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ORIGINAL ARTICLE

A study on Histopathological changes and neonatal outcomes in the placenta of COVID-19 positive mothers: A prospective study

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ABSTRACT

CONTEXT: It has been estimated that are about 168 million lab confirmed COVID 19 cases worldwide as of 28th may, 2021. Due to the high prevalence of this disease, it is of utmost importance to study its effect on the vulnerable population of pregnant women.

AIM: Aim of the study are 1: the histopathological changes in placenta of COVID19 mothers. 2: To correlate the histopathological changes with the fetal outcome in COVID 19 positive mothers.

MATERIALS & METHOD: Twenty five placentas of Covid 19 positive mothers were received in formalin with proper clinical history including age of the mother, gestational age, mode of delivery, complications during pregnancy and during labor, baby weight and APGAR score of the baby. The specimen were allowed to fix in neutral buffered formalin for a period of 48 hrs. The placentas were then grossly and histopathologically examined.

RESULTS: Out of 25 placentas, some showed features of maternal vascular malperfusion (MVM), particularly villous infarct, villous agglutination and intervillous and perivillous fibrin deposits. Some showed fetal vascular malperfusion features like avascular villi, stem vessel obliteration were also present in a few of the cases. Out of the 25 pregnancies, 21 babies were delivered live with normal birth weight. There were 4 spontaneous abortions ranging from 14 - 22 weeks.

CONCLUSION: As the placenta acts as a bridge between the mother and the developing baby, any insult to the placenta in the form of maternal or fetal vascular malperfusion may result in an adverse perinatal outcome.

KEYWORDS | placenta, COVID-19, histoplathology, malperfusion

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INTRODUCTION

THE COVID-19 PANDEMIC IS STILL ACTIVE IN many parts of the world. It is caused by the novel coronavirus SARS-CoV-2, an enveloped positive-stranded RNA virus. It has a characteristic spike protein. The host receptor for SARS-CoV-2 cell entry is the angiotensin-converting enzyme 2 (ACE2) receptor.[1] The expression and distribution of ACE2 receptor has been reported in heart, lungs and kidneys, which exhibit tissue-specific activity patterns.[2-4] Some of the previous studies have shown the expression of ACE2 receptor on the syncytiotrophoblast and the cytotrophoblast. It has also been found on the endothelium and the vascular smooth muscle of both primary and secondary villi of the placenta.[5] The primary site of infection is the respiratory system, but recent research shows that it is also capable of infecting other organs including the placenta.

The placenta is a key organ that fulfills

several critical roles as the interface between the mother and fetus and plays a major part in preventing maternal-fetal transmission of pathogens.[6] It has been reported that the novel coronavirus may pose a greater risk in pregnant women than in non-pregnant women and increase the risk for requiring intensive care and mechanical ventilation during pregnancy.[7,8] It also increases complications like preterm births.[9] Poor respiratory function due to infection in mother is likely to impact maternal and fetal oxygenation during pregnancy.

Although the syncytiotrophoblast (ST) of the villi, which lines the surface of the placenta, acts as a barrier to placental infection, viral infections of the placenta do occur most commonly with DNA viruses and histologically show evidences of chronic inflammation. Our study aims at identifying the histopathological changes in the placentas of mothers who had Covid-19 infection, which is a RNA virus.

METHOD AND MATERIALS

This was a prospective study done to identify the histopathological changes in the placentas obtained from mothers who tested positive for COVID-19 by RT-PCR. All the placentas of COVID-19positive mothers received in the Department of Pathology of a tertiary care center in South India between November 2020 and April 2021 were included in this study. Total of 25 placentas were received and fixed in 10% neutral buffered formalin for 48 hours before dissection due to the infectious nature of the disease. All relevant clinical information was retrieved from the medical case record of the patients. The placenta were examined for macroscopic changes. Sections were submitted from membrane rolls, the umbilical cord, the maternal and fetal surfaces of the placenta and from any obvious lesions. The sections underwent routine processing, embedding, sectioning at 4µm and staining with hematoxylin and eosin (H&E). Histological examination was performed in each of the sections submitted.

