SARS-CoV-2 placental infection is associated with massive perivillous fibrin deposition at the maternal-fetal interface: a preliminary study"

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Abstract

We observed an increased frequency of massive perivillous fibrin deposition (MPFD) ,during the second COVID-19 pandemic wave dominated by the alpha variant of SARS-CoV-2. MPFD associated with 100% RT-PCR positivity for SARS-CoV-2 and detection by immunohistochemistry. The alpha variant was identified in all placentas with MPFD that could be sequenced.

Keywords: Placenta, SARS-CoV-2, fibrin deposition

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Introdcution

The prevalence of COVID-19 in East London during December 2020 and January 2021 reached 7022 per 100,000 with 86% thought to be alpha variant (6). Our population's annual birth rate is ~16,000 births (2018-2019). During 2020, 1190 pregnant women had a positive SARS-CoV-2 swab on admission to labour wards in Barts Health of which 293 were during the second pandemic wave in December 2020.

During the second pandemic wave, there was an increase in the number of placentas referred with massive perivillous fibrin deposition (MPFD) associated with histiocyte-rich intervillous inflammation on microscopy. MPFD is a rare lesion of uncertain aetiology reported in <1% of all pregnancies (1, 2). Perivillous fibrin deposition is postulated to be a reaction to trophoblast necrosis where exposure of chorionic villous stroma to maternal blood stimulates the maternal clotting cascade (3). A case report by Linehan et al terms this entity 'SARS-CoV-2 placentitis' (4) and Marton et al described it as an emerging complication of SARS-CoV-2 infection, along with chronic histiocytic intervillositis (CHI), that causes placental insufficiency leading to poor pregnancy outcomes including severe intra-uterine growth restriction (IUGR) and stillbirth (5).

We evaluated the presence of SARS-CoV-2 in placental tissue (and its lineage) and correlated with histopathological findings and clinical outcomes.

Each year, >1500 placentas are referred to our histopathology department. Indications for referral include stillbirth, late miscarriage, neonatal admission to intensive care, premature birth, severe IUGR, and maternal severe pre-eclampsia.

During December 2020 and January 2021, 224 placentas were referred for histopathological assessment. Amongst these, 11 (5%) cases of MPFD were identified. This compared to zero cases in 199 placentas referred during the same months in the previous year (December 2019 and January 2020), and one case out of 160 placentas (0.6%) referred two years earlier (December 2018 and January 2019). Until December, there had been no cases of MPFD out of 1140 placentas referred for examination in 2020.

Thirty-three placentas were included for investigation: 30 were from COVID-19 positive mothers as per clinical information in request form: 11 with MPFD, 19 with normal or other histopathology abnormalities of which 17 had a positive CNTS and 2 had a positive history of COVID-19 during pregnancy. Three COVID-19 negative controls were also included (Figure 1a, *Supplementary Material 1*).

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Histological examination of the 11 MPFD cases revealed large areas of perivillous fibrin deposition with associated trophoblast necrosis and patchy dense intervillous inflammation. In all 11 cases, IHC showed granular positivity within the cytoplasm of syncytiotrophoblast (ST) (Figure 1b and 1c). Electron microscopy (EM) performed in one case of MPFD confirmed the location of virions in the cytoplasm of ST (Figure 1d and 1e).

The other 19 placentas from COVID-19 positive mothers showed a variety of non-MPFD pathologies: acute chorioamnionitis, maternal vascular malperfusion (MVM) plus acute chorioamnionitis, delayed villous maturation with acute chorioamnionitis (ACA) in one; foetal vascular malperfusion (FVM), chronic villitis of unknown aetiology, villous oedema and microscopically increased fibrin without intervillous inflammation. Three of these 19 placentas were from women who tested SARS-CoV-2 positive and did not show any significant histological abnormality. IHC for SARS-CoV-2 spike protein was negative in all 19 cases. EM performed in one of these cases did not identify virions.

Of the 3 placentas from COVID-19 negative women one showed ACA, one showed MVM and one had features of both MVM and FVM. IHC for SARS-CoV-2 spike protein was negative in all.

