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Low-level SARS-CoV-2 viremia coincident with COVID placentitis and stillbirth

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ABSTRACT

SARS-CoV-2 infection in pregnancy and COVID placentitis are associated with an increased risk of stillbirth. We sought to investigate the presence of maternal viremia in people with SARS-CoV-2 infection during pregnancy who had histologic placentitis versus those without placentitis. SARS-CoV-2 qRT-PCR was performed on plasma from 6 patients with COVID placentitis and 12 matched controls without placentitis. SARS-CoV-2 infection occurred between 4/2020–1/2021; the latency between SARS-CoV-2 diagnosis and delivery was 0–76 days. Two placentitis cases demonstrated viremia (1 stillbirth and 1 well infant), while 12/12 controls were negative. Future research may consider viremia as a possible marker of COVID placentitis.

1. Introduction

Recent reports have identified an increased risk of stillbirth in pregnant people infected with SARS-CoV-2 [1,2]. Perinatal pathologists have identified specific placental pathology, termed COVID placentitis, associated with a high risk of stillbirth and poor neonatal outcome [3,4]. COVID placentitis, characterized by histiocytic intervillositis, increased perivillous fibrin deposition, and villous trophoblast necrosis, has been associated with direct viral infection of the syncytiotrophoblast layer of the placenta. Placentitis represents a clinical dilemma to obstetric providers as it can only be diagnosed on examination of the placenta after delivery. We hypothesized that, due to the interface between syncytiotrophoblast and maternal blood, patients with COVID placentitis would be more likely to demonstrate viremia than SARS-CoV-2-infected patients without placentitis.

2. Methods

As part of an active prospective observational cohort study, we identified patients who had SARS-CoV-2 infection during pregnancy and delivered at Northwestern Medicine Prentice Women's Hospital in Chicago, IL, USA. We identified 6 patients diagnosed with COVID placentitis on pathologic exam and with maternal plasma samples collected and 12 matched controls who had SARS-CoV-2 infection without COVID placentitis. The control cases were matched for gestational age at birth and time between SARS-CoV-2 infection and delivery. COVID placentitis was diagnosed based on the presence of histiocytic intervillositis confirmed with immunohistochemical staining for CD68 and increased perivillous fibrin deposition in the context of maternal SARS-CoV-2 infection. The percentage of villous parenchyma involved was estimated using both gross and microscopic evidence of involvement.

Demographic and clinical data were collected through EMR review. Severity was defined according to National Institutes of Health criteria as asymptomatic, mild, moderate, severe, or critical illness [5]. After

Abbreviations: SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; COVID-19, Coronavirus disease 2019; qRT-PCR, quantitative reverse transcription polymerase chain reactions; RNA, ribonucleic acid; CDC, Centers for Disease Control and Prevention.

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Table 1
Data of patients with SARS-CoV-2 infection during pregnancy with and without COVID placentitis

*	Gestational Age at delivery	SARS-CoV-2 infection date	Symptoms	Placentitis	Latency‡	Viremia	N1 CT	RNAseP	Neonatal outcome Growth APGARs
P1^	37 0/7	4/2020	Mod	5%	62	–	ND	29.830	SGA
							ND	27.795	9/9
P2	29 4/7	11/2020	Asympt	Diffuse	1	+	35.542	28.869	AGA
							35.103	28.694	0/0
							35.421	28.559	
							34.876	28.678	
P3	39 0/7	11/2020	Mild	40%	29	+	35.022	31.185	AGA
							37.642	31.566	8/9
							36.522	30.984	
							37.508	30.972	
P4	39 5/7	12/2020	Mild	Diffuse	0	–	ND	35.397	AGA
							ND	36.793	7/9
P5	40 4/7	12/2020	Mild	10%	76	–	ND	35.198	AGA
							ND	35.684	9/9
P6	40 3/7	1/2021	Mild	10%	63	–	36.784	33.445	AGA
							ND	33.210	8/9
							36.651	33.380	
							ND	33.439	
							ND	33.576	
							ND	33.731	
C1	38 2/7	6/2020	Mild	n/a	59	–	ND	34.655	LGA
							ND	33.995	8/9
C2	38 2/7	6/2020	Mild	n/a	85	–	ND	33.853	AGA
							ND	33.751	9/9
C3	38 2/7	12/2020	Asympt	n/a	0	–	ND	34.642	AGA
							ND	34.348	9/9
C4	38 0/7	11/2020	Asympt	n/a	13	–	ND	33.274	SGA
							ND	32.113	9/9
C5	37 0/7	1/2021	Mild	n/a	31	–	ND	34.104	AGA
							ND	33.571	6/8
C6	34 2/7	12/2020	Mild	n/a	17	–	ND	31.719	AGA
							ND	31.786	8/9
C7	36 5/7	1/2021	Mild	n/a	5	–	ND	33.073	AGA
							ND	32.987	8/9
C8	40 2/7	12/2020	Mild	n/a	1	–	ND	32.875	AGA
							ND	32.536	1/8
C9	39 0/7	12/2020	Mild	n/a	76	–	ND	35.339	AGA
							ND	34.470	8/9
C10	39 1/7	10/2020	Mild	n/a	90	–	ND	34.881	AGA
							ND	33.641	8/9
C11	41 2/7	11/2020	Mild	n/a	56	–	ND	26.575	AGA
							ND	26.541	9/9
C12	39 6/7	1/2021	Mild	n/a	59	–	ND	35.518	AGA
							ND	34.769	8/9