RESULTS

A total of 25 placentas of RT-PCR confirmed COVID-19 positive mothers were examined. Out of which, 2 had clinical history of preeclampsia, 2 had hypothyroidism, 1 had oligohydramnios and the rest of the pregnant mothers had no specific clinical history.

21 of the pregnant mothers were in the third trimester at the time of diagnosis of COVID-19 and delivered in the third trimester.4 out of 25 mothers were diagnosed earlier in their pregnancy and had extremely preterm delivery(< 20 weeks of gestation) and underwent spontaneous expulsion in second trimester. Out of the 4, one had previous history of abortion. 15 of them were primi and the rest of them were of gravid 2, 3 and 4.

Majority of (18/25) mothers underwent lower segment cesarean section (LSCS) and only 3 had normal delivery. Out of 18 mothers,10 had previous LSCS. All babies born at term were with the birthweight ranging between 2.5 kg and 3.2 kg with one exception of 1.66 kg. In all these cases, the infants had 5 minutes Apgar score of 8 or 9, and were admitted in the well-baby nursery, and discharged home without apparent sequelae.

All the placental discs appeared grossly normal except for the presence of subtle pale areas in few placentas. All the cords contained three vessels with no knots or any other gross lesions. The cords showed either central or eccentric insertion. One placental disc showed thrombotic lesions in the vessels of the chorionic plate.

Histopathological examination showed varied features of maternal vascular malperfusion, fetal vascular malperfusion, acute and chronic inflammatory pathology and few other findings like perivillous fibrin deposits, villous edema, increased syncytial knots, dystrophic calcification etc.

As a feature of maternal vascular malperfusion, 11/25 cases had villous infarct of more than 30%, villous agglutination

CASE NO	AGE	GA	G	P	MODE OF DELIVERY	BIRTH WEIGHT	APGAR 1'	APGAR 5'	MATERNAL COMPLICATIONS
1	23	22 wks	2	1	Spontaneous expulsion	Dead	8/10	9/10	Hypothyroidism
2	33	38 wks	2	1	LSCS	2690	8/10	9/10	–
3	33	38wks +2d	2	1	LSCS	2630	8/10	9/10	–
4	32	39 wks	2	1	LSCS	2560	8/10	9/10	–
5	23	14 wks	1	1	Spontaneous expulsion	Dead	8/10	9/10	–
6	28	39 wks+ 5d	2	1	NVD	2930	8/10	9/10	–
7	30	38 wks 2d	3	1	LSCS	3220	8/10	9/10	Preeclampsia
8	27	40 wks	1	1	LSCS	3150	8/10	9/10	–
9	21	40 wks	1	1	LSCS	2490	8/10	9/10	Postdated, Oligo-hydraminos
10	25	39 wks +2d	2	1	LSCS	3162	8/10	9/10	–
11	28	38 w+ 4d	1	1	LSCS	1660	8/10	9/10	–
12	32	38 wks	1	1	LSCS	2970	8/10	9/10	–
13	32	38 wks	3	2	LSCS	3070	8/10	9/10	–
14	23	39 wks +6d	1	1	LSCS	3650	8/10	9/10	Eclampsia
15	23	40 wks	1	1	LSCS	3000	8/10	9/10	Hypothyroidism
16	28	39 wks+ 5d	2	1	NVD	2930	8/10	9/10	–
17	25	17wks+1d	1	1	Spontaneous expulsion	DEAD	8/10	9/10	–
18	25	39 wks +2d	2	1	LSCS	3200	8/10	9/10	–
19	24	37 wks+3d	4	0	LSCS	2700	8/10	9/10	–
20	34	37 wks+4d	3	1	LSCS	2780	8/10	9/10	–
21	25	38 wks+5d	2	1	LSCS	2600	8/10	9/10	–
22	29	37 wks+6d	1	1	NVD	2490	8/10	9/10	–
23	20	19 wks+3d	1	1	Spontaneous expulsion	DEAD	8/10	9/10	–
24	30	34 wks	3	1	LSCS	2620	8/10	9/10	Preeclampsia
25	24	38 wks	2	1	LSCS	3100	8/10	9/10	–

