

Yasminur Gabdulhakovna Turdybekova*, Irina L. Kopobayeva, Yevgeniy K. Kamyshanskiy and Anar A. Turmukhambetova

Comparative clinical and placental pathologic characteristics in pregnancies with and without SARS-CoV-2 infection

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Abstract

Objectives: To compare the clinical and morphological characteristics of the “mother-placenta-fetus” system in high risk pregnant women of three groups: no SARS-CoV-2 infection, mild SARS-CoV-2 infection, and severe SARS-CoV-2 infection.

Methods: A case-control study was performed for all deliveries, at 28 weeks’ gestation or greater, who had standard indications for placental pathologic examination. Three groups were formed: (1) control group (no SARS-CoV-2 infection), (2) mild SARS-CoV-2 infection, (3) severe SARS-CoV-2 infection. High-risk pregnancies were registered in all cases in the study groups. The examination of the placenta and the selection of fragments of placental tissue were carried out in accordance with the consensus recommendations of the Amsterdam Placental Workshop Group. The sections were subjected to standard processing and stained with hematoxylin and eosin according to the standard protocol. All cases were reviewed by two pathologists, which did not know any information on pregnancy outcome and clinical data. Statistical analysis was performed using SPSS, $p<0.05$ was considered statistically significant.

Results: Women with severe SARS-CoV-2 infection had an increased rate of multimorbidity including diabetes, chronic hypertension and obesity ($p<0.01$) compared with the other groups. Placentas at severe COVID-19 course were damaged by both chronic and acute injuries, in comparison to the mild and control groups ($p<0.001$). Also an important finding in severe COVID-19 was diffuse necrosis of the villous trophoblast – homogenization, diffuse circular eosinophilic masses surrounding the chorionic villi.

Conclusions: Women with multimorbidity are an “at-risk” subgroup for severe SARS-CoV-2 infection and greater likelihood of both placental damage and perinatal hypoxic-ischemic events. These results suggest that patient education, SARS-CoV-2 disease monitoring and preventive measures would be of benefit to this group.

Keywords: COVID-19 (SARS-CoV-2); high-risk pregnancy; placenta

Introduction

Since the start of the global COVID-19 pandemic, much attention has been paid to how SARS-CoV-2 affects pregnancy. Accumulated evidence suggests that pregnant women may be at increased risk of more severe COVID-19, and there has been an increase in maternal mortality worldwide [1, 2]. Many placental findings are associated with both symptomatic and asymptomatic COVID-19. They most often include nonspecific signs of maternal or fetal vascular malperfusion, villitis, or intervillitis. Although all of these have previously been associated with fetal morbidity, none of the lesions are specific to SARS-CoV-2 infection.

SARS-CoV-2 placental involvement has been documented in numerous studies [3–7]. In order to highlight the characteristic histomorphological features of SARS-CoV-2 placental infection, a classification scheme and criteria for placental lesions were developed [8]. However, the specificity of lesions and their relationship to perinatal and clinical features remain unknown.

The aim of the work was a comparative clinical and morphological characteristics of the “mother – placenta – fetus” system from high-risk pregnancy with mild and severe SARS-CoV-2 infection and without.

Materials and methods

Tissue samples and study cohort selection

The study was conducted by the case-control method in the Pathology department of NC JSC “Karaganda Medical University” from March 1, 2020 to November 1, 2021.

*Corresponding author: Yasminur Gabdulhakovna Turdybekova, Medical University of Karaganda, Karaganda, Kazakhstan, E-mail: Turdybekova@qmu.kz. <https://orcid.org/0000-0001-8742-6300>
Irina L. Kopobayeva, Yevgeniy K. Kamyshanskiy and Anar A. Turmukhambetova, Medical University of Karaganda, Karaganda, Kazakhstan. <https://orcid.org/0000-0003-1149-7879> (I.L. Kopobayeva). <https://orcid.org/0000-0001-8975-3612> (Y.K. Kamyshanskiy)

The objects of the study were all pregnant women with a gestation period of more than 28 weeks who gave birth in medical organizations of Karaganda, whose placentas were taken to the Pathology department for examination.

Placentas were sent for histological examination based on the following indications: (1) maternal problems, including diabetes, hypertensive disorders, thyroid disease, kidney disease, asthma, autoimmune diseases, coagulopathy and COVID-19 infection of a pregnant person; (2) pregnancy complications, including gestational diabetes, gestational hypertension, and premature birth; (3) fetal/neonatal problems, including stillbirth, neonatal death, cord blood pH <7.0, 5 min Apgar score <6, respiratory support >10 min, severe neonatal anemia (hematocrit <35 %), seizures, infection or sepsis; (4) placental macroscopic abnormalities including infarction, vascular thrombosis, retroplacental hematoma, abnormal coloration, turbidity, unpleasant odor, placenta mass, and umbilical cord lesions including thrombosis, twist, true knot, and absence of Wharton's jelly [9].

