



Effect of asymptomatic COVID-19 infection on the placenta in the third trimester of pregnancy: A prospective case-control study

Term gebeliklerde asemptomatik COVID-19'un plasentaya etkisi: Prospektif olgu-kontrol çalışması

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Abstract

Objective: To clarify the effect of asymptomatic coronavirus disease-2019 (COVID-19) positivity on the placenta in the third trimester of pregnancy.

Materials and Methods: This prospective, case-control study included 30 pregnant women diagnosed with asymptomatic COVID-19 between April 30, 2021 and July 20, 2021 who delivered after the 34th gestational week, and a control group of 30 pregnant women without COVID-19, who delivered between April 2021 and July 2021, matched to the study group regarding age, gestational age and body mass index. Outcomes were compared in terms of demographic characteristics, serum blood outcomes, neonatal results, complications and placental histopathological findings.

Results: The mean age of the study population was 28.8 years and the mean gestational week was 38.2 weeks. The C-reactive protein levels (38.2 mg/L vs 5.8 mg/L, $p=0.001$) and ferritin levels (266.4 µg/L and 40.5 µg/L, $p=0.001$) were significantly higher in the COVID-19-positive pregnant women. The lymphocyte level was significantly higher in the non-COVID-19 pregnant women ($p=0.040$). Mural hypertrophy was determined at a significantly higher rate in COVID-positive pregnant women (83.3% vs 30.0%, $p=0.001$). Multivariate regression analysis showed that only COVID-19 positivity increased the presence of mural hypertrophy in pregnant women with asymptomatic COVID-19 (4.716-fold, 95% confidence interval=1.012-22.251).

Conclusion: The results of this study demonstrated that asymptomatic COVID-19 had no significant effect on pregnancy and neonatal complications. However, mural hypertrophy in the placenta was found at a significantly higher rate in pregnant women with asymptomatic COVID-19.

Keywords: Asymptomatic, COVID-19, mural hypertrophy, placenta, pregnancy

Öz

Amaç: Term gebeliklerde asemptomatik koronavirüs hastalığı-2019 (COVID-19) pozitifliğinin gebelik ve plasenta üzerindeki etkisini araştırmak amaçlandı.

Gereç ve Yöntemler: Bu prospektif, olgu-kontrol çalışmasına 30 Nisan 2021 ile 20 Temmuz 2021 tarihleri arasında asemptomatik COVID-19 tanısı konan ve 34. gebelik haftasından sonra doğum yapan gebeler ile COVID-19 olmayan 30 gebeden oluşan bir kontrol grubu dahil edilmiştir. Kontrol grubu ile çalışma grubu yaş, gebelik yaşı ve vücut kitle indeksi açısından eşleştirildi. Sonuçlar demografik özellikler, serum kan sonuçları, neonatal sonuçlar, komplikasyonlar ve plasental histopatolojik bulgular açısından karşılaştırıldı.

Bulgular: Çalışma popülasyonunun ortalama yaşı 28,8 yıl ve ortalama gebelik haftası 38,2 hafta idi. COVID-19 pozitif gebelerde C-reaktif protein düzeyi (38,2 mg/L vs 5,8 mg/L, $p=0,001$) ve ferritin düzeyi (266,4 µg/L ve 40,5 µg/L, $p=0,001$) anlamlı olarak daha yüksekti. COVID-19 olmayan gebelerde lenfosit düzeyi anlamlı olarak daha yüksekti ($p=0,040$). Mural hipertrofi, COVID-19 pozitif gebelerde anlamlı olarak daha yüksek oranda belirlendi (%83,3'e karşı %30,0, $p=0,001$). Çok değişkenli regresyon analizi ile asemptomatik COVID-19'lu gebelerde sadece COVID-19 pozitifliğinin mural hipertrofi varlığını anlamlı oranda artırdığı gösterildi (4.716 kat, %95 güven aralığı=1.012-22.251).

PRECIS: Asymptomatic COVID-19 positive 3rd trimester pregnant woman has vascular pathology that may complicate pregnancy in the placenta.

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Received/Geliş Tarihi: 14.05.2022 **Accepted/Kabul Tarihi:** 30.06.2022

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Turkish Journal of Obstetrics and Gynecology published by Galenos Publishing House.

