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Microscopic and Macroscopic Findings of Placental Pathology in Pregnant Women with SARS-CoV-2

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Abstract

Viral infections are usually transmitted through the mother's bloodstream, and the mother's immune system responds by sending white blood cells into the intervillous space, which can then spread into the amniotic fluid. The purpose of this research was to examine the histopathological alterations linked to SARS-CoV-2 infection in placentas. Histopathological changes of the placentas of 61 pregnant women with SARS-CoV-2 who delivered between 2021 and 2022 were examined at Besat Hospital in Sanandaj, Iran. The size of the placenta was normal in all cases. However, 3 samples (4.9%) showed the presence of hematoma, while 14 samples (23.0%) had microcalcifications. Thrombofibrin was observed in 27 samples (44.3%), and villous edema was observed in 22 samples (36.1%). Necrosis and ischemic infarction were present in 26 samples (42.6%), and inflammation was observed in 22 samples (36.1%). Hemorrhage was found in 27 samples (44.3%) and retroplacental hematoma was present in only 3 samples (4.9%). Trophoblastic proliferation was observed in 1 sample. Maternal levels were normal in 55 samples (90.2%) and fetal levels were normal in all 61 samples (100.0%). Some of the placentas that were analyzed showed histopathological changes, primarily vascular and inflammatory, which suggest that the pregnant individuals were infected with SARS-CoV-2 at term. These changes could be linked to impaired placental function, fetal growth restriction, preeclampsia, and prematurity. However, more prospective studies are needed to confirm the type, prevalence, and prognosis of each of these changes.

Keywords: SARS-CoV-2, placenta, pregnant women, histopathology.

Introduction

The SARS-CoV-2 pandemic has brought attention to the potential role of the placenta in protecting against infection by the SARS-CoV-2 virus. The placenta is a vital and complex organ that plays a critical

role in the development of the fetus and can impact health from birth through childhood. The placenta typically acts as a barrier, preventing the transmission of infectious agents from the mother to the fetus. This is due to the trophoblastic epithelial lining on the surface of chorionic villi and the presence of macrophages in both the villous stroma (known as Hofbauer cells) and the decidua. However, some infectious agents can still pass through the placenta, leading to fetal abnormalities or direct damage to the placenta. This can result in fetal growth restriction or demise [1,2]. For example, Zika virus infection can cause microcephaly, while cytomegalovirus can cause villitis [3].

Multiple studies have suggested that Angiotensin Converting Enzyme (ACE) inhibitors and angiotensin receptor blockers (ARBs), which increase the expression of ACE2 receptors, can facilitate the entry and spread of the virus in human cells. These receptors are present all over the body, but are especially abundant in the lungs and reproductive organs, including the placenta, uterus, and maternal-fetal interface during pregnancy. Additionally, fetal tissues such as the heart and liver exhibit high expression of ACE2 receptors [4-7]. ACE2 receptors could potentially aid in the transmission of SARS-CoV-2 from a mother to her developing fetus. However, multiple studies have not found any conclusive evidence of vertical transmission from a mother with SARS-CoV-2 to her baby [8-11]. On the other hand, some research has suggested that SARS-CoV-2 may be responsible for fetal death during the first and second trimesters of pregnancy. Unfortunately, in certain cases of miscarriage, no examination of the placenta or testing for the presence of SARS-CoV-2 in the placenta or fetus was performed [12]. In another study, histological examination of placentas revealed inflammation [13], diffuse perivillous fibrin, infarcts, as well as the presence of macrophages and T cells [14, 15]. Considering the importance of the SARS-CoV-2 virus and its effects on the placenta, it is necessary to collect complete information in this field to take the necessary measures faster in the repetition of similar pandemic cases. Our objective was to investigate placental pathology in pregnant women with SARS-CoV-2.

Methods

Type of study

This research is a descriptive, cross-sectional study. In cross-sectional studies, the relationship between exposure and outcome is investigated at a specific point in time, which can be either past or present. It is important to evaluate both time frames simultaneously. Since the time of occurrence of the desired outcome is unknown in cross-sectional studies, and we only observe it during the study, we can determine its prevalence.

