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DETERMINATION OF THE VASCULAR LUMEN AREA INDEX OF CHORIONIC VILLOUS VESSELS IN COVID-19 DURING PREGNANCY

Abstract. Background. The causative agent of the SARS-CoV-2 virus infects cells that express angiotensin-converting enzyme 2 (ACE2). Endothelial activation leads to microcirculatory disturbances, proliferative changes in the vascular wall, fibroblast activation, and arteriolosclerosis. These processes result in malperfusion and the development of placental dysfunction. Structural changes in the vessels of chorionic villi represent compensatory mechanisms that ensure the birth of PCR-negative neonates with high Apgar scores. Identification of quantitative differences in the vessels of stem and intermediate chorionic villi in cases of live birth and antenatal asphyxia prompted us to conduct this study.

Objective. To determine the vascular lumen area index of stem and intermediate chorionic villi in pregnant women with COVID-19.

Methods. A total of 184 placentas from pregnancies complicated by COVID-19 were studied: Group I (n=150) — placentas from live births, and Group II (n=34) — placentas in cases of antenatal fetal asphyxia. Depending on the duration of the post-COVID interval (PCI — the time between maternal COVID-19 diagnosis and delivery), subgroups were formed. In subgroups I.2 (n=80) and II.2 (n=22), the PCI was 1–4 weeks, whereas in subgroups I.1 (n=70) and II.1 (n=12), it was 5–20 weeks. COVID-19 in pregnant women was confirmed by a positive polymerase chain reaction (PCR) test for SARS-CoV-2 RNA. For comparison, placentas (n=110) from physiological deliveries before the COVID-19 pandemic were examined. Immunohistochemical methods using monoclonal antibodies against CD31 and MSB staining, as well as statistical methods, were applied. Quantitative assessment of the studied structures was performed using pixel-based visualization of the structures differing in color after immunohistochemical staining with the ONLINE JPG TOOLS service. The vascular lumen area index (VLAI) was calculated according to the formula: VLAI = LA / VWA × 100, where VWA is the percentage area of the vascular wall and LA is the percentage area of the vascular lumen.

Results and conclusions. During the acute period of COVID-19 in pregnancy, both in live births and in antenatal fetal asphyxia, the lumen of vessels in stem villi was

reduced. This was observed in all cases of subgroup I.2 and in 91.7% of Group II. In subgroup I.1, vascular lumen narrowing was observed in 35.7% of cases ($p_{1-2}<0.001$; $p_{1-3}<0.001$; $p_{1-4}<0.001$). With increasing duration of the PCI in live births, vascular lumens demonstrated recovery. In the main groups, vascular wall thickness was increased: 22 [10; 32] in subgroup I.1, 35 [17; 48] in subgroup I.2, 23 [10; 35] in subgroup II.1, and 33 [15; 46] in subgroup II.2 versus 13 [6; 28] in the comparison group. These changes were associated with proliferative alterations in the vascular wall observed in the main groups during COVID-19 in pregnancy. Reduced vascular lumen in Group II and proliferative vascular changes resulted in a decreased VLAI. The lowest values were observed in antenatal fetal asphyxia (subgroups II.1 and II.2 — 10 [0.0; 38] and 2.4 [0.3; 2.9], respectively). In subgroup I.2, the VLAI was 18 [4.2; 23], whereas in subgroup I.1 it was 27 [25; 32], not differing from the corresponding value in the comparison group (Table 1).

Conclusions. A quantitative manifestation of malperfusion in COVID-19 associated with antenatal fetal death is a decreased vascular lumen area index of stem chorionic villi. Changes in this parameter reflect vascular remodeling.

Keywords: placenta, COVID-19, SARS-CoV-2, stem chorionic villi, vascular lumen area index.

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ВИЗНАЧЕННЯ ІНДЕКСУ ПЛОЩІ ПРОСВІТУ СУДИН ВОРСИН ХОРІОНА ПРИ КОРОНАВІРУСНІЙ ХВОРОБІ (COVID 19) У ВАГІТНОЇ

Анотація. Актуальність. Збудник вірусу SARS-CoV-2 уражає клітини, що експресують ангіотензинперетворюючий фермент 2 (angiotensin-converting enzyme 2 – ACE2). Активація ендотелію призводить до порушення мікроциркуляції, проліферативних змін судинної стінки, активації фібробластів та артеріолосклерозу. Це обумовлює мальперфузію та розвиток плацентарної дисфункції. Структурні зміни судин ворсин хоріона є проявом компенсаторних механізмів, що забезпечують народження ПЛР-негативних немовлят с високими балами за шкалою Апгар. Виявлення кількісних відмінностей судин стовбурових та проміжних ворсин хоріона у випадку живонародження та при антенатальній асфіксії спонукало нас провести дослідження.