The positive result of SARS-CoV-2 infection was based on: (1) the detection of SARS-CoV-2 RNA in a nasopharyngeal swab by PCR test at gestational age of 28 weeks or more (upon admission to labor/hospitalization for other indications/outpatient) and the manifestation of COVID-19 symptoms; (2) detection of IgG antibodies to SARS-CoV-2 virus in patients with a history of COVID-19 symptoms. Cases of multiple pregnancies, established fetal abnormalities and acute inflammatory placental lesions (acute chorioamnionitis and acute chorionitis, with or without an inflammatory response in fetal chorionic and umbilical vessels) were excluded from the study.

We formed three separate groups to study the clinical and placental characteristics of pregnant women with SARS-CoV-2 infection: (1) control group, (2) mild SARS-CoV-19 course, (3) severe SARS-CoV-19 course.

The group with mild SARS-CoV-19 course consisted of 56 women with a clinically mild course of SARS-CoV-2.

The group with severe SARS-CoV-19 course consisted of 39 women with a clinically severe course of SARS-CoV-2.

The severity of SARS-CoV-19 was determined as follows:

Mild: one or more symptoms, including fever, cough, headache, myalgia, nausea, vomiting, diarrhea, anosmia, ageusia, but without shortness of breath and X-ray/computed tomography (CT) of changes in the lungs.

Severe: one or more symptoms: dyspnoea on light exertion, according to CT data, the signs of bilateral viral lung damage with 50–75 % lung damage (CT-3) or >75 % lung damage (CT-4).

The control group consisted of 114 arbitrarily selected women with a negative PCR result of nasopharynx smear for SARS-CoV-2 and no symptoms of an infectious disease during pregnancy, who gave birth during the study period, with a complete medical record for the current pregnancy, whose placentas, were sent for histological examination.

The received data was depersonalized, as each subject was encoded accordingly.

Clinical data collection

Clinical data were obtained from the medical data of the mother and child in a comprehensive health information system.

Obstetric data included parity and gravidity, gestational age at infection and at delivery, mode of delivery, and the presence or absence of any pregnancy-related abnormalities (e.g., maternal origin: hypertension, thyroid disease or gestational diabetes mellitus; fetal/neonatal origin: fetal growth restriction (FGR) and macrosomia).

Neonatal data were also collected: neonatal sex, low Apgar score, cord blood acidosis, or the need for respiratory support during the first

few minutes of postnatal life in the neonatal intensive care unit, neonatal death.

All documented clinical maternal data and pregnancy characteristics of the studied placentas are presented in Table 1.

Histological examination

The examination of the placenta and the selection of fragments of placental tissue were carried out in accordance with the consensus recommendations of the Amsterdam Placental Workshop Group [10]. Placentas of pregnant women who gave birth in medical organizations of Karaganda were sent for the study. Placentas were sent for examination immediately after delivery. Placental tissue was fixed in 10 % formalin for 24–48 h and then processed according to standard biosecurity measures. Placentas were photographed from the maternal and fetal surfaces, measured, weighed without amniotic membranes, and the cut surfaces were examined. Fragments taken for histological examination included 2 rolls of amniotic membranes, 2 umbilical cord fragments, 2 full-height sections of the placenta, and representative samples of any lesions present. The sections were subjected to standard processing and stained with hematoxylin and eosin according to the standard protocol. All cases were reviewed by two pathologists, which did not know any information on pregnancy outcome and clinical data.

Definitions

The diagnosis of type 1 diabetes mellitus (DM1) and type 2 diabetes mellitus (DM2) was made on the basis of HbA_{1c} data and a glucose tolerance test for plasma glucose [11].

Chronic arterial hypertension is systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg at two measurements when measuring up to 20 weeks of pregnancy [12].

Obesity was diagnosed on the base of pre-pregnancy BMI data [13].

All diseases were in remission at the time of the study.

The diagnosis of GDM was established on the base of data from a glucose tolerance test for plasma glucose levels [11]. Preeclampsia was defined as a blood pressure level ≥140/90 mmHg after 20 weeks of pregnancy with proteinuria higher than 0.3 g per day or with severe symptoms in a woman with previously normal blood pressure [14].