Sonuç: Bu çalışmanın sonuçları asemptomatik COVID-19'un gebelik ve yenidoğan komplikasyonları üzerinde anlamlı bir etkisinin olmadığını göstermiştir. Bununla birlikte, asemptomatik COVID-19'lu hamile kadınlarda plasentada mural hipertrofi önemli ölçüde daha yüksek oranda bulundu.

Anahtar Kelimeler: Asemptomatik, COVID-19, mural hipertrofi, plasenta, gebelik

Introduction

The new coronavirus disease-2019 (COVID-19), which was first determined at the end of 2019, spread rapidly around the world within a few months and was declared a pandemic by the World Health Organisation on March 11, 2020⁽¹⁾. As little is currently known about COVID-19 in pregnancy, the consequences of infecting pregnant women with COVID-19 and the potential risks of vertical transmission have become a major concern. In a previous large series of studies, it has been shown that pregnant women aged 15-44 years were diagnosed with COVID-19 infection at a rate of 9%, which was higher than the rate of 5% in the general female population of reproductive age⁽²⁾. Cardona-Pérez et al.⁽³⁾ reported that severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) real time-polymerase chain reaction (PCR) positivity was determined in 29% of pregnant women, of whom 86% were asymptomatic. In another study, it was reported that 5.7% females diagnosed with COVID-19 were pregnant and showing symptoms⁽⁴⁾. In two cohort studies in Turkey, universal screening was applied to all the patients who presented for childbirth, and asymptomatic SARS-CoV-2 carriers were determined at the rates of 7.7% and 1.4%^(5,6).

Among these asymptomatic patients admitted for labor and childbirth, the global rates of SARS-CoV-2 positivity were reported to range between 0.45% and 19.9%⁽⁷⁾. Breslin et al.⁽⁸⁾ observed that symptoms developed during labor or in the postpartum period in 71% of asymptomatic cases who presented for childbirth.

In a meta-analysis that included 42 studies of 438,548 pregnant women, COVID-19 was associated with an increased risk of preterm birth, pre-eclampsia, and stillbirth, compared with women who were SARS-CoV-2 negative during pregnancy⁽⁹⁾. In another study, Allotey et al.⁽¹⁰⁾ emphasized that pregnant women with COVID-19 had significantly higher risks for preterm delivery, requirements for the intensive care unit and maternal death compared with pregnant women without COVID-19. Furthermore, Tasca et al.⁽¹¹⁾ claimed that high perfusion of the placenta with COVID-19 infection could increase the inflammatory response and oxidative stress, which could cause undesirable events in pregnant women.

The placenta may be affected by the virus in pregnant women with COVID-19 positivity. Although the probability of vertical transfer seems to be low, it has been indicated that the fetus can be potentially affected by the placenta infection⁽¹²⁾. Vertical transfer of SARS-CoV-2 determined in pregnancy has not yet been clarified and research on the effects of the viral pathogen on the placenta is ongoing.

Many studies have focussed more on examining the placental effects of COVID-19 in symptomatic pregnant patients and have attempted to explain the effect of this on the pregnancy. These studies have revealed some common morphologies histologically and there has been observed to be a predominance of vascular malperfusion (VM) and placental infection with histiocytic intervillitis increased perivillous fibrin, and villous trophoblast necrosis^(13,14). In contrast, there are very limited data related to how the effect is formed in asymptomatic COVID-19-positive pregnant patients.

This study aimed to clarify the effect of asymptomatic COVID-19 on the placenta by comparing the placentas of pregnant women with asymptomatic COVID-19 and pregnant women without COVID-19 in the third trimester.

Materials and Methods

This study was conducted as a prospective, case-control study. One of the primary study endpoints was the rate of placental vascular pathology, which was expected to be higher in asymptomatic COVID-19-positive pregnant women. With reference to a study by Jaiswal et al.⁽¹⁵⁾, sample size to provide α 0.5, and power of $(1-\beta)$ 80, was calculated as a minimum of 60 study participants (30 COVID-positive asymptomatic pregnant women during delivery and 30 COVID-negative pregnant women during delivery in the control group) to be sufficient to produce statistically significant results.

The COVID-19-positive group included asymptomatic pregnant women who were positive after the routine PCR test performed for patients who were hospitalized for delivery and who had no suspicious complaints. A control group was formed from patients who were admitted to the hospital for delivery within the same period with a negative routine PCR test, matched according to age, gestational week and body mass index (BMI). The patients in the control group were tested for antibodies (Weimi Diagnostic, Guangzhou Weimi Bio-Tech, Guangzhou, China) to exclude the possibility of having asymptomatic COVID-19 during pregnancy. Those with a negative result were deemed not to have had COVID-19 and were included in the control group.