Метою було визначення індексу площі просвіту судин стовбурових та проміжних ворсин хоріона при коронавірусній хворобі (COVID 19) у вагітної.

Методи. Досліджували 184 плаценти при COVID-19 у вагітної: I група (n=150) – плаценти при народженні живого плода та II група (n=34) – при анте-

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натальній асфіксії. В залежності від тривалості постковідного інтервалу (ПІ – проміжок часу між діагностуванням COVID-19 у матері та пологами) були сформовані підгрупи. В підгрупах I.2 (n=80) та II.2 (n=22) ПІ склав 1-4 тижні, в підгрупах I.1 (n=70) та II.1 (n=12) – 5-20 тижнів. COVID-19 у вагітної підтверджено позитивним результатом полімеразної ланцюгової реакції (ПЛР) на наявність РНК вірусу SARS-CoV-2. Для порівняння даних досліджували плаценту (n=110) при фізіологічних пологах до епідемії COVID-19. Використовували муногістохімічний з використанням моноклональних антитіл проти CD31, MSB та статистичні методи дослідження. Для кількісного визначення досліджуваних структур впроваджували якісну попіксельну візуалізацію досліджуваної структури, які різняться за кольором при їх імуногістохімічному фарбуванні з використанням сервісу ONLINE JPG TOOLS. З подальшим обчисленням індексу площі просвіту (ІПП) за формулою: ІПП= ПП /ПСС×100, де: ПСС – відсоток площі стінки судини; ПП – відсоток площі просвіту.

Результати та підсумок. В гострому періоді COVID-19 у вагітної при живонародженні та при антенатальній асфіксії плода просвіт судин стовбурових ворсин виявився зменшеним. В усіх спостереженнях підгрупи I.2 та 91,7% групи II. В підгрупі I.1 просвіт судин був зменшений у 35,7% (p_{1-2}<0,001; p_{1-3}<0,001; p_{1-4}<0,001). Зі збільшенням тривалості ПІ при живонародженні просвіт судин відновлювався. В основних групах відмічалося збільшення товщини судинної стінки в підгрупі I.1 –22 [10; 32]; в I.2 – 35 [17; 48], в II.1 – 23 [10; 35]; і II.1 – 33 [15; 46] проти 13 [6; 28] у групі порівняння. Це обумовлювалося проліферативними змінами в стінці судини, які виявлялися в основних групах при COVID-19 у вагітної. Зменшення просвіту судин в групі II та проліферативні зміни спричиняли зменшення ІПП. Найменше значення показника виявилося при антенатальній асфіксії плода (підгрупи II.1 та II.2 – 10 [0,0; 38] та 2,4 [0,3; 2,9] відповідно. В підгрупі II.2 ІПП склав 18 [4,2; 23]. В II.1 – 27 [25; 32] та не відрізнявся від аналогічного показника групи порівняння (таблиця 1).

Висновки. Кількісний прояв мальперфузії при коронавірусній хворобі (COVID 19) у випадку антенатальної загибелі плода – зменшення індексу площі просвіту стовбурової ворсини хоріона. Зміна показника свідчить про ремоделювання судини.

Ключові слова: плацента, COVID-19, SARS-CoV-2, стовбурові ворсини хоріона, індекс площі просвіту судини.

Problem statement. The SARS-CoV-2 virus affects cells expressing angiotensin-converting enzyme 2 (ACE2) [1,2]. Endothelial activation leads to microcirculatory disturbances and proliferative changes in the vascular wall, resulting in fibroblast activation and the development of arteriolosclerosis [3–7]. These changes lead to malperfusion and the development of placental dysfunction [8]. Structural alterations in the vessels of chorionic villi represent compensatory mechanisms that ensure the birth of PCR-negative neonates with high Apgar scores [9,10]. The identification of quantitative differences in the vessels of stem and intermediate chorionic villi in cases of live birth and antenatal asphyxia prompted us to conduct this study.