Multimorbidity: Multimorbidity was defined in our study as the co-existence of two or more chronic conditions, at least one of which was represented by DM1, DM2, CAH or obesity.

Recurrent pregnancy loss was defined as the loss of two or more pregnancies up to 24 weeks of pregnancy, including pregnancy loss both after spontaneous conception and after assisted reproductive technologies [15].

The diagnosis of fetal growth restriction (FGR) was established at an estimated fetal weight below the 10th percentile for gestational age [16]. Macrosomia is a fetus whose weight by the end of the intrauterine period is 4,000 g or more [17].

Perinatal hypoxic-ischemic event: A perinatal hypoxic-ischemic event was defined in our study as any clinical sign of developing fetal/newborn hypoxia/perinatal hypoxic-ischemic event, such as abnormal ultrasound dopplerography, meconium-stained amniotic fluid, low Apgar score, cord blood acidosis or the need for respiratory support during the first few minutes of postnatal life [18, 19].

Table 1: Clinical characteristics of groups with high-risk pregnancies.

Parameter	Control SARS-CoV-19 negative (n=114)	Mild degree SARS-CoV-19 positive (n=56)	Severe degree SARS-CoV-19 positive (n=39)	p-Value ^a	p-Value ^b	p-Value ^c
Gravidity, median (IQR)	2 (2)	2 (1)	2 (2)	0.595	0.887	0.779
Parity, median (IQR)	1 (1)	1 (1)	2 (2)	0.318	0.517	0.230
Gestational age, weeks (mean ± SD)	36.9 ± 2.6	36.2 ± 2.9	36.4 ± 1.9	0.102	0.026	0.840
Gestational age at the moment of infection diagnosis, weeks (mean ± SD)	—	33.0 ± 2.2	33.5 ± 1.8	—	—	0.161
Maternal age, years (mean ± SD)	28.3 ± 6.1	32.7 ± 4.8	33.5 ± 5.1	0.833	<0.001	<0.001
Race, n (%)						
Asian	79 (69.3)	35 (62.5)	28 (71.8)	0.376	0.770	0.346
White	35 (30.7)	21 (37.5)	11 (28.2)			
Other	—	—	—			
Drugs, n (%)						
Cigarettes	8 (7.0)	5 (8.9)	1 (2.5)	0.660	0.308	0.210
Alcohol	—	—	—			
Other	—	—	—			
Hospitalizations during pregnancy, n (%)						
Yes	35 (30.7)	41 (73.2)	24 (61.5)	<0.001	<0.001	0.229
No	79 (69.3)	15 (26.8)	15 (38.5)			
Unknown	—	—	—			
Habitual miscarriage, n (%)	12 (10.5)	6 (10.7)	2 (5.1)	0.820	0.492	0.556
Pathology according to cervical smear examination, n (%)	7 (6.1)	3 (5.4)	1 (2.6)	0.887	0.654	0.883
Positive tests for STIs, n (%)	3 (2.6)	1 (1.8)	1 (2.6)	0.845	0.577	0.641
Group B streptococcal infections, n (%)	4 (3.5)	5 (8.9)	2 (5.1)	0.264	0.978	0.766
Gynecological operations, n (%)	13 (11.4)	8 (14.3)	5 (12.8)	0.592	0.813	0.839
Prior cesarean delivery, n (%)	7 (6.1)	6 (10.7)	4 (10.2)	0.292	0.391	0.943
Complicated previous pregnancy, n (%)	8 (7.0)	7 (12.5)	5 (12.8)	0.370	0.430	0.789
Mode of delivery, n (%)						
Vaginal delivery	70 (61.4)	31 (55.4)	1 (2.6)	0.451	<0.001	<0.001
Scheduled cesarean delivery	23 (20.2)	13 (23.2)	7 (17.9)	0.649	0.763	0.422
Emergency cesarean delivery	21 (18.4)	12 (21.4)	31 (79.5)	0.642	<0.001	<0.001
IVF-induced pregnancy, n (%)	—	—	1 (2.6)	—	—	—
Maternal death, n (%)	1 (0.9)	—	7 (17.9)	0.384	<0.001	<0.001
Baby's sex, n (%)						
Female	51 (44.7)	26 (46.4)	17 (43.6)	0.836	0.901	0.785
Male	63 (55.3)	30 (53.6)	22 (56.4)			

SD, standard deviation; IQR, interquartile range; BMI, body mass index; STIs, sexually transmitted infections; IVF, in vitro fertilization. ^aComparison of the control group (SARS-CoV-19 Negative) and the group with mild COVID-19 (SARS-CoV-19 Positive). ^bComparison of the control group (SARS-CoV-19 Negative) and the group with severe COVID-19 course. ^cComparison of the group with mild COVID-19 course and the group with severe COVID-19 course. Bold values represent p<0.05 was considered statistically significant.

