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Research Article

Ki-67 Expression Level in placentas with COVID-19 Infected Women

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Abstract

Objective: The SARS-CoV-2 virus, which emerged in December 2019 in Wuhan, China, spread very quickly in a short time and was declared a pandemic by the World Health Organization in March 2020. Physiological and immunological changes during pregnancy cause complications in respiratory tract infections. Complications by COVID-19 lead to a systemic effect that causes maternal and fetal mortality and morbidity. In this study, we aimed to investigate histopathological changes and Ki-67 expression in placentas of women with positive COVID-19 infection.

Study Design: Placentas of 10 samples COVID-19 positive and 10 samples Covid-19 negative pregnant patients who were hospitalized in the Gynecology and Obstetrics Clinic of Dicle University Faculty of Medicine were included in the study. Placental tissues were fixed in 10% formaldehyde (24 hours) and processed for routine paraffin wax tissue staining. Hematoxylin Eosin dye and Ki-67 immunohistochemical staining were performed.

Results: Decidual cell degeneration, increased numbers of syncytial nodes, dilatation in vessels, degenerated villi were observed in the placentas of COVID-19 positive pregnant women. COVID-19 damage regular histology of placental structures. Ki-67 expression was intensely increased in COVID-19 positive placentas compared to COVID-19 negative placentas.

Conclusion: We suggest that COVID-19 causes some abnormalities in the histology of placentas and cell death, which leads to new cell proliferation.

Keywords: COVID-19, placenta, villi, Ki-67, histopathology

1. INTRODUCTION

The new coronavirus called Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) caused global pandemic beginning in late 2019 in Wuhan, China, through all over the world. The World Health Organization (WHO) called the disease as Coronavirus Disease 2019 (COVID-19)[1]. COVID-19 infection is a multi-system disease and predominantly affected the lungs. In severe cases, cytokine storms occur causing abnormal cellular immune responses, abnormal coagulation, respiratory and cardiovascular failure, multiple organ damage, and eventually death[2]. Novel coronavirus enters the cell via its S protein utilizing angiotensin converting enzyme 2 (ACE2), in the human lung. However, ACE2 enzyme is expressed in many organs of human, such as intestine, brain, placenta, especially in placental villi and its associated structures[3]. Although it has been two years of COVID-19 pandemic, there are still no exact literature information about placenta and COVID-19 infection. During pregnancy, COVID-19 may pass through possible maternal-fetal transmission [4,5].

Congenital infection of SARS-CoV-2 is extremely rare despite many cases of COVID-19 during pregnancy. To demonstrate placental infection, viral localization in placental tissue should be demonstrated by molecular techniques. Vertical transmission has been observed in two SARS-COV-2 cases to date, and viral localization in the placenta has been detected. Current pathological studies have focused on the expression of ACE2 or TMPRSS2 protein in lung tissue. Pregnancy increases the risk of adverse obstetric and neonatal outcomes from many respiratory viral infections. The maternal immune system is altered during pregnancy to prevent fetal rejection and aid fetal development. Some viral infections cause a more severe or prolonged illness in pregnant women. The SARS-related coronavirus has resulted in high rates of maternal death, miscarriage, and premature birth. Numerous influenza studies have shown an increased risk of maternal morbidity and mortality compared with non-pregnant women [6,7]. SARS-CoV-2 is highly contagious and can be passed from person to person through multiple transmission routes. There has been a rapid increase in knowledge about the genetic, virological, epidemiological, and clinical aspects of COVID-19. However, few reports have been published describing the risks and specific effects of SARS-CoV-2 among pregnant women and newborns. Controversy continues as to whether SARS-CoV-2 can be transmitted from an infected mother to her baby in the womb [8,9].

In this study, the placental histopathological structures were examined in the placentas of pregnant patients with COVID-19 positive by histochemical and immunohistochemical techniques.

2. METHODS

This study was approved by Republic of Turkey, Ministry of Health and local ethics committee (date: 17.12.2021, record

number: 24916). This study was supported by Dicle University Research Projects Unit (DUBAP) with the Project number (TIP.21.010).