The aim of the study was to determine the vascular lumen area index of stem and intermediate chorionic villi in pregnant women with COVID-19.

Materials and methods.

A total of 184 placentas from pregnancies complicated by COVID-19 were studied: Group I (n=150) — placentas from live births, and Group II (n=34) — placentas in cases of antenatal fetal asphyxia. Depending on the duration of the post-COVID interval (PCI — the time between maternal COVID-19 diagnosis and delivery), subgroups were formed. In subgroups I.2 (n=80) and II.2 (n=22), the PCI was 1–4 weeks, whereas in subgroups I.1 (n=70) and II.1 (n=12), it was 5–20 weeks. COVID-19 in pregnant women was confirmed by a positive polymerase chain reaction (PCR) test for SARS-CoV-2 RNA. For comparison, placentas (n=110) from physiological deliveries before the COVID-19 pandemic were examined.

Immunohistochemical methods using monoclonal antibodies against CD31, MSB staining modified according to Zerbino-Lukasevych, and statistical methods were applied. After fixation of placental tissue samples in 10% neutral formalin, processing through ascending concentrations of alcohols, paraffin embedding, preparation of 5-μm tissue sections, and immunohistochemical staining, microscopic specimens were photographed using a digital camera mounted on a microscope. Using the SNIPPING TOOL, the studied vessel was isolated from the image, and the vascular lumen was colored differently from the histological staining of surrounding structures (yellow); the tissues surrounding the vessel were also colored differently (Fig. 1). Quantitative assessment of the studied structures was performed using pixel-based visualization of structures differing in color after immunohistochemical staining with the ONLINE JPG TOOLS service. The vascular lumen area index (VLAI) was subsequently calculated according to the formula:

VLAI = LA / VWA × 100, where VWA is the percentage area of the vascular wall and LA is the percentage area of the vascular lumen (utility model patent). № u202305769; https://sis.nipo.gov.ua/uk/search/detail/1837802/).

Fig. 1. Screenshot of the ONLINE JPG TOOLS service: on the left — the uploaded image; on the right — the colors used for percentage determination. A — microscopic section of an arteriole with a narrowed lumen in a stem chorionic villus in antenatal fetal death at 35 weeks of gestation following maternal COVID-19 at 23–24 weeks of pregnancy. The lumen and background are colored differently from the colors of the stained tissue structures in the image (yellow and white, respectively). MSB, ×200. B — percentage distribution of colors in the service: lumen area — 0.8%. The

sum of the percentages of pink color shades corresponds to the percentage area of the vascular wall.

Presentation of Main Findings. Placentas from pregnancies complicated by COVID-19 at 19–40 weeks of gestation were studied in cases of live birth and antenatal fetal asphyxia.

The vascular response of the placenta to injury was associated with the effects of SARS-CoV-2. Pathomorphological vascular changes depended on the severity of injury, including the extent of endothelial necrosis, thrombosis, and placentitis during the acute phase of the disease [11], as well as on the rate of response to injury. The intensity of inflammatory changes in the placenta (chorioamnionitis, intervillositis) predominated during the acute phase of the disease in antenatal fetal asphyxia (+++++) compared with subgroup I.2 (+++). With increasing duration of the post-COVID interval, the intensity of inflammatory changes decreased (+) (Table 1).

Table 1

Pathomorphological changes of the placenta in COVID-19 during pregnancy and in the comparison group.