Neonatal infection: Neonatal infection is defined as a clinical diagnosis, established in the neonatal intensive care unit. Antenatal death is the intrauterine death of a structurally normal fetus. Neonatal death is a death among live births during the first 28 days of life. Placental injury patterns were defined according to the Amsterdam criteria [10].

Maternal vascular malperfusion (MVM): Maternal vascular malperfusion (MVM) is a complex of histopathological signs of damage to the villi and/or decidua vasculature of the mother in the pathology of maternal perfusion of the placental bed: central or peripheral infarcts of the placental villi with an area >10 %, acute atherosclerosis of the arteries of

the basal plate and/or decidual arteries, hypertrophy of the muscular layer of the arteries of the basal plate, thickening of the muscular wall of the arterioles in the amniotic membranes [20–22].

Fetal vascular malperfusion (FVM): Fetal vascular malperfusion (FVM) is the complex of signs, indicating reduced or no perfusion of the fetal villous parenchyma: two or more medium or large foci of avascular villi/villi with stromal vascular karyorrhexis or three or more foci of small avascular villi/villi with stromal vascular karyorrhexis, thrombi in the lumen of large fetal vessels and/or stem villi with vascular obliteration [23–26].

Chronic inflammatory damage to the placenta: Chronic inflammatory damage to the placenta is a lesion of the chorionic villi, characterized by the accumulation of small lymphocytes with a stromal reaction in the terminal chorionic villi and an increased number of fetal macrophages (Hofbauer cells) [27, 28].

Massive deposits of intravillous fibrinoid: Massive deposits of intravillous fibrinoid are fibrinoid deposits in the intervillous area, enveloping the chorionic villi [29] with obliteration of the intervillous area.

Additionally, necrosis of the villous trophoblast was assessed – necrotic damage to the syncytiotrophoblast of the chorionic villi (determined by the presence of a bright pink coagulation necrosis band at the border of the chorionic villus).

The degree of patterns distribution was assessed at three levels: (1) diffuse, involving >30 % of the parenchyma of the area of the histological section; (2) focal, involving <30 % of the parenchyma of the area of the histological section; (3) no damage.

Ethical approval

The study was approved by the Ethics Committee of the Medicine School of NC JSC “Karaganda Medical University” (Approval link: 18/04/2020). Written informed consent was obtained from all patients before being included in the study.

Statistical analysis

Data are presented as mean \pm SD for scale continuous variables and frequency \pm percent for categorical variables. Differences between groups were calculated using the Mann–Whitney U-test, χ^2 test with Yates continuity correction, and Fisher’s exact test; $p<0.05$ was considered statistically significant. Statistical analysis was performed using SPSS (v.25.00, IBM Statistics, Armonk/NY, USA).

Results

Table 1 presents the main characteristics of the study groups. The three groups were comparable in all assessed parameters. Women in the study groups did not differ in the number of pregnancies and births, gestational age and race. However, women in the severe SARS-CoV-2 group were older, more obese, more likely to undergo antenatal hospitalization and emergency caesarean section.

Clinical and morphological characteristics of the maternal, fetal and placental compartments of the “mother – placenta – fetus” system of the studied groups are presented in Figure 1.

Comparative characteristics of mothers and pregnancy course

Comparative characteristics of mothers and pregnancy course are shown in Table 2.

Severe degree SARS-CoV-19

More than one co-morbid condition was seen in 82.1 % of women with severe SARS-Co-V-2. The rates of co-morbid conditions were high, ranging from 20.5 % for type 1 diabetes to 41.0 % for obesity. Diseases associated with pregnancy (GDM and preeclampsia) were registered in 25.6 and 17.9 % of cases, respectively.

Mild degree SARS-CoV-19

Multimorbidity was detected in 37.5 % of women with a mild course of SARS-Co-V-2, ranging from 10.7 % for type 1 diabetes to 23.2 % for obesity. Diseases associated with pregnancy (GDM and preeclampsia) were observed in 17.9 and 16.1 % of cases, respectively.

Control SARS-CoV-19 negative

The control group had multimorbidity in 29.8 % of cases (34 women) and the rates of morbid conditions ranged from 11.4 % for type 1 diabetes to 25.4 % for obesity. Diseases associated with pregnancy (GDM and preeclampsia) were observed in 15.8 and 6.1 % of cases, respectively.

Comparative characteristics of fetuses/newborns

Comparative characteristics of fetuses/newborns are shown in Table 2.