Demographic and clinical information of the two groups was obtained from the hospital information system (PANATES® hospital information systems) and patient records. Ethics committee approval was obtained from the local "Medical Ethics and Institutional Review Board" with ID number E-48670771-514.10/136 in April 19, 2021, and the study was completed in accordance with Helsinki Declaration principles. All the pregnant women provided informed consent to participate in the study. The pregnant women diagnosed with asymptomatic COVID-19 and those without COVID-19 were accepted as

candidates for the study between April 30, 2021 and July 20, 2021, and those who delivered after the 34th gestational week were included in the study. Exclusion criteria were defined as a history of COVID-19 during pregnancy, the presence of multiple pregnancy, the presence of any chronic disease during pregnancy (neurological disease, heart disease, renal disease, immune disorder, etc.) or the use of drugs (not including pregnancy vitamin supplements and iron preparations) due to such diseases, a history or diagnosis of hypertension during pregnancy, diabetes mellitus, fetal growth retardation, infections (eg., toxoplasmosis, rubella, cytomegalovirus, herpes simplex), placental anomalies, vascular pathology determined on obstetric Doppler ultrasonography performed during pregnancy (abnormal pulsatility index, uterine artery notch etc.), placenta previa, fetal anomaly diagnosis, or incomplete data in the records.

The demographic characteristics of all pregnant women including age, gestational age (weeks), presence of gestational diabetes mellitus, maternal-obstetric complications such as amniotic volume disorders, intrapartum-postpartum adverse results, such as massive bleeding, new onset hypertension, dyspnea, nasal O₂ requirement, intensive care admission that may be related to COVID-19, BMI and laboratory results including C-reactive protein (CRP), ferritin level, white blood cell (WBC) level, neutrophil and lymphocyte levels were recorded. Neonatal data including the 5-min APGAR score, requirement for neonatal intensive care unit, pH of umbilical cord blood, and placental weight and diameter were also noted.

Histopathological Evaluation

All placental samples were stored in the pathology department and a single experienced gynecopathologist (A.Y.A.) performed all the histopathological and morphological analyses to minimize possible bias. The pathologist was blinded to clinical information, including the COVID-19 status. Placentas were fixed with 10% neutral buffered formalin and the Amsterdam Placental Workshop Group 2016⁽¹⁶⁾ classification was used to determine macroscopic and microscopic lesions. Two full thickness sections from the umbilical cord insertion site and a total of twelve sections including placental membranes and umbilical cord, were taken for examination. The pathological findings examined included ectasia and thrombosis of the vascular cord, infarcts, retroplacental hemorrhage, chorangiomas, distal villous hypoplasia, accelerated villous maturation, decidual arteriopathy, thrombosis, avascular villi, villous stromal karyorrhexis, mural hypertrophy, stem villous obliteration, delayed villous maturation, funisitis, chorioamnionitis, villitis, and perivillous fibrin deposition.

Statistical Analysis

Data obtained in the study were analyzed statistically using the IBM SPSS Statistics Version 25 (NY, USA). Shapiro-Wilk test and Q-Q plot tests were performed as the normality test. The Independent t-test was used for comparisons between

the groups of normally distributed variables, and the Mann-Whitney U test was used for not normally distributed data. The chi-square (χ^2) probability distribution was applied in analyzing categorical variables and Fishers' Exact probability test was used when the assumptions for conducting a chi-square test were not met. Quantities are shown as mean \pm standard deviation. A logistic regression model was performed using odds ratios to investigate confounding factors in the observed association. The data were resolved at 95% confidence interval level and $p < 0.05$ was considered statistically significant.

Results

According to the study design, 30 pregnant women with asymptomatic COVID-19 and 30 pregnant women without COVID-19 were enrolled in the study. The mean age of the study population was 28.8 years and the mean gestational week was 38.2 weeks. The mean placental weight and diameter was 587.5 g and 18.0 cm, respectively. Cesarean section (C/S) was performed in 39 (65%) patients. A total of 51 infants were born with a 5-min APGAR score more than 7, and the mean pH of umbilical cord blood 7.3. The demographic characteristics of the study population, serum blood outcomes, placental findings and neonatal results are summarized in Table 1.