Table 1: Clinical and Neonatal details of covid 19 positive mothers

and perivillous fibrin deposition. Decidual arteriopathy in the form of fibrinoid necrosis, mural hypertrophy, decidual artery perivasculitis and calcification were seen in few of the cases. Retroplacental hematoma was seen in one case. (Fig. 1)

Out of 25 cases, features of fetal vascular malperfusion were seen in 12 cases, 3/25 cases had avascular villi, fetal vessel mural fibrin deposition.[4] cases had stem vessel obliteration, one case had chorionic plate thrombus, 4 cases had chorangiosis and one had chorangioma. (Fig. 2)

Maternal inflammatory response in the form of acute chorioamnionitis was diagnosed in 6 cases. 2 were of stage 3 and grade 3 and all others were stage 1 and stage 2. Chronic inflammatory pathology like chronic villitis and chronic chorionitis were seen in 2 of the cases. Peri villous fibrin deposits were seen in 11 cases,increased syncytial knots were seen in 9 cases, intervillous thrombus was seen in 3 cases and dystrophic calcification of more than 10% was seen in 10 cases. (Fig. 3)

DISCUSSION

The pathology of placentas of COVID-19 positive patients has not been specifically addressed so far. The cesarean section (CS) rate in our study for women with confirmed COVID-19 infection is (18/25) 72%. According to other studies, the same has been reported as ranging from 42.9% to as high as 91–92%.[10] As per Di Mascio in his systematic review, cesarean section was not needed for mild and moderate Covid-19 infections but the procedures were performed in maternal interest, due to concern of sudden maternal decompensation and other comorbidities like Preeclampsia, eclampsia and oligohydramnios as observed in our study.

Maternal vascular malperfusion can reduce or completely interrupt the uteroplacental circulation, resulting in placental infarcts which consist of collapsed maternal intervillous

spaces and necrotic villi, abruptio placentae, and ischemic lesions.[11] Peripheral infarcts on the maternal side of the placenta are common at term and, if small, usually not clinically significant. Perinatal morbidity is related with infarcts of >5 percent of the placental mass or greater than 3 cm in diameter.[12,13] If 20 percent or more of the placenta is affected, infarcts are considered clinically severe (e.g. associated with fetal growth restriction/still birth).[14]

In our observation, findings that correlate with maternal vascular malperfusion include, maternal floor infarct of more than 10% of parenchyma and villous agglutination were seen in (11/25) 44%. Only 3 of them had clinical history of preeclampsia and eclampsia. Features of decidual arteriopathy including mural hypertrophy and calcifications were seen in (3/25) 12% and (8/25) 32% respectively. Increased syncytial knots (>1 in 3 to 5 terminal villi and/or >10 nuclei per knot) were present in 40% with few cases of increased intervillous fibrin deposition. Maternal hypertensive disorders, including gestational hypertension and preeclampsia are the major risk factors for MVM,[15,16] which were observed in 8% in our study. The manuscript by Shanes *et al.*[17] describes an increased prevalence of MVM in their series of 16 placentas delivered to women with COVID-19.

Fetal vascular malperfusion (FVM) is an indicator of fetal well-being and outcome. The most striking histological abnormality in our study was the occurrence of extensive avascular fibrotic villi in 12% of placentas. Avascular villi are usually the results of fetal vascular damage. Obliterated or collapsed vessels could be identified in 20% of the stem villi and larger intermediate villi, but the smaller intermediate and terminal villi showed hyalinised stroma. Fetal vessel thrombus has been associated with maternal diabetes, 14 coagulation disorders 15 and pre-eclampsia.[16] In our study only 3 patients i.e., 12% had history of pre-eclampsia. Chorangiosis were seen in 20% of placenta. Only one case showed a feature of chorangioma which was a microscopic lesion. Most chorangiomas are incidental but benign findings, but as size increases, there is an increasing risk of adverse outcome due to high output heart failure.[18] Notably, a higher concentration of chorangioma is associated with reduced oxygen saturation.