Severe degree SARS-CoV-19: Perinatal hypoxic-ischemic event occurred in 38.5 % of cases in the group with severe COVID-19 course. The number of children with diagnosed FGR and macrosomia was 23.2 and 19.6 %, respectively. Neonatal infection was diagnosed in 15.4 %. The cases of antenatal death and neonatal death were 10.3 and 5.3 %.

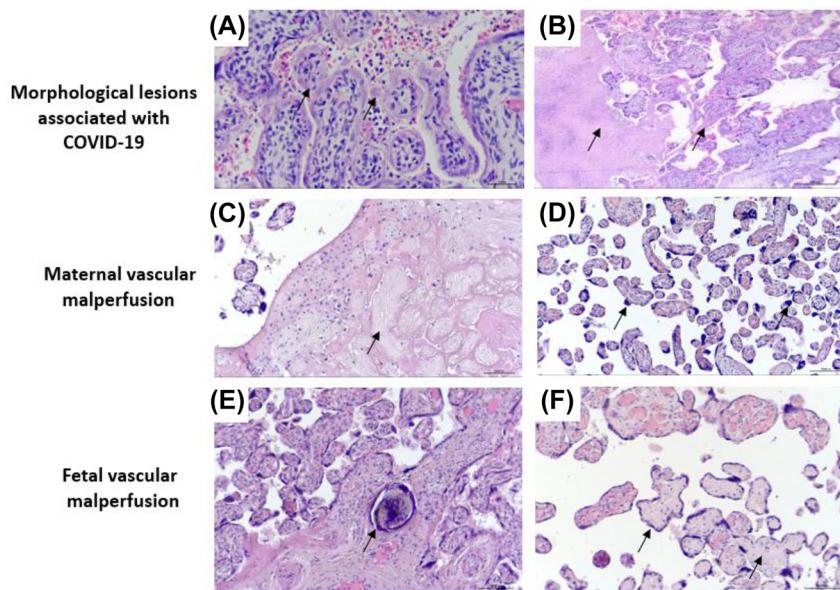


Figure 1: Histomorphological placental injury patterns in the submitted groups. Morphological placental lesions associated with COVID-19: (A) necrosis of syncytiotrophoblast (band of coagulative necrosis) of bright pink color at the border of chorionic villi (arrow) with perivillous fibrin deposition. An intervillous area is filled with mononuclear inflammatory cells and fibrin (histiocytic intervillousitis), H&E, magnification: $\times 200$; (B) massive perivillous fibrin deposition (arrow) and inflammatory lymphohistiocytic infiltrate, H&E, magnification: $\times 100$. Non-specific and heterogenic morphological placental lesions in the observed groups: (C) sharply delineated chorionic villus infarction, intervillous space collapse, intervillous fibrin, eosinophilia of the villous trophoblast and chorionic villous stroma (arrow), H&E, magnification: $\times 100$; (D) acceleration (accelerated maturation) of distal villi with diffuse rarefaction of the intervillous area, increased number of syncytial nodules formed by clusters of the villous trophoblast nuclei (arrow), H&E, magnification: $\times 100$; (E) occlusive thrombosis of the macrovessel of the stem villi (arrow) with mineralization indicating a long duration of the pathological process, H&E, magnification: $\times 100$; (F) focus of adjacent avascular distal villi with stromal sclerosis (arrow), H&E, $\times 100$.

Mild degree SARS-CoV-19: In the group with mild COVID-19 course, perinatal hypoxic-ischemic event occurred in 19.6 % of cases. The number of children with diagnosed FGR and macrosomia was 7.1 and 19.6 %, respectively. Neonatal infection was diagnosed in 8.9 %. The cases of antenatal death and neonatal death were 3.6 and 1.8 %.

Control SARS-CoV-19 negative: The control group was characterized by the incidence perinatal hypoxic-ischemic event (21.1 % of cases). The number of children with diagnosed FGR and macrosomia was 14.9 and 9.6 %, respectively. Neonatal infection was registered in 10.5 % of cases. The cases of antenatal death and neonatal death were 3.5 and 1.8 %.

Comparative characteristics of placental lesions

The frequency of single and combined histopathological lesions of the placenta is presented in Table 2.

