year and eight months, from November 2020 to July 2022. It included 23 COVID-19-positive pregnant females admitted for safe confinement during the first and second COVID-19 waves. Thirty COVID-19-negative pregnant women admitted for safe delivery during the same period served as controls. Placentas were collected, processed, and stained according to standard protocols. Descriptive data were interpreted as frequencies and percentages, and associations were tested using the Chi-square test. A p-value <0.05 was considered statistically significant.

Results: The study included 23 cases (mean gestational age: 37 weeks and 5 days) and 30 controls (mean gestational age: 38 weeks and 6 days). The prevalence of Maternal Vascular Malperfusion (MVM) and Foetal Vascular Malperfusion (FVM) was found to be higher among cases than controls.

Conclusion: Compared to controls, COVID-19-positive placentas showed a higher prevalence of both MVM and FVM. This might be attributable to the hypercoagulable state associated with COVID-19. Further research is needed to explore the potential effects of intrauterine inflammation on neonates exposed to COVID-19.

Keywords: Foetal vascular malperfusion, Maternal vascular malperfusion, Pregnant mothers

INTRODUCTION

In March 2020, the World Health Organisation (WHO) declared the COVID-19 a global pandemic. Since the index case in Wuhan, China, in December 2019, a multitude of COVID-19 infections were reported. A WHO assessment estimates that as of August 8, 2022, 581,686,197 people had been infected worldwide, and resulted in 6,410,961 deaths. India reported 44,161,899 of these cases and 526,730 deaths [1]. The coronavirus's effects on several organs have been extensively studied. However, the impact of COVID-19 on pregnant women and their perinatal outcomes remains less clear. Pregnant women with COVID-19 have been documented to experience increased risks of adverse perinatal outcomes related to placental infection, including miscarriage, preeclampsia, preterm birth, and stillbirth [2]. Limited studies have explored vertical transmission of the virus across the placenta and its impact on pregnancy outcomes since the onset of the pandemic [3-6]. The histomorphological abnormalities in the placentas of mothers testing positive for COVID-19 have been the focus of a few studies. These studies have reported a range of findings in females infected with SARS-CoV-2 [7]. Research in this area can significantly improve the health outcomes for both mother and foetus, as the implications of maternal SARS-CoV-2 infection on placental histopathology are not fully understood. In this study, the aim was to investigate histopathological changes, clinical outcomes, delivery outcomes, and pathological findings caused by SARS-CoV-2 infection and attempted to compare these with controls and also to evaluate foetal outcomes.

MATERIALS AND METHODS

This was a cohort study conducted between November 2020 and July 2022 at the Department of Obstetrics and Gynaecology and the Department of Pathology at KIMS, Bhubaneswar, Odisha, India. The study received due approval from the Institutional Ethics Committee (Ref. No: KIIT/KIMS/IEC/468/2020). It included 23 COVID-19 positive mothers admitted to the Department of Obstetrics and Gynaecology for safe care during the first and second COVID-19 waves. Thirty COVID-19 negative pregnant women, admitted for safe delivery during the same period, served as controls.

Inclusion criteria: All expectant mothers who gave consent and were tested positive for coronavirus Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) and were admitted to the Department of Obstetrics and Gynecology for safe delivery were included in the study.

Exclusion criteria: Women with twin or multiple births and those with other co-morbidities were excluded from the study. Initially, 24 women were included, but one was excluded due to pre-existing hypothyroidism, resulting in 23 cases. None of the pregnancies in present study were twins or other multiple pregnancies.

Clinical information and haematological examination reports were collected. Only one participant, who was 32 weeks pregnant, gave birth prematurely. After birth, the placentas were removed and fixed in formalin for 48 hours to preserve the tissue and minimise the risk of infection. The placental sampling was performed following the guidelines of the Amsterdam Placental Workshop Group [8]. One section each was taken from the umbilical cord and membrane, and at least three sections were taken from the placental body. Additional pieces were taken from visibly abnormal areas. All sections underwent standard Haematoxylin and Eosin (H&E) staining procedures. Lesions were identified using the standards outlined in the Amsterdam Placental Workshop Group consensus statement for placental lesions. Every newborn born to a mother who tested positive or negative for COVID-19 was monitored until discharge. Clinical information, anthropometric data, and APGAR scores were collected [5].

STATISTICAL ANALYSIS

Data were compiled and tabulated in Excel and analysed using Statistical Package for Social Sciences (SPSS) statistical software, version 21.0. Descriptive data were interpreted as frequencies and percentages. Associations were tested using the Chi-square test. A p-value <0.05 was considered to be statistically significant.