Viral infections like TORCH [Toxoplasma, others, rubella, cytomegalovirus (CMV), herpes] are very common during pregnancy. Among those infections CMV is the leading cause of prenatal viral infections. In a study conducted by Jenna M Iwasenko *et al.* Fetal thrombotic vasculopathy was the only histopathological abnormality associated in 60% CMV-infected placentas.[19]

In another case control study , placental and fetal findings were interpreted in 62 fetal deaths in which CMV, Herpes and Parvoviruses were detected through PCR. Fetal hydrops and chronic villitis were evident in all those cases. [20] whereas in another study on Herpes simplex virus infected placentas the microscopic findings were remarkable for the absence of both inflammation and characteristic viral inclusions.[21]

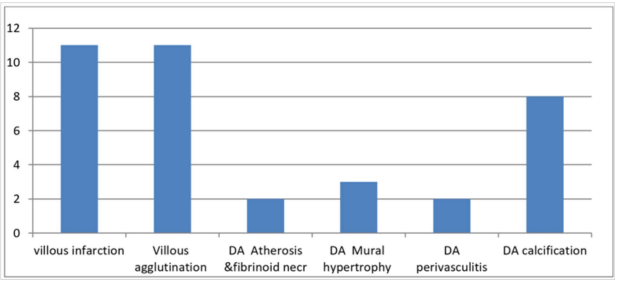
In a study on Zika virus, the placenta demonstrated prominently enlarged, hydropic chorionic villi with hyperplasia and focal proliferation of Hofbauer cells. No acute or chronic villitis, villous necrosis, remote necroinflammatory abnormalities, chorioamnionitis, funisitis, or hemorrhages were present.[22]

HIV-associated placental findings are well described which include acute chorioamnionitis, low placental weight, and maternal vascular malperfusion and lower rates of chronic villitis.[23]

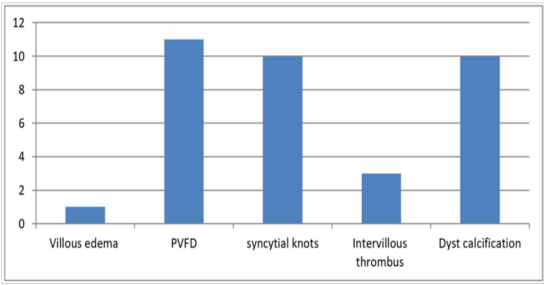
As covid-19 is also a viral infection, we expect inflammatory pathology in placentas of infected mothers. Acute inflammatory pathology in the form of acute chorioamnionitis of various stages and grades were present in 24% of placentas and chronic inflammatory pathologies, particularly chronic villitis or chronic intervillositis and deciduitis were present in only 8%. There are only case reports identifying