*P = placentitis, C = control.
‡ Latency = time from SARS-CoV-2 PCR/symptoms to pregnancy outcome (stillbirth, delivery).
^ P1 maternal sample at time of acute infection, others from delivery admission.
Growth categories: SGA = small for gestational age, AGA = appropriate for gestational age, LGA = large for gestational age.

birth, maternal blood was retrieved from as discards from the clinical laboratory. Blood was centrifuged, and plasma aliquots were stored at −80° Celsius. This study was approved by the Institutional Review Board of Northwestern University (STU00212232) with a waiver of informed consent obtained.

Viral RNA was extracted from clinical specimens utilizing the QIAamp Viral RNA Minikit (Qiagen, cat. no. 52906). Testing for SARS-CoV-2 presence was performed by qRT-PCR with the CDC 2019-nCoV RT-PCR Diagnostic Panel utilizing the N1 probe in SARS-CoV-2 and RNASE P probe for sample quality control as previously described (IDT, cat. no. 10006713) [6]. Specimens were run in technical duplicate with additional replicates performed to verify putative amplification. All specimens with an N1 probe cycle threshold (Ct) less than or equal to 35 were considered positive. Specimens with an N1 probe Ct value between 35 and 40 were considered positive only if all replicates amplified and on-target amplification was verified by TOPO cloning (CloneJET PCR Cloning Kit, ThermoFisher, cat. no. K1231) and Sanger sequencing of the N1 qPCR product.

3. Results

In this cohort, SARS-CoV-2 infections occurred between April 2020 and January 2021, prior to the emergence of variants of concern in Chicago (including Delta). Time between a positive SARS-CoV-2 test and delivery ranged from 0 to 76 days (Table 1). Of the 6 patients with COVID placentitis, 1 had asymptomatic SARS-CoV-2 infection, 4 were mild, and 1 had moderate COVID-19 severity. One placentitis case resulted in stillbirth at 29 4/7 weeks gestational age.

Of the 6 patients with placentitis, SARS-CoV-2 was amplified from maternal blood in 2 cases, including the case of stillbirth, while 0 controls were viremic at delivery. Cloning and Sanger sequencing of the qRT-PCR products confirmed specific on-target amplification of SARS-CoV-2 in these 2 samples. One additional case with placentitis showed amplification below the level of detection in 2 out of 6 replicates and was thus interpreted as negative. Of the 2 cases with confirmed low-level viremia, one woman was asymptomatic and delivered a stillborn infant 1 day following a positive test, while the other woman was mildly symptomatic and delivered a well infant.

4. Discussion

SARS-CoV-2 infection has affected over 173,500 pregnant persons reported to the Centers for Disease Control and Prevention as of February 2022 and remains a significant issue globally [7]. Concerning findings of placentitis and higher incidence of stillbirth are among the many adverse outcomes of SARS-CoV-2 infection during pregnancy. Placentitis and stillbirth are not limited to patients with overt or severe infection, thus clinicians remain without tools to identify pregnancies with placentitis or high risk of stillbirth until after birth.

Viremia in SARS-CoV-2 infection is rare. Approximately 1% of individuals with SARS-CoV-2 infection have detectable virus in their blood. While data on pregnant patients is scarce, one study of 127 pregnancies detected no cases of viremia [8]. Shook et al. recently reported SARS-CoV-2 viremia of ~10 genomes/ml in two cases of SARS-CoV-2 B.1.617.2 (delta) variant associated placentitis and stillbirth [9].

Our study expands this small and important body of literature by demonstrating viremia in pregnant patients in the pre-Delta era and in both stillbirth and those with mild, non-lethal placentitis. Given the wide clinical availability of SARS-CoV-2 PCR testing, viremia could represent a target for screening pregnant patients with SARS-CoV-2 infection for potential COVID placentitis and risk stratification of stillbirth.

However, there are several limitations to this study. First, the Ct values reported here are near or below the commonly used limit of detection (Ct value 35) for the CDC SARS-CoV-2 qRT-PCR assay used here. To mitigate the risk of false positive detection, we performed at least 4 technical replicates of each tentative positive sample, sequence verified on-target amplification, and included matched controls, none of which amplified. Nevertheless, the risk of false positives in widespread deployment remains possible. Additionally, while viremia was present in all 3 stillbirth cases reported (2 from Shook et al., 1 from this series), viremia was not detected in some placentitis cases we tested, including one case with diffuse placental involvement on pathological examination. Thus, while the presence of viremia may portend an increased risk of placentitis-mediated stillbirth, the absence of detectable viremia may not exclude placentitis. Notably, 2 of the 6 cases of placentitis had >60 day latency between infection and maternal sample. Thus, transient viremia at the time of SARS-CoV-2 infection cannot be excluded. Further near-term research is needed to consider low-level viremia as a possible marker of COVID placentitis.

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Declaration of competing interest

The authors have no conflicts of interest to declare.

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