RESULTS

A total of 30 placentas from women who gave birth in the hospital during the same period and had negative COVID-19 test results were compared to 23 placentas from women with COVID-19. Eleven cases were collected during the first wave, and the remaining 12 cases during the second wave. There was no statistically significant difference in the mean maternal age between the two groups (p-value=0.423). All participants were asymptomatic, delivered via Lower Segment Caesarean Section (LSCS), and had term pregnancies, with the exception of one COVID-19 positive mother who gave birth prematurely at 32 weeks of gestation. Both cohorts had deliveries in the third trimester. Most of the patients exhibited elevated total white cell counts, C-Reactive Protein (CRP), and D-dimer levels at the time of delivery, suggesting active infection [Table/Fig-1]. At one and five minutes after birth, the majority of newborns had normal APGAR scores, with most deliveries occurring through LSCS [Table/Fig-2,3]. One newborn was admitted to the NICU due to prematurity and under weight (32 weeks and 2 days, 1.6 kg). After successful management and discharge, follow-up was lost. All babies born tested negative for COVID-19.

Group	Mean total leukocyte count (×10³/microlitre) (Normal range: 4-11×10³/microlitre)	Mean CRP (mg/L) (Normal range: <3 mg/L)	Mean D-dimer (micron/mL) (Normal range: <0.5 micron/mL)
COVID-19 positive group	11.2	12.24	3.36
COVID-19 negative group	10.3	7.67	1.97

[Table/Fig-1]: Laboratory findings of COVID-19 positive and negative groups.

Variable	COVID-19 +ve (n=23)	COVID-19 -ve (n=30)	p-value
Mean birth weight (in Kgs)	2.62 (2.50-3.10)	2.61 (2.38-3.10)	0.506
APGAR at birth (Mean)	8 (7-8)	8 (7.75-8.00)	0.271
Length (cm)±SD	50.95±2.00	51.13±1.93	0.738
Head circumference (cm)±SD	32.19±1.70	31.83±1.82	0.469

[Table/Fig-2]: Clinical outcomes of babies born to COVID-19 positive and negative mothers.

Gestational age			Chi-square, p-value
Group	<37 w	≥37 w	6.72, 0.0095
COVID-19 positive (n=23)	8	15	
COVID-19 negative (n=30)	2	28	
Mode of delivery			**Chi-square, p-value**
Group	LSCS	NVD	6.183, 0.0129
COVID-19 positive (n=23)	23	0	
COVID-19 negative (n=30)	23	7	

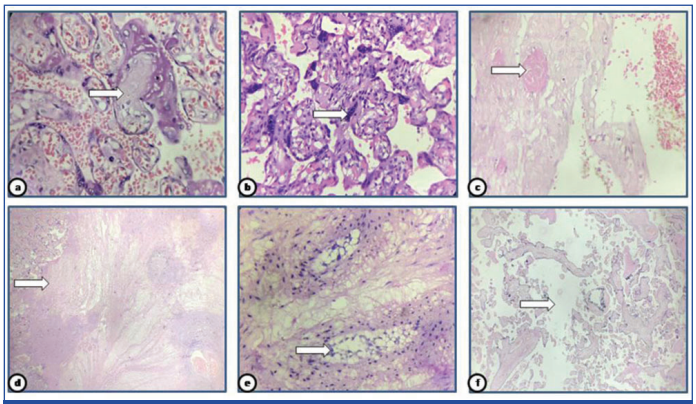
[Table/Fig-3]: Gestational age and mode of delivery of COVID-19 positive and negative mothers.

Placental Pathology

Microcalcification of the placenta was observed in all cases (23/23) and in the majority of the control group (25/30). MVM [Table/Fig-4] indicated by perivillous and intervillous fibrin deposition, was present in all cases (23/23), compared to a prevalence of 6.6% in the control group [Table/Fig-5a]. An increase in syncytial knots was evident in the majority of placentas (16/23) [Table/Fig-5b]. Other findings, such as fibrin thrombi (3/23) [Table/Fig-5c], maternal floor infarcts (7/23) [Table/Fig-5d], decidual vasculopathy (7/23) [Table/Fig-5e], and distal villous hypoplasia (5/23) [Table/Fig-5f], were observed in some cases. Signs of FVM [Table/Fig-6], such as syncytial karyorrhexis (4/23) [Table/Fig-7a], chorangiosis (5/23) [Table/Fig-7b], villous agglutination (4/23) [Table/Fig-7c], stem villous vessel obliteration [Table/Fig-7d], and thrombosis in the foetal circulation, were seen in a subset of cases.