Comparisons of the COVID-positive and COVID-negative pregnant women revealed that age, gestational age, BMI, WBC level, neutrophil level, 5-min. APGAR score, the pH of umbilical cord blood and maternal blood pressures were comparable ($p=0.059$, $p=0.412$, $p=0.499$, $p=0.311$, $p=0.647$, $p=0.071$, $p=0.649$ and $p=0.404$, respectively). Placental weight and placental diameter was similar between the groups ($p=0.346$ and $p=0.930$). CRP level (38.2 mg/L vs 5.8 mg/L, $p=0.001$) and ferritin level (266.4 μ g/L and 40.5 μ g/L, $p=0.001$) were significantly higher in the COVID-19-positive pregnant women. Lymphocyte levels were significantly higher in pregnant women without COVID-19 ($p=0.040$), but the neutrophil/lymphocyte ratio was not statistically significant ($p=0.157$) (Table 2). The requirement for maternal nasal O₂ was significantly higher in the COVID-19-positive pregnant women ($p=0.024$). Complications were detected in one pregnant woman without COVID-19 and in four pregnant women with COVID-19 ($p=0.706$). The types of complications are listed in Table 2.

In the pregnant women with asymptomatic COVID-19, mural hypertrophy (83.3%), distal villous hypoplasia (63.3%) and perivillous fibrin deposition (56.7%) were the most common histological findings and in pregnant women without COVID-19, perivillous fibrin deposition (76.7%), distal villous hypoplasia (40.0%) and infarcts (33.3%) were most common. Only mural hypertrophy was significantly common in COVID-19-positive pregnant women (83.3% vs 30.0%, $p=0.001$) (Figure 1). No statistically significant difference was determined between the groups with respect to the other pathological findings (Table 3). Univariate analysis revealed that higher CRP value and COVID-19 positivity was significantly common in pregnant

women with mural hypertrophy ($p=0.014$ and $p<0.001$) and increased the mural hypertrophy risk 1.066-fold and 11.667-fold, respectively. The multivariate regression analysis showed that only COVID-19 positivity increased the presence of mural hypertrophy in pregnant women with asymptomatic COVID-19 [4.716-fold, 95% confidence interval (CI)=1.012-22.251] (Table 4).

Discussion

This study aimed to investigate the effect of COVID-19 on the placenta by comparison with COVID-19-negative third trimester pregnant women. The study results showed mural hypertrophy to be significantly more common in the placenta of COVID-19-positive pregnant women. Although specific placental histopathological changes in patients infected with COVID-19 have been suggested in some previous studies, there have been no appropriate control groups in those studies^(11,17,18). The current study results showed vasculopathy in maternal-fetal placental vessels as a significant finding in the

cohort of asymptomatic SARS-CoV-2-positive patients, which was consistent with the findings of some previous studies⁽¹⁹⁾. Additionally, it was determined that CRP and ferritin levels were significantly higher and lymphocyte levels were lower in pregnant women with asymptomatic COVID-19 positivity, as well as an increased need for maternal nasal O₂ during delivery and an increase in the rate of cesarean section delivery.

Angiotensin-converting enzyme 2 is expressed in the human placenta, primarily in syncytiotrophoblasts and cytotrophoblasts and secondarily in placental villi. It is also found in the arterial and venous endothelium of the umbilical cord, thereby rendering it theoretically possible that COVID-19 can spread to and affect the placenta from the mother⁽²⁰⁾. Placental trophoblast atherosclerosis represents a series of morphological spectra compatible with placental pathology and may be associated with different pathogenic mechanisms such as viral, or inflammatory responses related to trophoblast cell death and vascular remodeling. Mural hypertrophy can be evaluated as a histopathological result of vascular changes known as placental atherosclerosis or VM⁽²¹⁾.