Parameters	COVID-19 (N=82)					p
Groups	Live births (Group I) (N=150)		Antenatal asphyxia (Group II) (N=34)		Comparison (n=110)	
	I.1 (n=70)	I.2 (n=80)	II.1 (n=12)	II.2 (n=22)		
Chorioamnionitis/intervillitis/ Severity of manifestation	67 (95.7) [90.9–100]/ 2 (2.9) [0.3–9.9]/ +	80 (100) [95.5–100]/ 18 (22.5) [13.3–31.7]*/ +++	3 (25) [2-55]/0 0,0 [0,0-14,6]/ +	22 (100) [91,7-100]/ 22(100) [91,7-100]*/ +++++	0 (0.0) [0–2.7]	p_{1-2}<0,001 p_{3-4}<0,001
Arteriolar endothelial necrosis; n (%) [95 % CI];	4 (6.1) [0.2–11.9]	76 (95.0) [90.2–99.8]*	2 (16,7) [1,1-45,3]	100 [91,7-100]*	0 (0.0) [0–2.7]	p_{1-2}<0,001 p_{2-3}<0,001 p_{1-4}<0,001 p_{2-5}<0,001 p_{4-5}<0,001
Narrowing of the lumen of the blood vessels in the stem villi; n (%) [95 % CI]	25 (35,7) [25–48]	80 (100) [95.5–100]*	11 (91,7) [61-99,8]	21 (91,7) [67,1-100]*	0 (0.0) [0–2.7]	p_{1-2}<0,001 p_{1-3}<0,001 p_{1-4}<0,001
Proliferation of the muscular layer of the arterioles of the stem villi; n (%) [95 % CI]	47 (67.1) [56.1–78.1]*	65 (81.3) [72.7–89.8]*	4 (33,3) [8,6-64,6]*	10 (45,5) [24,5-67,3]*	0 (0.0) [0–2.7]	p<0,001
Wall thickness of the stem villus arterioles (μm)	22 [10; 32]*	35 [17; 48]*	23 [10; 35]*	33 [15; 46]*	13 [6; 28]	p_{1-2}<0,001 p_{2-3}<0,001 p_{3-4}<0,001 p_{2-5}<0,001 p_{4-5}<0,001
VLAI	27 [25; 32]	18 [4,2; 23]	10 [0,0; 38]*	2,4 [0,3; 2,9]*	26,5 [21; 30]	p_{1-2}<0,001 p_{2-3}<0,001 p_{1-3}<0,001 p_{4-5}<0,001 p_{2-5}<0,001

Примітка: *p<0,01 (Kruskal–Wallis test)

Necrotic changes of the vascular endothelium were detected during the acute phase of the disease in 95% of live births and in all cases of antenatal fetal asphyxia (Table 1). Endothelial necrosis manifested as decreased CD31 expression in the endothelium of chorionic villous arterioles (Fig. 2A) and complete absence of expression in obliterated arterioles in antenatal fetal death (Fig. 2B).

MSB staining of the vascular wall demonstrated a color change from red to blue with increasing duration of the post-COVID interval. The blue coloration indicated an increased amount of connective tissue fibers (Fig. 2C, D).

During the acute phase of COVID-19 in pregnancy, both in live births and antenatal fetal asphyxia, the lumen of vessels in stem villi was reduced. This finding was observed in all cases of subgroup I.2 and in 91.7% of Group II. In subgroup I.1, vascular lumen narrowing was observed in 35.7% of cases ($p_{1-2} < 0.001$; $p_{1-3} < 0.001$; $p_{1-4} < 0.001$). With increasing duration of the post-COVID interval, vascular lumens recovered in cases of live birth.