Severe degree SARS-CoV-19: In the group with a severe course of COVID-19, acute hypoxic placental injury was detected in 74.4 % of cases: diffuse – in 20.5 %, focal – in 53.8 %. Chronic

hypoxic damage to the placenta according to MVM-type was observed in 25 placentas (64.1 %), according to FVM type – in 9 placentas (23 %). Focal damage according to MVM- and FVM-type was 48.7 and 10.2 %, diffuse – 10.2 and 12.8 %, respectively. Focal and diffuse chronic inflammatory lesions of the placenta were observed in 7.8 and 15.4 % of the studied placentas, and blood circulation disorders in the intervillous space – in 12.8 and 17.9 % of placentas, respectively. Necrosis of the villous trophoblast was found in 8 placentas: 4 – focal and 4 – diffuse. Combined and cross placental injuries were observed in 74.4 % of all studied placentas.

Mild degree SARS-CoV-19: In the group with a mild course of COVID-19, acute hypoxic placental injury was detected in 28.5 % of cases: diffuse – in 10.7 %, focal – in 14.3 %. Chronic hypoxic placental injury of MVM-type was observed in 24 placentas (42.9 %), of FVM type – in 11 placentas (19.6 %). Focal damage according to MVM- and FVM-type was 28.6 and 14.3 %, diffuse – 8.9 and 10.7 %, respectively. Focal and diffuse chronic inflammatory lesions of the placenta were registered in 7.1 and 10.7 % of the studied placentas, and blood circulation disorders in the intervillous space – in 12.5 and 10.7 % of placentas, respectively. Focal necrosis of the

Table 2: Clinical and morphological characteristics of the maternal, fetal and placental compartments ($p < 0.05$).

		Variable, n, %	SARS-CoV-19	Mild course	Sever course	p-Value ^a	p-Value ^b	p-Value ^c
			negative (n=114)	SARS-CoV-19 positive (n=56)	SARS-CoV-19 positive (n=39)			
Clinical data	Maternal	DMI	13 (11.4)	6 (10.7)	8 (20.5)	0.894	0.154	0.186
		DMII	14 (12.3)	7 (12.5)	9 (23.1)	0.968	0.104	0.176
		CAG	15 (13.2)	11 (19.6)	12 (30.8)	0.270	0.013	0.213
		Obesity	29 (25.4)	13 (23.2)	16 (41.0)	0.752	0.066	0.064
		The other	31 (27.2)	17 (30.4)	17 (43.5)	0.667	0.108	0.102
		GDM	18 (15.8)	10 (17.9)	10 (25.6)	0.733	0.170	0.360
		Preeclampsia	7 (6.1)	9 (16.1)	7 (17.9)	0.072	0.143	0.810
		Multimorbidity ^d	34 (29.8)	21 (37.5)	32 (82.1)	0.315	<0.001	<0.001
Clinical data	Fetal	IGR	17 (14.9)	4 (7.1)	9 (23.1)	0.148	0.242	0.055
		Macrosomia	11 (9.6)	11 (19.6)	8 (20.5)	0.069	0.136	0.876
		Perinatal hypoxic-ischemic event	24 (21.1)	11 (19.6)	15 (38.5)	0.831	0.032	0.043
		Neonatal infection	12 (10.5)	5 (8.9)	6 (15.4)	0.745	0.417	0.334
		Antenatal death	4 (3.5)	2 (3.6)	4 (10.3)	0.674	0.224	0.375
		Neonatal death	2 (1.8)	1 (1.8)	2 (5.3)	0.546	0.577	0.749
Morphological variables	Placenta	Acute hypoxic damage of the placenta						
		Focal, n=40	11 (9.6)	8 (14.3)	21 (53.8)	0.368	<0.001	<0.001
		Diffuse, n=19	5 (4.4)	6 (10.7)	8 (20.5)	0.214	0.006	0.303
		Chronic hypoxic damage of the placenta						
		MVM						
		Focal, n=49	14 (12.3)	16 (28.6)	19 (48.7)	0.009	<0.001	0.046
		Diffuse, n=17	3 (2.6)	8 (14.3)	6 (15.4)	0.011	0.012	0.885
		FVM						
		Focal, n=15	5 (4.4)	6 (10.7)	4 (10.2)	0.214	0.342	0.789
		Diffuse, n=17	7 (6.1)	5 (8.9)	5 (12.8)	0.728	0.321	0.544
		Chronic inflammation of the placenta						
		Focal, n=13	6 (5.3)	4 (7.1)	3 (7.8)	0.887	0.872	0.766
		Diffuse, n=21	9 (7.9)	6 (10.7)	6 (15.4)	0.748	0.296	0.719
		Disorders of blood circulation in the intervillous area						
		Focal, n=15	3 (2.6)	7 (12.5)	5 (12.8)	0.027	0.041	0.789
		Diffuse, n=15	2 (1.8)	6 (10.7)	7 (17.9)	0.010	<0.001	0.313
		Necrosis of villous trophoblast						
		Focal, n=14	6 (5.3)	4 (7.1)	4 (10.3)	0.887	0.476	0.872
		Diffuse, n=4	—	—	4 (10.3)	—	—	0.015
		Combined and cross damages of the placenta (2 or more patterns)	21 (18.4)	16 (28.5)	29 (74.4)	0.132	<0.001	<0.001