In the present study, only mural hypertrophy was significantly common in the pregnant women with COVID-19, and asymptomatic COVID-19 positivity increased the risk of mural hypertrophy in the placental vascular bed 4.716-fold. Jaiswal et al.⁽¹⁵⁾ compared the placentas of COVID-19-positive and COVID-19-negative women and showed that there were significantly higher maternal-fetal malperfusion changes in pregnant women with COVID-19. In contrast, Blasco Santana et al.⁽²²⁾ analyzed the placenta histology in 29 patients and concluded that COVID-19 did not change any histological properties of the placenta. Moreover, it was claimed in that study that the placenta formed a barrier against the spread of COVID-19. In the review, which evaluated the histopathological findings in 3rd trimester placentas with infected maternal SARS-CoV-2, evidence of fetal and maternal VM was reported at the rate of 35% (95% CI: 27.7%-43.0%) and 46% (95% CI 38.0%-54.0%) cases, respectively⁽²³⁾. In a recent study by Patberg et al.⁽²⁴⁾ on asymptomatic pregnant women, VM rates were found to be higher than those of the control group (31.3% vs 3.6%, $p<0.0001$). Although these rates are lower than those of the current study cohort, they are compatible with this study. However, the reason for the lower rates could be attributed to the absence of blinding and control groups in most of the studies. The considerable variation in these studies limits the comparisons that can be made with the current study findings. Theoretically, SARS-CoV-2 can affect placental development during pregnancy either directly through infection or indirectly through inflammation it elicits. Although recent studies seem to support vertical transmission of the SARS-CoV-2 in contrast to previous studies, this feature has not yet been comprehensively described in the literature⁽²⁵⁾. Additionally, the presence of SARS-CoV-2 RNA in placental tissue has still not been clearly demonstrated. In parallel with the findings of Patberg et al.⁽²⁴⁾, the present study results

Table 1. Demographic data for all patients

	Mean ± SD/n (%)
Age (years)	28.8±5.9
Gestational age (weeks)	38.2±1.7
BMI (kg/m ²)	24.0±2.8
CRP (mg/L)	22.0±30.5
Ferritin (µg/L)	153.4±288.9
WBC (10 ³ /µL)	10.4±3.6
Neutrophil (10 ³ /µL)	8.0±3.2
Lymphocyte (10 ³ /µL)	1.8±0.7
Neutrophil/lymphocyte ratio	5.1±2.7
5-min. APGAR score	
≤7	9 (15.0%)
>7	51 (85.0%)
pH of umbilical cord blood	7.3±0.1
Insertion anomaly	3 (5.0%)
Placental weight (g)	587.5±119.3
Placental diameter (cm)	18.0±2.5
Type of delivery	
Vaginal delivery	21 (35.0%)
C/S	39 (65.0%)
Nasal O ₂ requirement	6 (10.0%)
Gestational diabetes mellitus	1 (16.7%)
NICU requirement	9 (15.0%)
BMI: Body mass index, CRP: C-reactive protein, WBC: White blood cell, C/S: Cesarean/section, SD: Standard deviation, NICU: Neonatal intensive care unit	

Table 2. Comparison of demographic and clinical data between groups

	COVID (+) n=30	COVID (-) n=30	p-value*
Age (years)	30.3±5.7	27.4±5.9	0.059
Gestational age (weeks)	38.0±1.6	38.4±1.8	0.412
BMI (kg/m ²)	23.7±3.2	24.2±2.4	0.499
CRP (mg/L)	38.2±36.6	5.8±3.8	0.001
Ferritin (µg/L)	266.4±378.0	40.5±23.1	0.001
WBC (10 ³ /µL)	9.9±3.5	10.9±3.7	0.311
Neutrophil (10 ³ /µL)	7.9±3.0	8.2±3.5	0.647
Lymphocyte (10 ³ /µL)	1.6±0.7	1.9±0.5	0.040
Neutrophil/lymphocyte ratio	5.6±2.6	4.6±2.8	0.157
5-min. APGAR score			
≤7	7 (23.3%)	2 (6.7%)	0.071
>7	23 (76.7%)	28 (93.3%)	
pH of umbilical cord blood	7.3±0.1	7.3±0.1	0.649
Placental weight (gr)	572.9±100.0	602.2±136.1	0.346
Placental diameter (cm)	18.0±2.6	18.0±2.3	0.930
Type of delivery			
Vaginal delivery	6 (20.0%)	15 (50.0%)	0.015
C/S	24 (80.0%)	15 (50.0%)	
Fetal distress	6 (25%)	6 (40%)	0.478
Mother request	7 (29.1%)	1 (6.6%)	0.121
Previous cesarean section	5 (20.8%)	7 (46.6%)	0.153
Non-progress of labour	3 (12.5%)	1 (6.6%)	1.0
Intrapartum bleeding	1 (4.16%)	0	1.0
Maternal nasal O ₂ requirement	6 (20%)	0	0.024
Neonatal PCR positive tests	0 (0%)		
Gestational diabetes mellitus	1 (3.3%)	0	0.492
NICU requirement	7 (23.3%)	2 (6.7%)	0.145
Maternal Blood Pressure			
SP (mmHg)	121.0±7.8	123.0±9.8	0.385
DP (mmHg)	81.0±4.8	83.0±5.2	0.127
Insertion anomaly	0 (0%)	3 (10.0%)	0.237
Maternal-obstetric complications n (%)	4 (13.3%)	1 (3.3%)	0.353
Uterine atony	1 (3.3%)	0	
Oligohydramnios	1 (3.3%)	1 (3.3%)	
Polyhydramnios	1 (3.3%)	0	
Premature rupture of membranes	1 (3.3%)	0	