Description of the surveyed population

The study included pregnant women during the 2021-2022 SARS-CoV-2 pandemic, regardless of symptoms. The study included patients who tested positive for SARS-CoV-2, excluding women who experienced stillbirth. The sample size of 61 patients was considered sufficient.

Assessments

As this was a retrospective study, the researchers assured that patient information would remain confidential. The Ethics Committee of the Kurdistan University of Medical Sciences approved this study with the code IR-MUK.REC.1401.231. The study allowed the scientific use of patient information while preserving their privacy.

In order to study with a light microscope, after fixing in formalin and preparing paraffin moulds, slices with a thickness of 5 microns were obtained using a microtome. The slices prepared from both groups were compared and examined histologically after H&E staining with light microscopy.

The pathologist evaluated the placenta's macroscopic features such as maternal and fetal surface, size, lobulation, membrane transparency, hematoma, and umbilical cord length. The microscopic features were

examined for trophoblastic cells, maternal and fetal villi, fibrinoid deposition, local inflammation, basal plate necrosis, fetal vessels in the chorionic plate, fibrosis, fetal art, vascular system, syncytial knot, chorangiosis, meconium color, and microcalcification. All demographic variables from patient files were extracted and analyzed.

Statistical Analysis

After collecting the data, we used SPSS v21 to calculate mean and standard deviation for quantitative variables, and frequency and percentage for qualitative variables.

Results

The study results indicated that the average age of the mothers included was 29.89+/-6.34 years, and the average gestational age was 38.00+/-2.46 weeks. The length of the umbilical cord was measured to be 28.00+/-9.75, and the duration of the SARS-CoV-2 was found to be 29.11+/-9.72, as shown in **Table 1**.

Table 1. Characteristics of pregnant women with COVID-19.

	N	mean	SEM
Age (y)	61	29.89	6.34
Gestational age (w)	61	38.00	2.46
Days of COVID-19 infection	61	29.11	9.72
Placental length (cm)	61	28.00	9.75

According to the study, 95.1 percent of the participants were housewives, while only 4.9 percent were employees. 14.8 percent of the participants were illiterate, and 54.1 percent had a diploma. Additionally, 6.24 percent of the participants had a bachelor's degree, and 6.6 percent had completed a postgraduate program. The study also included information on the number of pregnancies for each patient, which is shown in **Table 2**.

Table 2. Demographic information of pregnant women with COVID-19.

Information of patient		n	percent
Job	Housewife	58	95.1
	Employee	3	4.9
Education	illiterate	9	14.8
	diploma	33	54.1
	cycle	15	24.6
	Bachelor's degree	4	6.6
Number of pregnancies	1	25	41.0
	2	17	27.9
	3	13	21.3
	4	4	6.6
	6	2	3.3

In this study, all samples showed normal placenta size. Hematomas were observed in only 3 cases (4.9%) of the samples, while normal maternal levels were found in 55 cases (90.2%) of the samples, and normal fetal levels were observed in all 61 cases (100%). Microcalcification was found in 14 cases (23%), thrombofibrin in 27 cases (44.3%), villous edema in 22 cases (36.1%), necrosis and ischemic infarction in 26 cases (42.6%), and inflammation in 22 cases (36.1%). Hemorrhage was observed in 27 cases (44.3%) of the samples, while retroplacental hematoma was observed in only 3 cases (4.9%). Additionally,

trophoblastic proliferation was only observed in 1 case of the samples. Syncytial konting was found in 23 samples (37.7%) (**Table 3** and **Figure 1**).

Table 3. Placental characteristics of pregnant women with COVID-19.