Features of maternal vascular malperfusion	COVID-19 positive group (n=23), n (%)	COVID-19 negative group (n=30)	p-value
Fibrin thrombi	3 (13)	0	0.042
Perivillous and intervillous fibrin	23 (100)	2 (6.6)	<0.001
Maternal floor infracts	7 (30.4)	0	0.001
Decidual vasculopathy	7 (30.4)	0	0.001
Distal villous hypoplasia	5 (21.7)	0	0.007
Tenny parker change	16 (69.5)	3 (10)	0.00

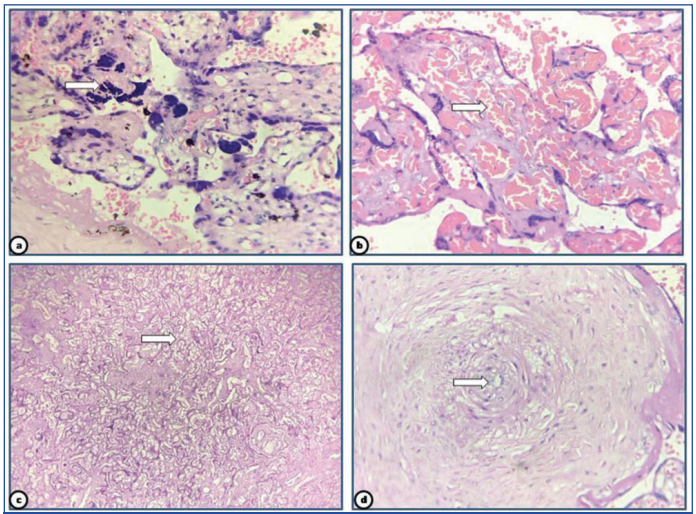
[Table/Fig-4]: Comparison between COVID-19 positive and negative groups based on maternal vascular malperfusion.



[Table/Fig-5]: a) Perivillous and intervillous fibrin (H&E, 40 X); b) Tenny parker change (H&E, 40 X); c) Fibrin thrombi (H&E, 40X); d) Maternal floor infract (H&E, 40 X); e) Decidual vasculopathy (H&E, 40 X); f) Distal villous hypoplasia (H&E, 40X).

Features of Foetal Vascular Malperfusion (FVM)	COVID-19 positive group (n=23)	COVID-19 negative group (n=30)	p-value
Thrombosis in foetal circulation	1 (4.3)	0	0.249
Stem villous vessel obliteration	1 (4.3)	0	0.249
Villous agglutination	4 (17.3)	0	0.018
Syncytial karyorrhexis	4 (17.3)	0	0.018
Chorangiosis	5 (21.7)	0	0.007

[Table/Fig-6]: Comparison between COVID-19 positive and negative groups based on foetal vascular malperfusion.

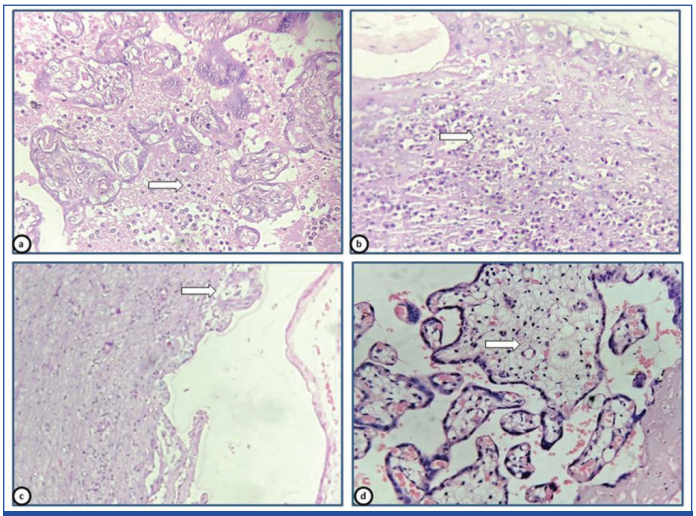


[Table/Fig-7]: a) Syncytial karyorrhexis (H&E, 40X); b) Chorangiosis (H&E, 40X); c) Villous agglutination (H&E, 40X); d) Stem villous vessel obliteration (H&E, 40X).

Inflammatory features [Table/Fig-8] including intervillositis (5/23) [Table/Fig-9a], deciduitis (18/23) [Table/Fig-9b], chorioamnionitis (16/23) [Table/Fig-9c], and villitis (8/23) [Table/Fig-9d] were also identified in varying proportions.