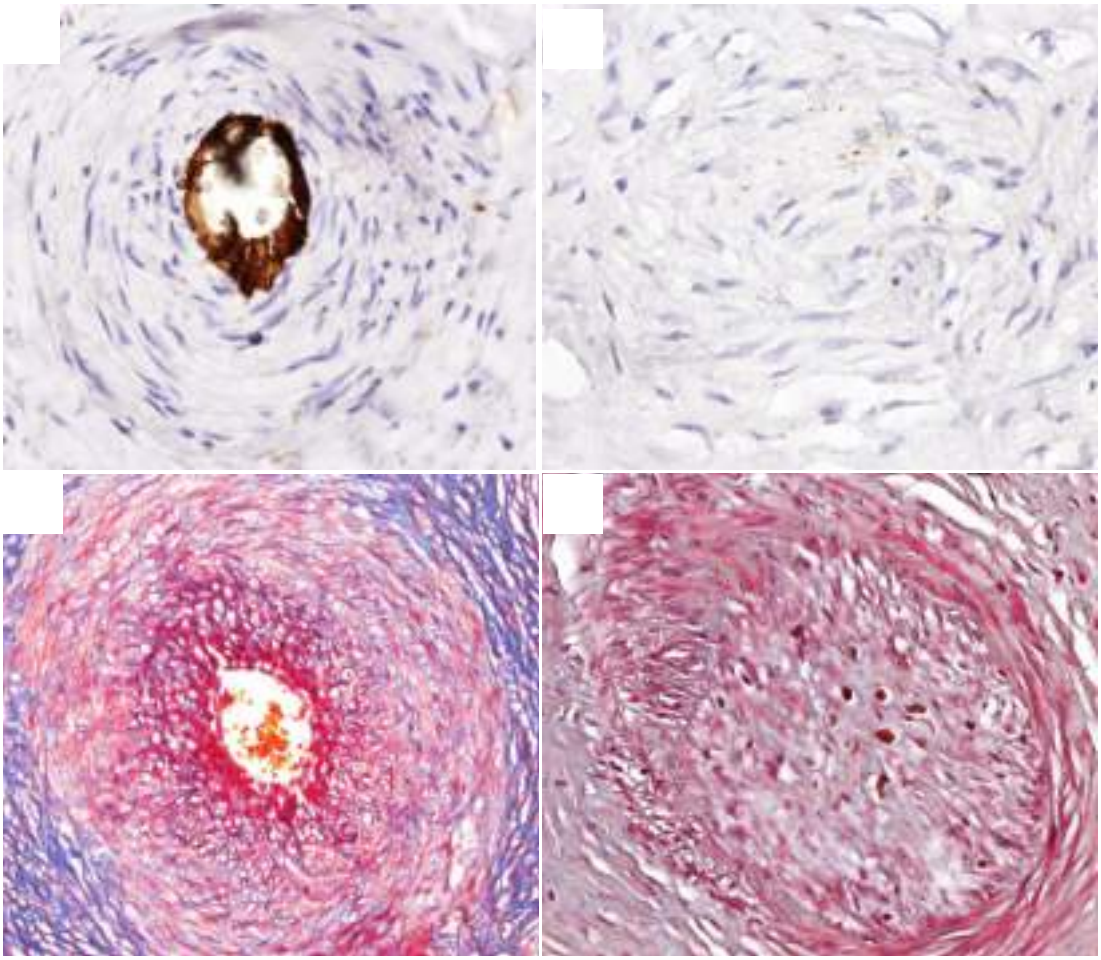

Fig. 2. Structural changes in vessels of stem chorionic villi in COVID-19 during pregnancy in cases of antenatal fetal asphyxia. A, C — subgroup II.2. B, D — subgroup II.1. A, B — expression of monoclonal antibodies against CD31 in the endothelium of chorionic villous arterioles. C, D — MSB staining modified according to Zerbino D.D. and Lukasevych L.L., ×400.

In the main groups, vascular wall thickness was increased: 22 [10; 32] in subgroup I.1, 35 [17; 48] in subgroup I.2, 23 [10; 35] in subgroup II.1, and 33 [15; 46] in subgroup II.2 versus 13 [6; 28] in the comparison group. These changes were associated with proliferative alterations in the vascular wall observed in the main groups during COVID-19 in pregnancy (Table 1).

A reduction in vascular lumen in Group II together with proliferative vascular wall changes resulted in a decreased vascular lumen area index (VLAI). The lowest values of this indicator were observed in antenatal fetal asphyxia (subgroups II.1 and II.2 — 10 [0.0; 38] and 2.4 [0.3; 2.9], respectively). In subgroup I.2, the VLAI was 18 [4.2; 23], whereas in subgroup II.1 it reached 27 [25; 32] and did not differ from the corresponding value in the comparison group (Table 1).

According to published data, vascular wall remodeling under the influence of SARS-CoV-2 is mediated not only by endothelial cells but also by pericytes. It has been shown that pericytes exposed to the viral spike protein (Sp) undergo phenotypic and morphological changes accompanied by increased expression of contractile and myofibrogenic proteins, including α-smooth muscle actin, fibronectin, and collagen, leading to alterations in blood flow [12,13].

Disorder of microcirculation and endothelial activation act synergistically, resulting in vascular lumen narrowing (stenosis and obliteration) as well as inflammatory cell infiltration in organs affected by COVID-19. This may contribute to an excessive immune response that can lead to long-term tissue damage and/or death [14].

Conclusions. A quantitative manifestation of malperfusion in COVID-19 associated with antenatal fetal death is a reduction in the vascular lumen area index of stem chorionic villi. Changes in this parameter reflect vascular remodeling and structural adaptation of the placental vascular bed.

Author Contributions:

The author confirms their contribution to the work: concept and study design, data collection, analysis and interpretation of results, verification, reviewing, and editing – T. V. Savchuk.

The author confirms their contribution to the work: concept and study design, verification, reviewing, and editing – I.V. Leshchenko.

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