Pathological findings		n	percent
Length (cm)	Normal	61	100.00
	Abnormal	0	0
Hematoma	Positive	3	4.9
	Negative	58	95.1
Maternal surface staining	Normal	55	90.2
	Dark red	3	4.9
Fetal surface staining	Violet	61	100.00
	Greenish-brown	0	0
Microcalcification	Positive	14	23.00
	Negative	47	77.00
Thrombi–Fibrin	Positive	27	44.3
	Negative	34	55.7
Villitis	Positive	22	36.1
	Negative	39	63.9
Presence of necrosis and placental infarction	Positive	26	42.6
	Negative	35	57.4
Inflammation	Positive	22	36.1
	Negative	39	63.9
Hemorrhages	Positive	27	44.3
	Negative	34	55.7
Retroplacental hematoma	Positive	3	4.9
	Negative	58	95.1
Trophoblastic proliferation	Positive	1	1.6
	Negative	60	98.4
Syncytial knotting	Positive	23	37.7
	Negative	38	62.3

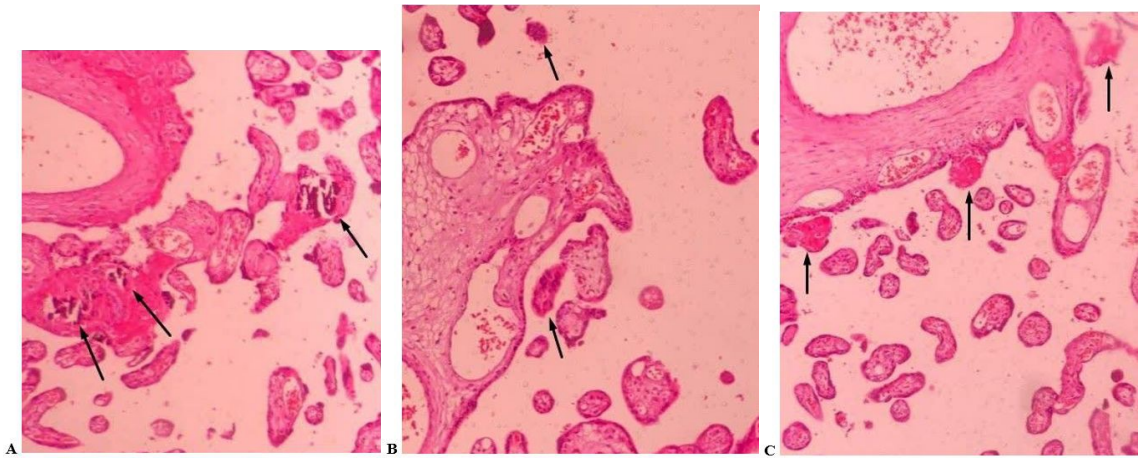

Figure 1. Key histopathological findings in the placental tissue of pregnant women with SARS-CoV-2 infection (**A**) Microcalcification (**B**) Syncytial knotting and (**C**) Thrombi-Fibrin.

Discussion

Pregnant and postpartum women faced higher mortality rates and were admitted less frequently to intensive care units than the general population during the SARS-CoV-2 pandemic. To evaluate the impact of SARS-CoV-2 on placental histology, a study was conducted. Previous research showed that women infected with SARS-CoV-2 experienced maternal complications such as oligohydramnios, polyhydramnios, and premature rupture of membranes, as well as placental abnormalities like placental abruption, placenta previa, and abnormal cord insertion [16,17]. However, detailed descriptions of placental abnormalities are limited. Therefore, this study aimed to provide a comprehensive account of the main histopathological findings in the placentas of SARS-CoV-2-infected women.

SARS-CoV-2, like other viruses, has been found to invade placentas in pregnant women who are infected [18]. Although some macroscopic placental abnormalities and inflammatory lesions have been associated with SARS-CoV-2 infection, there is no conclusive evidence of a typical SARS-CoV-2 -specific pattern of placental pathology [15,19-21].