All 11 placentas with MPFD were positive for SARS-CoV-2 RNA by RT-PCR (Figure 1a). Six of these were successfully sequenced and all were SARS-CoV-2 alpha variant positive. Mean cycle threshold (CT) values for MPFD placentas was 23.8 for E gene (ranges 16.9-35.6, SD 5.59) and 23.3 for N gene (range 18.4-34.4, SD 4.9) versus CT 34 (ranges 28.4-37.5, SD 3.17) and CT 34.15 gene (range 27.4-39.4, SD 3.24) for E and N genes respectively in non-MPFD, RT-PCR positive placentas. One of the placentas was tested on a different platform so it was excluded. Although placenta tissue was not standardized and our RT-PCR is not quantitative, CT values are included as an indication of possible differences in viral load in MPFD vs non-MPFD placentas. For the 6 placentas that failed

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sequencing, there were combined nose and throat swabs (CNTS) collected perinatally: 2 were positive for SARS-CoV-2 RNA but sequencing was unsuccessful.

Of the 19 non-MPFD placentas (including the normal placentas) from SARS-CoV-2 positive mothers, 14 were RT-PCR positive (73%), none were successfully sequenced. CNTS were collected peri-delivery for 18 patients of which 16 were RT-PCR positive. Seven of the 16 positive CNTS were successfully sequenced: 6 had the alpha variant and one was B.1.177. None of the 16 abnormal placentas were IHC positive for spike protein. Of the 3 normal placentas from SARS-CoV-2 positive mothers, 2 were RT-PCR positive for SARS-CoV-2 RNA, none successfully sequenced (Figure 1a).

In COVID-19 mothers, the difference between MPFD and non-MPFD placentas PCR positivity plus IHC positive confirmation was statistically significant (*p=0.0001*, Fisher exact 2-tailed test).

Amongst the 11 placentas with MPFD there were six live births and 5 stillbirths: seven placentas were associated with birthweight < 10th centile. Severity of COVID-19 (7) was not associated with MPFD findings. Thrombophillia screening was not performed for the most patients as not clinically indicated. Classically MPFD is associated with low birth weight and significant recurrence risk in subsequent pregnancies. The main aetiology is thought to be immunologic with increased risk in women with autoimmune diseases (8). An infectious aetiology has been reported, including coronaviruses (8, 9, 10).

Our findings have demonstrated the presence of SARS-CoV-2 in placentas from COVID -19 positive mothers in association with trophoblast necrosis, intervillous inflammation, and MPFD. MPFD could be the result of a sequence of immunologically driven pathological events with deposition of young fibrin accompanying the inflammation caused by the presence of SARS-CoV-2. Our series does not suggest a correlation between MPFD and

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increased transplacental infection. There was only one neonatal COVID 19 infection, in concordance with the infrequent report of vertical transmission of COVID-19 (11).

In the meta-analysis of Sharps et al, IHC identified ST as the main infected cells. Our findings are concordant with this. The SARS-CoV-2 spike protein antibody we used showed diffuse or patchy positivity in the cytoplasms of ST which are often the first cells showing hypoxic changes in placenta (12). Similar to pneumocytes in the lung, ST are interface cells in placenta and fibrin in MPFD is similar to the hyaline membranes described in the lung in post-mortem (PM) studies (11).

The high frequency of the alpha variant in successfully sequenced placentas reflects the high prevalence of this variant in the UK during the time of collection (PHE 2020) suggesting that many of the of the RT-PCR positive placentas were likely to have these variant. The significant difference of RT-PCR detection of SARS-CoV-2 RNA confirmed by IHC in placentas with MPFD versus any other histological diagnosis suggests a role of the virus in the development of this disease. The confirmation by IHC of all RT-PCR positive MFPD placentas suggests that active replication and expression of viral proteins may drive the inflammation causing MPFD.