2.1. *Placental tissue histological processing*

The umbilical cords of 10 samples COVID-19 positive and 10 samples COVID-19 negative pregnant patients who were hospitalized in the Gynecology and Obstetrics Clinic of Dicle University Faculty of Medicine were included in the study. Patients with chronic and systemic diseases were not included in the study. After the placental tissues were fixed in 10% formaldehyde (24 hours) and passed through ascending alcohol series, and then incubated in paraffin wax. Later, the tissues were embedded in paraffin blocks and 5-µm thick sections were taken from the blocks with a microtome (catalog no: Leica RM2265, Wetzlar, Germany) and Hematoxylin Eosin and Ki-67 immunohistochemical staining were performed.

2.2. *Hematoxylin-Eosin Staining*

In order to remove the excess paraffin, placental tissues sections were kept in xylene and then passed through descending alcohol series and washed in distilled water. Hematoxylin stain for 8 minutes and eosin stain for 4 minutes were applied to the sections, respectively. The sections were quickly immersed in increasing alcohol series and washed in absolute alcohol for 2 minutes. Finally, the sections were mounted with mounting medium.

2.3. *Anesthesia and Surgical Procedures*

Placental sections were brought to distilled water and washed in phosphate buffer solution (PBS) 3 times for 5 minutes. Sections were placed in EDTA buffer solution (pH: 8.0, catalog no: ab93680, Abcam, Cambridge, USA) and heat-induced epitope retrieval was performed. Then, the sections were left at room temperature for 20 minutes and were taken back into PBS. Hydrogen peroxide solution (catalog no: TA-015-HP, ThermoFischer, Fremont, CA, USA) was dropped onto the sections and waited for 20 minutes. Then, it was washed again with PBS for 3 times for 5 minutes and kept in Ultra V Block (catalog no: TA-015-UB, ThermoFischer, Fremont, CA, USA) solution for 7 minutes. Sections were incubated overnight at +4°C with Ki-67 antibody. Biotin-containing secondary antibody (catalog no: TP-015-BN, ThermoFischer, Fremont, CA, USA) was dropped onto the sections and incubated for 14 minutes at room temperature. After washing with PBS, streptavidin-peroxidase (catalog no: TS-015-HR, ThermoFischer, Fremont, CA, USA) was dropped for 15 minutes. Diaminobenzidine (DAB) (catalog no: TA-001-HCX, ThermoFischer, Fremont, CA, USA) was dropped onto the sections. After the tissues were kept in PBS for 15 minutes, counterstained with Harris hematoxylin, then the sections were mounted with mounting medium Entellan (catalog no: 107961, Sigma-Aldrich, St. Louis, MO, United States) and visualized under Zeiss Imager A2 photomicroscope.

3. RESULTS

3.1. *Histopathological findings*

Pyknosis in decidual cell nuclei, vacuolization, an increase in syncytial nodes (arrowhead) and fibrinoid tissue, vascular dilatation and degeneration of villi were observed in the placentas of COVID-19 positive pregnant women (Figure 1). COVID-19 damaged regular histology of placental structures.