*Independent t-test, Mann-Whitney U test and Fisher's Exact test, where appropriate. Data are given as mean ± SD, n (%). BMI: Body mass index, CRP: C-reactive protein, WBC: White blood cell, C/S: Cesarean/section, NICU: Neonatal intensive care unit, SP: Systolic pressure, DP: Diastolic pressure, COVID: Coronavirus disease, PCR: Polymerase chain reaction

suggested that even though SARS-CoV-2 could not be examined with PCR in the placental tissues, considering PCR positivity in the newborns, the maternal immune response in the placenta of asymptomatic women was of secondary vascular changes similar to that of symptomatic patients.

The correlation between placental findings and their impact on clinical features is a controversial issue. According to Linehan et al.⁽²⁶⁾ perivillous fibrinoid accumulation and necrotic trophoblast remnants were described as placental findings in pregnant women with COVID-19, however reported that no complications developed in either the mothers or the newborns. In another study, Chen et al.⁽²⁷⁾ identified massive infarction, diffuse fibrinoid deposition, and local increases in syncytial nodes in the placental pathologies of pregnant women with

COVID-19, but no adverse events were seen in the mothers and infants either during pregnancy or postpartum. Hsu et al.⁽²⁸⁾ reported chronic villitis, hypertrophic arteriopathy and extravillous trophoblast islands in the placentas of COVID-19-positive patients, but these findings had no negative clinical effects on mother or infant. These studies showing that the placental vascular structure was affected were conducted on patient populations with predominant clinical COVID-19 symptoms, and this raises the question of the relationship between disease severity and the effect on the placenta in asymptomatic cases. The impact of COVID-19 on maternal and fetal health remains a topic of research interest. Tasca et al.⁽¹¹⁾ compared pregnant women with COVID-19 positivity with healthy pregnant women and suggested that COVID-19 does

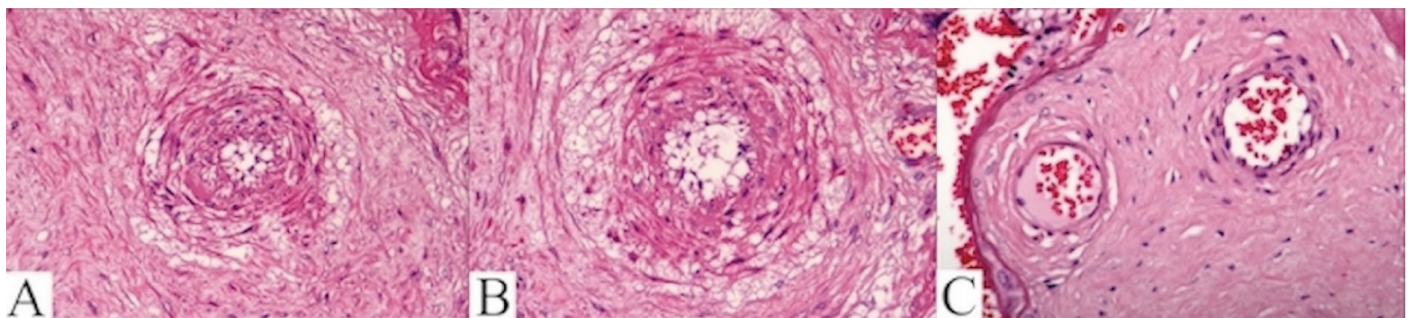


Figure 1. A-B: Mural hypertrophy of the vessel wall in placentas of COVID pregnancies. HE x400. C: Normal small sized vessels of placenta from a healthy control. HE x400