In this study, we conducted both macroscopic and microscopic examinations of the placenta samples collected. The results showed that the size of the placenta was normal in all cases. However, 3 samples (4.9%) showed the presence of hematoma, while 14 samples (23.0%) had microcalcifications. Thrombofibrin was observed in 27 samples (44.3%), and villous edema was observed in 22 samples (36.1%). Necrosis and ischemic infarction were present in 26 samples (42.6%), and inflammation was observed in 22 samples (36.1%). Hemorrhage was found in 27 samples (44.3%) and retroplacental hematoma was present in only 3 samples (4.9%). Trophoblastic proliferation was observed in 1 sample. Maternal levels were normal in 55 samples (90.2%) and fetal levels were normal in all 61 samples (100.0%). Syncytial knoting was found in 23 samples (37.7%).

Some cases of maternal SARS-CoV-2 infection have been linked to changes in the placenta. These changes include atheroma in decidua vessels, poor blood supply, and placental vasculopathy, foci of placental infarction, chorioangiomas, and inflammatory infiltrates with edema in placental villi [22]. The cells of the female reproductive system and the fetus-placental unit, including syncytiotrophoblasts, cytotrophoblasts, and endothelial cells, express ACE2 and TMPRSS2, the primary mediators of SARS-CoV-2 entry [23]. This widespread expression of ACE2 and TMPRSS2 in the placenta can make it more susceptible to the novel coronavirus during maternal infection, leading to an inflammatory response with vasoconstrictive, proliferative, and angiogenic effects. These phenomena can endanger the growth of the placenta and lead to adverse consequences during pregnancy, such as preeclampsia, premature delivery, and an increased incidence of stillbirth [9,24]. In addition, poor maternal vascular perfusion may be associated with more significant risks of impaired placental function and fetal growth restriction [25]. It is crucial to recognize the placenta as a vital organ for mother-fetal communication, the primary barrier against pathogens between mother and fetus, and responsible for maintaining and balancing endocrine-immunological factors for normal fetal development [11].'

Shanz et al's 2020 study on pregnant women with SARS-CoV-2 found that placentas from the third trimester showed a greater incidence of abnormal or damaged maternal vessels, intervillous thrombi, and other features of MVM compared to the control group. However, there was no significant increase in inflammation [26]. In our study, 36.1% of the samples exhibited villous edema, while inflammation was observed in 22 cases (36.1%) of the samples. Gao et al. (2021) conducted a study on eight placentas from third-trimester pregnant women to detect SARS-CoV-2 RNA. All patients were treated and no cases of vertical transmission were found. The study revealed that all cases had maternal vascular malperfusion (MVM) and some other issues [27]. In our study, all 61 cases had normal fetal levels, while microcalcification was observed in 23% and thrombofibrin was found in 44.3%. Tasca et al. (2021) conducted a study on 64 pregnant women with SARS-CoV-2. The study found no significant difference in the placental morphology between healthy and infected mothers. The use of antiviral drugs, chloroquine, LMWH, or antibiotics did not improve the fetal-to-placental weight ratio. However, treated females showed a greater delay in villous maturity, although not significantly so [28].

Pathologists indicate that the presence of calcification in the mature placenta, both at microscopic and macroscopic levels, is a normal and simple degenerative process that is not typically linked to any maternal or fetal diseases [29]. Fibrin deposition in the placenta can be caused by thrombin and circulating dermatan sulfate proteoglycan. Excessive fibrin can lead to growth restriction, preterm labor, and other complications [30]. Villitis of unknown etiology (VUE), or chronic villitis, is a placental injury. VUE is an inflammatory disease involving the chorionic villi (placental villi). VUE is a recurrent condition and can be associated with intrauterine growth restriction (IUGR) [31].

Conclusion

The analysis of placentas through histopathology indicates that there may be a connection between acute infections caused by the COVID-19. The results are consistent with similar studies conducted on different populations, highlighting the potential of SARS-CoV-2 to trigger histological modifications. Therefore, further comprehensive studies in this field are recommended.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author contribution

All authors contributed equally to this article.

Ethical approval

This study was conducted based on the ethical protocol of the institution where the research was conducted. Informed consent was obtained from the patient and all patient information is protected.

Declaration of competing interest

There are no conflicts of interest.

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