DMI, type 1 diabetes mellitus; DMII, type 2 diabetes mellitus; CAH, chronic arterial hypertension; GDM, gestational diabetes mellitus; IGR, intrauterine growth retardation; MVM, maternal vascular malperfusion; FVM, fetal vascular malperfusion. ^aComparison of the control group (SARS-CoV-19 Negative) and the group with mild COVID-19 (SARS-CoV-19 Positive). ^bComparison of the control group (SARS-CoV-19 Negative) and the group with severe COVID-19 course. ^cComparison of the group with mild COVID-19 course and the group with severe COVID-19 course. ^dThe co-existence of two or more chronic conditions at least one of which was represented by DMI, DMII, CAH or obesity.

villous trophoblast was revealed in 4 placentas (7.1%). Combined and cross-over damage of the placenta was observed in 28.5 % of all studied placentas.

Control SARS-CoV-19 negative: In the control group, acute hypoxic injury of the placenta was detected in 18.4 % of cases:

diffuse – in 9.6 % of placentas, focal – in 4.4 % of cases. Focal damage of MVM- and FVM-type was 12.3 and 2.6 %, diffuse – 4.4 and 6.1 %, respectively. Focal and diffuse inflammatory lesions of the placenta were revealed in 5.3 and 7.9 % of the studied placentas, and blood circulation disorders in the intervillous space – in 2.6 and 1.8 % of placentas, respectively. Focal necrosis

of the villous trophoblast was found in 6 placentas (5.3 %). Combined and cross-over placental injuries were observed in 18.4 % of all studied placentas.

Discussion

A comparative clinical and morphological characterization of the “mother – placenta – fetus” system of pregnancies associated with COVID-19 and pregnancies without signs of an infectious disease was carried out. Clinical data in the study sample showed that the group with a severe course of SARS-CoV-2 was statistically significantly different from other groups in terms of multimorbidity, which is most common in severe cases of COVID-19 infection ($p<0.01$). At the same time, no differences in diseases and pathology of pregnancy were found in the group with a mild course of COVID-19 infection and in the control group.

These multimorbidity included 2 or more diseases, at least one of which was represented by DMI, DMII, CAH or obesity. Multimorbidity was noted in 32 cases (82.1 %) in the group with severe SARS-CoV-2 course in comparison with the group with mild COVID-19 course and the control group ($p<0.001$). This is consistent with the results of previously published studies of clinical features and outcomes in non-pregnant patients [30–33]. The etiology of the increased risk of severe disease in pregnant women with multimorbidities is currently unknown, but appears to be related to a pro-inflammatory state [34, 35] and a weakened immune response [36]. We believe, that the multimorbidity state is significantly enhanced at SARS-CoV-2, which aggravates the condition of the pregnant woman, therefore multimorbidity is a risk factor for the severe course of SARS-CoV-2 in pregnant women.

It was revealed, that perinatal hypoxic-ischemic event was more common in the group with severe COVID-19 course ($p<0.01$), while there were no differences between the group with mild COVID-19 course and the control group in terms of perinatal outcomes. We believe that this is due to the fact that COVID-19-induced damage is a predisposing background condition and the result of adverse conditions in the antenatal period, which increases the vulnerability of the nervous and cardiovascular systems of the body in postnatal life.

Childbirth and the transition from intrauterine to independent existence are a stress factor for the fetus and newborn, and against the background of an existing infection of the mother, they can cause a breakdown in compensatory-adaptive processes, which leads both to episodes of perinatal hypoxic-ischemic event and to postnatal collapse of the vascular and respiratory systems. In