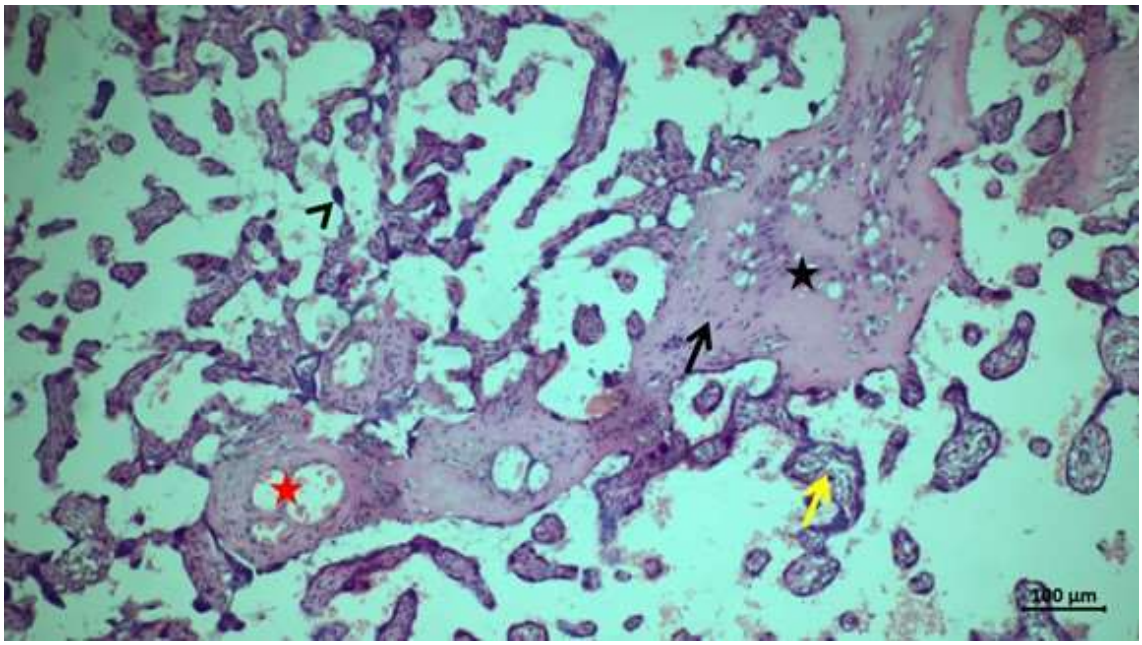

Figure 1: Hematoxylin Eosin staining of placentas with positive COVID-19 infection. Pycnotic decidual cell nuclei (black arrow), syncytial nodes (arrowhead), fibrinoid tissue (black star), vascular dilatation (red star) and degenerated chorionic villi (yellow arrow)

3.1. *Immunohistochemical findings*

Ki-67 expression was generally negative in the placentas of COVID-19 negative pregnant women (Figure 2). Ki-67 expression was positively observed in a few trophoblast cells while it was negative in trophoblast layer, villous stroma and vascular structures.

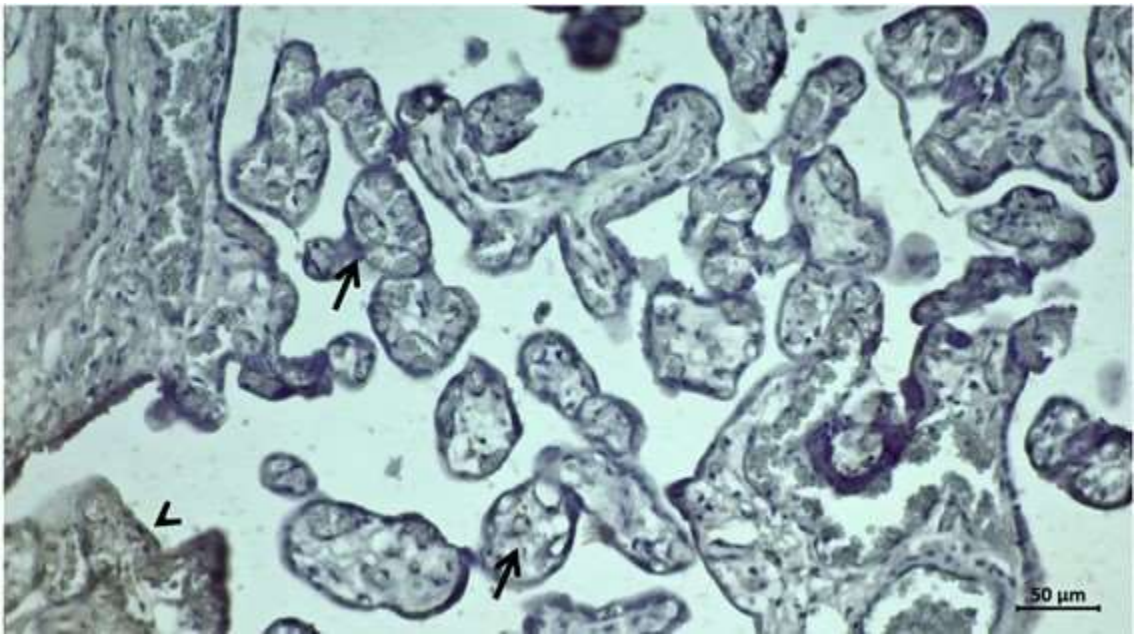

Figure 2: Ki-67 immune activity in COVID-19 negative placentas. Positive (arrowhead) and negative Ki-67 expression (black arrows).