CASE NO	MVM	FVM	OTHERS
1	Villousinfarction, decidual artery mural, hypertrophy perivasculituis and calcification	Avascularvilli, stem vessel Obliteration Obliteration	Chorionic deciduitis, CIP
2	Villous infarction	–	Syncytial knots, intervillous thrombi, dystrophic calcification
3	-	–	–
4	Villous agglutination	–	Villous edema, PVFD, syncytial knots, dystrophic calcification
5	Villous agglutination, decidual artery calcification	Fetal vessel mural fibrin, Chorangiosis	Villous edema, PVFD, syncytial knots, dystrophi calcification
6	Villous agglutination, decidual artery Mural hypertrophy	Stem vessel obliteration	Maternal inflammatory response, villous edema, syncytial knots
7	Villous infarction, Villous agglutination, Decidual artery perivasculitis	Fetal vessel mural fibrin, chorionic plate infarct	PVFD
8	–	–	–
9	–	–	–
10	–	–	–
11	Villous infarction, decidual artery calcification, retroplacental hematoma	–	Maternal inflammatory response, syncytial knots
12	Villous infarction, villous agglutination, decidual artery calcification	Chorangiosis	PVFD, syncytial knots
13	Villous agglutination	–	Maternal inflammatory response, PVFD, syncytial knots
14	Villous agglutination, decidual artery Mural hypertrophy and calcification	Avascular villi, fetal vessel mural fibrin, stem vessel obliteration, chorangiosis hypercoiled umbilical chord	Maternal inflammatory response, PVFD, syncytial knots, dystrophic calcification
15	–	Chorangiosis	–
16	Villous agglutination	–	PVFD, dystrophic calcification
17	Villous infarction, villous agglutination, decidual artery atherosisand calcification,		PVFD
18	Villous infarction, decidual artery calcification	–	Intervillous thrombi, Dystrophic calcification
19	Villous infarction, decidual artery atherosis	stem vessel obliteration	Maternal inflammatory response, intervillous thrombi
20	–	–	–
21	Villous infarction, villous agglutination, decidual artery calcification	–	PVFD, syncytial knots, dystrophic calcification
22	Villous agglutination	Stem vessel obliteration, chorangiosis	CIP, PVFD, syncytial knots
23	Villous infarction	–	Maternal inflammatory response, chronic deciduitis
24	Villous infarction	–	Dystrophic calcification
25	–	–	–

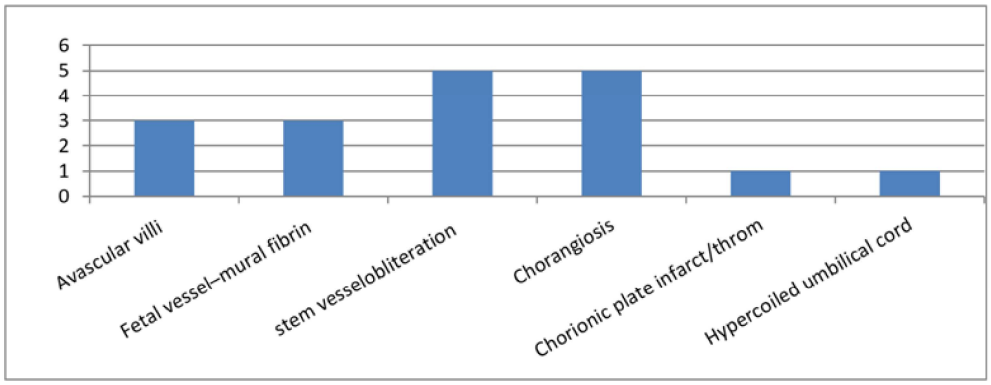
Table 2: Microscopic features in placentas of Covid-19 positive mothers.



Graph 1: Showing the distribution of various placental changes.



Graph 2: Showing the distribution of various maternal vascular malperfusion changes.



Graph 3: Showing the distribution of various maternal vascular malperfusion changes.