As the amount of tissue used in RT-PCR is not standardised, comparing CT values is of limited utility. Nevertheless, the mean CT values in MPFD placentas, compared with non-MPFD and normal placentas from COVID-19 positive mothers were 10 CTs lower suggestive of more viral RNA being present in the MPFD placental tissue. When normal placentas and non-COVID-19 pregnancies were excluded, there as a significantly higher incidence of stillbirth amongst pregnancies with MPFD (Fischer exact test The Fischer two-tailed $p = 0.0265$) vs pregnancies with other non-MPFD abnormalities. This increase in stillbirth remained significant when all cases in the series were included *($p = 0.0274$).* This contrasts with reports of no increases in the rate of stillbirth in England during the first pandemic wave (13).

Limitations of our study include small case numbers and selection bias as we specifically selected all MPFD cases for molecular testing. IHC detecting other viral proteins like nucleoprotein are necessary to rule out false negative results (14).Due to the retrospective nature of this study, women have not been recalled for thrombophilia screening or additional testing.

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Our results suggest that SARS-CoV-can cause MPFD. During the alpha wave of the pandemic, we observed an increase in MPFD cases. We believe alpha may present a higher risk for MPFD. However, we have not been able to confirm this. Future studies should include SARS-CoV-2 negative patients as disease controls and placental tissue from patients infected with different variants of SARS-CoV-2 to allow direct comparison of MPFD with alpha versus other variants.

Disease progression in the placenta seems similar to other organs, particularly the lungs. As placenta is an easily retrievable organ, future mechanistic studies could allow better understanding of the pathophysiology of COVID-19 infection. Standardization of RT-PCR in placental tissue will also allow for better estimation of viral load.

The risk to pregnancies posed by emerging SARS-CoV-2 variants like Omicron must be explored further.

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Notes:

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Figure 1. Placenta tissue testing results. a) Flowchart showing testing of samples in this case seriesb) Histological appearance of MPFD; c and d) IHC staining with anti- SARS-CoV-2 spike protein antibodies, c: low power field showing multifocal patchy staining / ST, d: high power field showing the apical surface of a ST with numerous cytoplasmic cisternae containing virus nucleocapsids and intracytoplasmic granules reflecting viral spike protein presence; e and f) Ultrastructural features of SARS-CoV-2 by EM, e: high power field showing a single membrane-bound vacuole containing round or oval structures with internal electron dense granules, f: arrows indicate structures consistent with coronavirus nucleocapsids.

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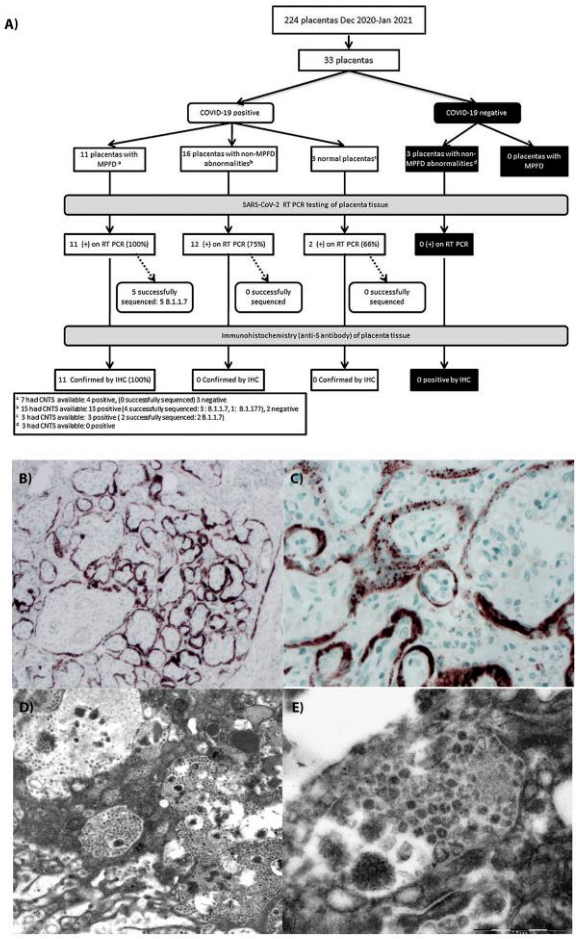
Figure 1



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