Table 3. Comparison of pathological findings between groups

	COVID (+) n=30	COVID (-) n=30	p-value*
Cord vascular ectasia	13 (43.3%)	8 (26.7%)	0.176
Cord vascular thrombosis	2 (6.7%)	2 (6.7%)	1.000
Placental infarcts	14 (46.7%)	10 (33.3%)	0.292
Retroplacental hemorrhage	2 (6.7%)	0 (0%)	0.150
Chorangiosis	3 (10.0%)	8 (26.7%)	0.095
Distal villous hypoplasia	19 (63.3%)	12 (40.0%)	0.071
Accelerated villous maturation	1 (3.3%)	5 (16.7%)	0.085
Decidual arteriopathy	1 (3.3%)	2 (6.7%)	1.000
Thrombosis	2 (6.7%)	1 (3.3%)	1.000
Avascular villi	12 (40.0%)	8 (26.7%)	0.273
Villous stromal karyorrhexis	0	0	
Mural hypertrophy	25 (83.3%)	9 (30.0%)	0.001
Stem villous obliteration	6 (20.0%)	5 (16.7%)	0.739
Delayed villous maturation	0	0	
Funisitis	0	0	
Chorioamnionitis	1 (3.3%)	2 (6.7%)	1.000
Villitis	0	0	
Perivillous fibrin deposition	17 (56.7%)	23 (76.7%)	0.100

All data are given as n (%). *Fisher's Exact test, COVID: Coronavirus disease

not have unfavorable effects in terms of delivery mode, placental weight, newborn weight and 5 min. APGAR scores. Similarly, Jaiswal et al.⁽¹⁵⁾ suggested that the presence of COVID-19 did not negatively influence maternal complications, except fever, which may be the result of COVID-19. In this study, no significant differences were determined regarding maternal-obstetric complications and fetal-neonatal parameters including birthweight, NICU admissions, and 5 min. APGAR scores, which were similar in both groups, despite the higher rate of placental abnormalities in the COVID-19-positive group. The lack of association between these underlying conditions in this study may be due to secondary. Type II error resulting from a small sample size, and therefore, further studies with larger sample sizes may help identify associations. Controversially, these results may suggest that the placenta acts as a protective biological filter, or actively produce factors that may protect the fetus in utero. Furthermore, in mothers infected with SARS-CoV-2, the fetus may also be protected by vertical transmission of immunoglobulins from the mother to the fetus through the blood supply⁽²⁹⁾. However, the current study findings suggest that damage to the placenta develops with possible adverse effects on the neonates, warranting follow-up for possible side effects.

The rate of delivery by cesarean section was significantly higher in COVID-19-positive pregnant women, which may be related

to “maternal desire associated with reduced contact with the patient and want a more organized intervention”. However, there was a significant increase in the need for maternal nasal O₂ support in asymptomatic cases. Similar to previous studies⁽⁸⁾, this may have been related to an increase in the oxygen requirement during vaginal or cesarean delivery.

As a secondary objective, a significant difference was observed in lymphopenia and CRP and ferritin elevation when asymptomatic COVID-19 patients were compared with control subjects. Although these findings are consistent with the literature^(30,31), they show a correlation with the severity of the disease and suggest that there is a change in laboratory parameters before the development of symptoms in asymptomatic cases. Additionally, no relationship was observed between mural hypertrophy and laboratory parameters in this study. As there are no studies in the literature that have investigated the relationship between placental pathologies and laboratory parameters, there is an obviously need for more research on this subject.

In the study, which included asymptomatic COVID-19-positive pregnant women, mural hypertrophy was a striking finding with respect to placental VM findings. The determination of VM in the placenta has been associated especially with the comorbidities of hypertension and preeclampsia in pregnancy⁽³²⁾. However, as these comorbidities were excluded