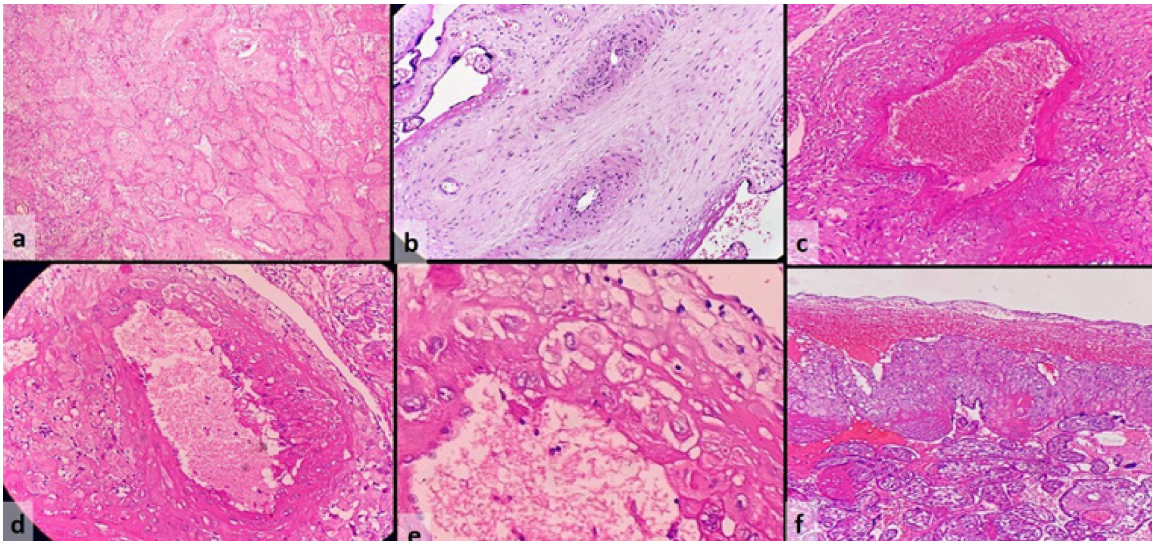


Figure 1: a) Massive villous infarct, H&E, x200, b) Mural hypertrophy, H&E, x200, c) Decidual arteriopathy, H&E, x200, d) Decidual atherosis, H&E, x200, e) Decidual atherosis H&E x200, f) Retroplacental Hemorrhage, H&E, x200

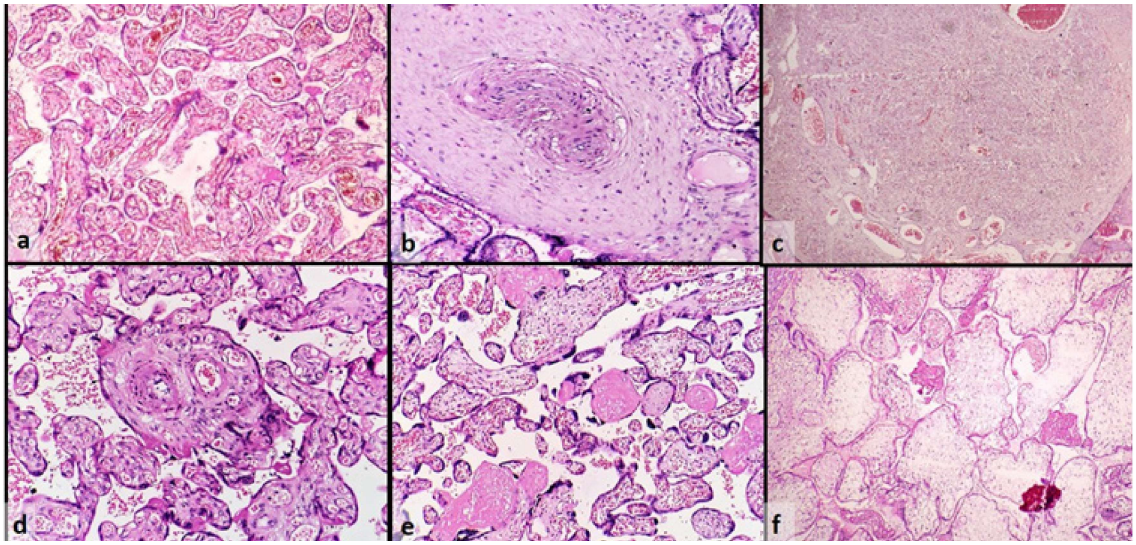


Figure 2: a) Massive villous infarct, H&E, x200, b) Mural hypertrophy, H&E, x200, c) Decidual arteriopathy, H&E, x200, d) Decidual atherosis, H&E, x200, e) Decidual atherosis H&E x200, f) Retroplacental Hemorrhage, H&E, x200