Features of inflammation	COVID-19 positive group (n=23)	COVID-19 negative group (n=30)	p-value
Villitis of unknown aetiology	8 (34.7)	0	<0.001
Intervillositis	5 (21.7)	0	<0.001
Deciduitis	18 (78.2)	2 (6.7)	<0.001
Chorioamnionitis	16 (69.5)	1 (3.3)	0.007

[Table/Fig-8]: Inflammatory features in COVID-19 positive and negative groups.



[Table/Fig-9]: a) Intervillositis (H&E, 40X), b) Deciduitis (H&E, 4X); c) Chorioamnionitis (H&E, 40 X); d) Villitis (H&E, 40X).

DISCUSSION

Present study compiled cases from women who were tested and found to be COVID-19 positive, tracking the outcomes for both mothers and foetuses post-delivery, as well as examining placental pathology. Consistent with findings by Shanes ED et al., and Di Mascio D et al., [3,9], all COVID-19 positive mothers were asymptomatic at the time of delivery. However, most had elevated D-dimer, CRP, and total WBC counts, which suggested persistent infection, corroborating the haematological changes noted by Zhang C et al., [10]. All deliveries were conducted via LSCS, resulting in viable live births (male=14, female=9), and the maternal/foetal outcomes were normal and asymptomatic at discharge. Pregnancies with COVID-19 are more likely to result in caesarean sections, often due to hypoxia-related complications, as reported by Menter T et al., and Di Mascio D et al., [4,9]. The placentas in present study had an increased prevalence of thrombohaemorrhagic changes and inflammatory findings. Microvasculopathy can manifest as maternal or foetal vascular malperfusion (MVM or FVM, respectively). Signs of MVM were identified, particularly perivillous and intervillous fibrin deposits, in all 23 cases (p-value <0.001).

Present study revealed fewer instances of low-grade villitis compared to earlier research [5,11], which identified villitis under the umbrella of increased Uteroplacental Exchange (VUE) as a frequent finding. Prochaska E et al., observed FVM as their primary finding, in contrast to present study that primarily identified MVM [12]. Singh N et al., also reported increased microcalcifications, fibrin, syncytial knots, and villous agglutination [6]. Joshi B et al., highlighted a greater prominence of MVM characteristics over FVM [13], and Surekha MV et al., found features indicative of maternal hypoxia, similar to present study findings [14]. None of the patients experienced pregnancy-related complications, and all mothers were asymptomatic. Neonates born to these mothers were active and cried immediately after birth.

Except for one case of premature delivery, all births were at term. Notably, the placenta in this premature case showed florid intervillositis among other signs of maternal and foetal malperfusion, potentially due to alterations in the maternal placental blood flow from hypoxic conditions. Similarly, Schwartz DA and Morotti D addressed foetal risk and placental pathology, finding that while only a few infants are affected, placental infection remains a potential risk factor [15]. The findings of this study may support the hypothesis that SARS-CoV-2 affects coagulation. However, pregnancy itself alters coagulation, and whether SARS-CoV-2 is the sole cause of these changes requires further investigation. Viral infections in mothers have been associated with adverse obstetric outcomes, with cases of preterm deliveries and low birth weight newborns following SARS-CoV and MERS-CoV infections [16]. Despite concerns about vertical transmission, comprehensive studies on the impact of SARS-CoV-2 on newborn outcomes are limited.

The strength of present study lies in the use of a suitable control group of women and placentas. Placentas are typically sent for pathological examination when necessary. Control group, comprising COVID-19 negative mothers with nearly identical clinical profiles who delivered around the same time, provided a compelling comparison with placental materials not influenced by the pregnancy's outcome. Nonetheless, present study does have inherent limitations.

Limitation(s)

In this study, the presence of viral RNAs or viral proteins in the placenta were not assessed, leaving us without direct evidence of SARS-CoV-2 infection within the placental tissue. Consequently, present study does not definitively establish whether SARS-CoV-2 infection contributes to the development of placental pathology. Additionally, nearly all the newborns were healthy, precluding the need for testing that could exclude the possibility of vertical transmission of COVID-19. Apart from HIV and Hepatitis viruses, the subjects were not screened for Toxoplasmosis, Rubella, Cytomegalovirus, and Herpes simplex virus (TORCH) infections. Furthermore, authors missed the opportunity to monitor the long-term outcomes of one preterm infant was missed.

CONCLUSION(S)

This study presents an analysis of placental pathology from 23 mothers infected with COVID-19. A comparison with controls revealed an increased incidence of MVM and FVM, which may be associated with the hypercoagulable state induced by COVID-19 infection. Further studies are essential to enhance the understanding of the impact of COVID-19 on neonates and the potential long-term effects on both mothers and children. Nonetheless, based on present study findings, it appears that COVID-19 infection did not inherently increase the risk of morbidity and mortality in mothers and their newborns.

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