Table 4. Factors affecting the formation of mural hypertrophy in vessel walls

	Mural Hypertrophy		Univariate		Multivariate	
	Negative (n=26)	Positive (n=34)	OR (95% CI)	p-value	OR (95% CI)	p-value
Age	27.73±6.53	29.65±5.43	1.058 (0.968 - 1.156)	0.217	0.996 (0.872 - 1.137)	0.950
Pregnancy week	38.32±1.69	38.11±1.71	0.927 (0.683 - 1.260)	0.630	1.083 (0.700 - 1.675)	0.720
BMI	24.52±2.59	23.6±2.92	0.886 (0.733 - 1.070)	0.209	0.806 (0.613 - 1.061)	0.124
CRP	7.92±11.08	32.77±36	1.066 (1.013 - 1.122)	0.014	1.032 (0.964 - 1.104)	0.365
Ferritin	46.06±27.64	235.54±364.16	1.011 (0.999 - 1.023)	0.065	1.001 (0.992 - 1.011)	0.775
WBC	10.72±3.87	10.15±3.42	0.956 (0.828 - 1.104)	0.540	0.940 (0.731 - 1.208)	0.630
NLR	4.41±2.69	5.62±2.65	1.209 (0.966 - 1.513)	0.097	1.241 (0.906 - 1.699)	0.179
Type of delivery						
Normal	12 (57.1)	9 (42.9)	Reference		Reference	
Cesarean	14 (35.9)	25 (64.1)	2.381 (0.805 - 7.039)	0.117	1.612 (0.302 - 8.596)	0.576
Complications						
Negative	20 (40.8)	29 (59.2)	Reference		Reference	
Positive	6 (54.5)	5 (45.5)	0.575 (0.154 - 2.144)	0.410	0.303 (0.046 - 2.021)	0.218
COVID-19						
Negative	21 (70)	9 (30)	Reference		Reference	
Positive	5 (16.7)	25 (83.3)	11.667 (3.384 - 40.22)	<0.001	4.716 (1.012 - 22.251)	0.048

Data are given as n (%) or mean ± SD. OR: Odds ratio, CI: Confidence interval, BMI: Body mass index, CRP: C-reactive protein, WBC: White blood cells, NLR: Neutrophil to lymphocyte ratio, COVID-19: Coronavirus disease, SD: Standard deviation

in this study, this suggests that the finding of VM could be specific to COVID-19⁽³³⁾. Since there was no difference between the groups in terms of comorbidities during pregnancy and neonatal follow-up, it was thought that the presence of placental vascular pathology could have been related to a placental effect even in asymptomatic cases and perhaps not enough time had passed for negative outcomes to have formed.

Strong aspects of this study can be considered to be that it is the first prospective case-control study to review the placental findings at the time of delivery of asymptomatic COVID-19-positive pregnant women. Additionally, hypertensive patients were excluded as that could have caused placental vascular pathologies, and asymptomatic COVID-19 patients were included, which is the most common but least studied patient population⁽³³⁾.

Study Limitations

However, the study also had some limitations; the number of patients was relatively low, although it was within acceptable limits for the power of the study. A second limitation was the focus on only the effects of COVID-19 during pregnancy and the neonatal period, and the lack of short-term and long-term results. Thirdly, this study examined the experience of a single centre, and therefore, further studies including data from more than one academic centre will undoubtedly make a significant contribution to explaining the impact of COVID-19 on pregnancy and the placenta. A final limitation could be considered to be that the sensitivity of the antibody tests used in the selection of the case and control groups has been reported to be 50-70%⁽³⁴⁾.

Conclusion

This study demonstrated that asymptomatic COVID-19 positivity in the perinatal period had no significant effect on the pregnancy or neonatal complications. However, mural hypertrophy in the placenta was detected at a significantly high rate in the COVID-19 patients. Although concerns about placental vasculopathy increase in COVID-19-positive pregnant women, further studies may clarify the importance of histological placental findings on maternal and neonatal health.

Acknowledgment

We would like to thank Prof. Dr. Veli Mihmanlı for his approval for the “signature to be obtained from the clinic head,” which is mandatory for the application of the ethics committee.

Ethics

Ethics Committee Approval: Ethics committee approval was obtained from the local “Medical Ethics and Institutional Review Board” with ID number E-48670771-514.10/136 in April 19, 2021, and the study was completed in accordance with Helsinki Declaration principles.

Informed Consent: All the pregnant women provided informed consent to participate in the study.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: O.Ş., A.Y.A., E.A., H.B., Ö.Y., Concept: O.Ş., A.Y.A., E.A., H.B., Ö.Y., Design: O.Ş., A.Y.A., E.A., H.B., Ö.Y., Data Collection or Processing: O.Ş., A.Y.A., E.A., H.B., Ö.Y., Analysis or Interpretation: O.Ş., A.Y.A., E.A., H.B., Ö.Y., Literature Search: O.Ş., A.Y.A., E.A., H.B., Ö.Y., Writing: O.Ş., A.Y.A., E.A., H.B., Ö.Y.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

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