VIRUSES	VASCULOPATHY	CHRONIC VILLITIS	HYDROPIC VILLI	PVFD	ACUTE CHORIOAMNIONITIS	DECIDUITIS
CMV[19,20]	+	+	-	+	-	-
Herpes[20,21]	-	+	-	-	-	-
Parvovirus B19[20]	-	+	-	-	-	-
Zika virus[22]	-	-	+	-	-	-
HIV[23]	+	+	-	-	+	-
Covid 19	+	+	-	+	+	+

Graph 1: Microscopic features of placentasin various other viral infections

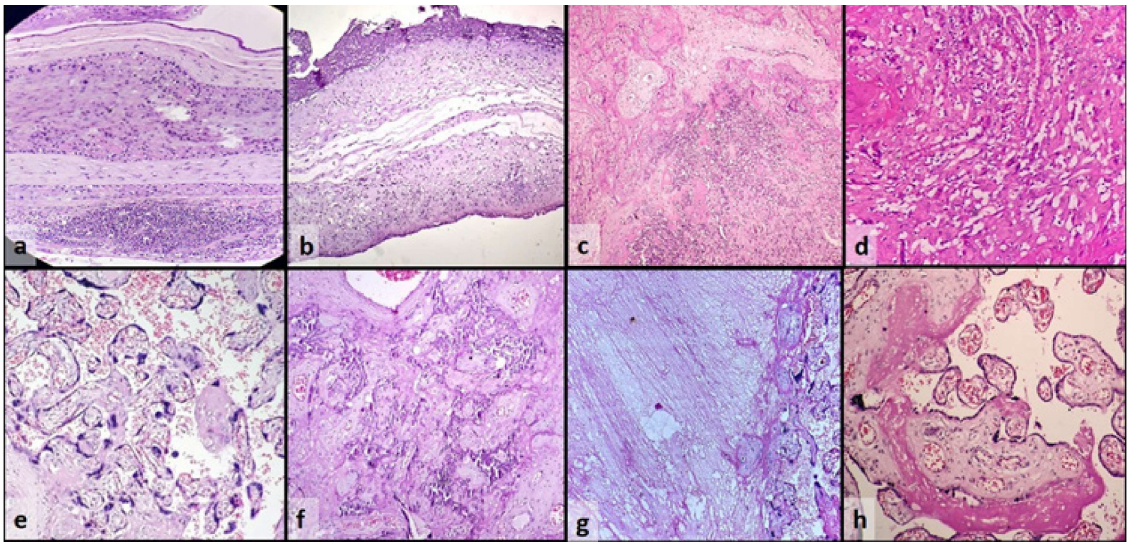


Figure 3: a) Massive villous infarct, H&E, x200, b) Mural hypertrophy, H&E, x200, c) Decidual arteriopathy, H&E, x200, d) Decidual atherosis, H&E, x200, e) Decidual atherosis H&E x200, f) Retroplacental Hemorrhage, H&E, x200

a chronic inflammatory process so far.[24,25,26] This is a brief report of initial findings seen in placentas of Covid-19 infected mothers.

In viral infections, the conventional light microscopic findings along with other ancillary techniques like immunohistochemical / histochemical, in situ hybridization as well as PCR are necessary to establish a diagnosis thereby the pathological findings can be correlated. This study is subject to some limitations due to relatively low number of patients observed. Further larger studies are necessary to determine the reproducibility and significance of these initial findings.

CONCLUSION

COVID-19 can potentially cause some abnormalities in the placenta of pregnant ladies and can lead to adverse pregnancy outcomes including reduced fetal growth, stillbirth, pre-eclampsia, and premature birth. We report various placental pathology observed in 25 patients with Covid-19 infection. No pathognomonic features are identified; however, there are increased rates of maternal vascular malperfusion features, suggesting an abnormal maternal circulation. These findings provide mechanistic insight into the observed epidemiologic associations between COVID-19 in pregnancy and adverse perinatal outcomes. Collectively, these findings suggest that increased antenatal surveillance for pregnant women diagnosed with SARS-CoV-2 could also be warranted. IJFMP

Conflict of Interest:
The author has made no acknowledgment in this article.
Conflict of Interest:
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