published sources, opinions on the impact of SARS-CoV-2 infection during pregnancy on the fetus are contradictory [37–39]: no direct relationship has been established, but there is a relationship with the clinical severity of the maternal condition, which suggests that the pathophysiological mechanisms of hypoxic damage are caused by perfusion disorders with mother's side. Placentas in the group with severe COVID-19 had a higher incidence of MVM with more diffuse degree of involvement (Table 2). In placentas from pregnancies with COVID-19, signs of hypoxic damage of MVM-type (heart attacks, maternal vascular microangiopathy) were more common. Theoretically, infection SARS-CoV-2 during pregnancy could affect placental function either directly through viral infection or indirectly through inflammation of the maternal compartment or changes in uterine oxygenation. Some authors [5, 40–46] believe that SARS-CoV-2 can cause a severe systemic inflammatory response with subsequent MVM-type hypoxic damage. The cause may also be nonspecific damage caused by an acute condition, possible pathophysiological mechanisms of which have been described earlier [47]. On the other hand, maternal hypertensive disorders, including gestational hypertension and preeclampsia, included in the study groups, are the main risk factors for MVM development [48, 49]. We believe that in the studied groups, placental damage is most often associated not with direct damage to the placenta by COVID-19, but with secondary damage to the maternal compartment: through damage to maternal vessels, infarctions, detachments, and hemorrhages.

No statistically significant differences were found between the groups in terms of the frequency of occurrence of signs of damage to the fetal-vascular component, which indirectly indicates the absence of direct COVID-19-associated fetal damage. However, it is possible that the histopathological findings of FVM in COVID-19 may depend on the duration of infection and the gestational age at the time of lesion, which should be studied in future studies.

In the placentas of the group with a severe course of COVID-19, histopathological signs of diffuse necrosis of the villous trophoblast were revealed, in comparison with the control group. These placentas were characterized by focal/diffuse loss of color of the nuclei of chorionic villus syncytiotrophoblast, thinning and discontinuity of the trophoblast, and collapse of the intervillous space. This sign was observed in 4 placentas (10.3 %), while the sensitivity of this sign was extremely low, due to the low frequency of occurrence – no more than 10 %. Focal manifestations were detected in 6 cases in the control group. On the one hand, this may be due to the presence of a clinically latent course of COVID-19 in the control group, on the other hand, this sign is nonspecific and can

develop in other diseases, including preeclampsia, which is consistent with the findings of other authors [6, 50–54].

The placentas of the COVID-19 groups more often showed, in addition to necrosis of the villous trophoblast, intervillous thrombi and fibrinoid deposits enveloping the chorionic villi with obliteration of the intervillous space, which may reflect a hypercoagulable state caused by COVID-19 infection or be associated with damage to syncytiotrophoblasts. A combination of diffuse villous trophoblast necrosis and chronic histiocytic intervillitis was registered in all placentas with diffuse trophoblast necrosis associated with an unfavorable perinatal outcome, which probably reflects the possible pathogenesis of coronavirus penetration through the placental barrier. Virus gains access to fetal endothelial cells, chorionic villus nuclei, and the vascular system of the placenta at diffuse necrotic damage to the continuous layer of syncytiotrophoblasts, which can lead to damage to the fetal and placental compartments. In this context, it is important that neither chronic lymphohistiocytic intervillitis nor lymphocytic basal deciduitis were increased in patients with COVID-19 compared with the control group, so it is not possible to judge direct infection of the fetus based on the histopathological picture.

Thus, no specific COVID-19-associated damage to the “mother – placenta – fetus” system was identified. However, we revealed a number of clinical and morphological features of the “mother – placenta – fetus” system depending on the severity of COVID-19. The main ones are multimorbidity: 2 or more diseases, one of which was type 1 or 2 diabetes mellitus, chronic arterial hypertension or obesity. Based on the presented clinical data, perinatal outcome was not statistically significantly different between groups with COVID-19, with the exception of a higher incidence of perinatal hypoxic-ischemic event at severe COVID-19 course than in the control group and the group with mild COVID-19 course.

We found that placentas at severe COVID-19 course were most often characterized by diffuse cross-injury to the placenta. Placentas were damaged by both chronic and acute injuries, in comparison to the mild and control groups ($p<0.001$). Also an important finding in severe COVID-19 was diffuse necrosis of the villous trophoblast – homogenization, diffuse circular eosinophilic masses surrounding the chorionic villi. This symptom was may indirectly indicate infection of the fetal-placental compartment with COVID-19. These results suggest that there is placental injury in SARS-CoV-2, but the further studies are needed to establish their connection with possible adverse perinatal outcomes.

The strengths of this study include the comparative characterization of clinical and morphological factors and signs of COVID-19-associated mother-placenta-fetus system from high-

risk pregnancies in terms of perinatal outcome. We believe that the high-risk group with multimorbidity is a subgroup at risk for severe SARS-CoV-2 course, with an increased incidence of severe damage to the «mother – placenta – fetus» system. Taken together, these results indicate the need to strengthen the monitoring of women at risk with multimorbidity conditions, the prevention of SARS-CoV-2 in them, and the stratification of risk groups for complications in newborns.

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