Intense Ki-67 expression was observed in syncytial nodes, Hoffbauer cells and trophoblast cell layer of the placentas in pregnant women with COVID-19 positive (Figure 3). COVID-19 was caused degeneration of trophoblast and stromal cells, leading to cellular proliferation via elevating Ki-67 expression

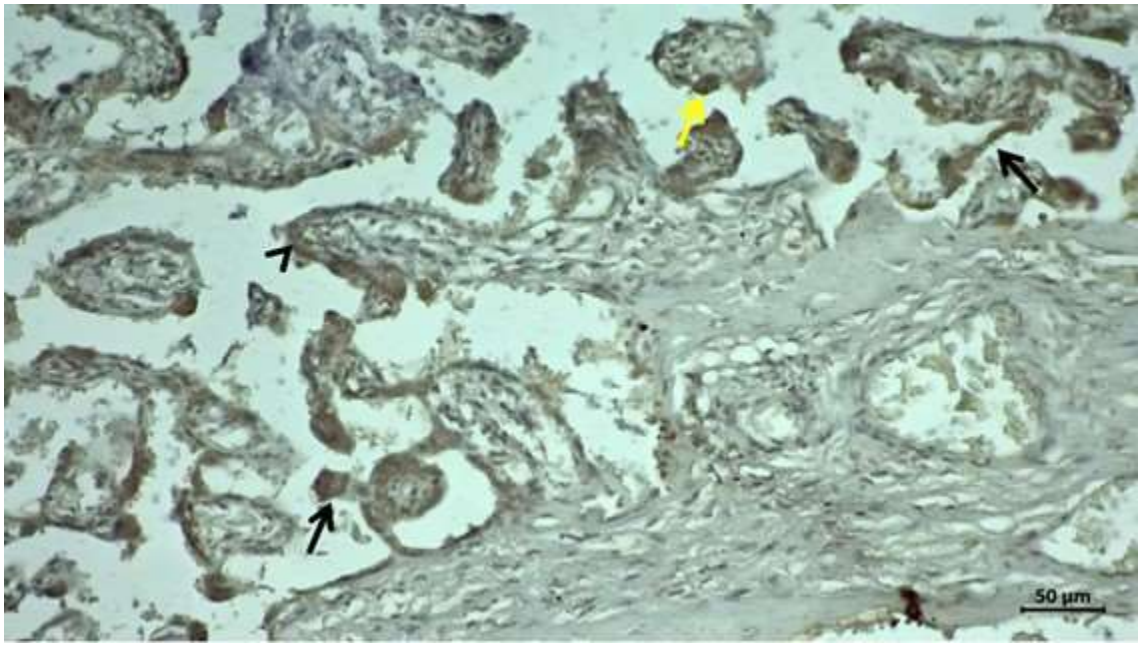

Figure 3: Intense Ki-67 expression in syncytial nodes (black arrow), Hoffbauer cells (yellow arrow) and trophoblast cell layer (arrowhead).

4. DISCUSSION

Like the previous SARS and Middle East Respiratory Syndrome (MERS), the coronavirus pandemic is known to cause maternal or perinatal distress such as maternal infections, maternal death, and spontaneous abortion during pregnancy [10]. Similarly, in a recent study by Di Mascio et al., conditions such as miscarriage, premature birth, stillbirth, and preeclampsia have been reported in relation to COVID-19 disease [11]. Therefore, histopathological examination of the placenta will make important contributions to the protection of both maternal and fetal health. There is still limited data on the impact of the current COVID-19 outbreak on women affected during pregnancy, their newborns, and the pediatric population. Therefore, diagnosis and treatment of a disease in pregnancy requires great importance to prevent maternal and neonatal complications related to this disease. In the studies, SARS-CoV-2 genome was found in the vaginal mucosa of a pregnant woman and term placenta. In addition, the presence of specific anti-SARS-CoV-2 IgM and IgG antibodies in the umbilical cord blood and milk samples of pregnant women has been reported. Similarly, studies have been conducted

showing that vertical transmission is possible in the uterus in SARS-CoV-2 positive pregnant women. Finally, it explains the inflammatory response triggered by SARS-CoV-2 infection in pregnant women at both systemic and placental levels [12].

In many studies, it has been shown that the placentas of SARS-CoV-2 cause changes in placental structures by affecting microvascular changes and inflammation. A study of sixteen placentas from pregnant women with SARS-CoV-2 viral infection showed maternal vascular mal-perfusion, decidual arteriopathy, atherosis, fibrinoid necrosis, and mural hypertrophy of membrane arterioles. The authors also reported peri villous fibrin deposition in some placental tissues, intervillous thrombus, villous edema, and chorangiosis. Among these findings, chorangiosis is associated with a decrease in maternal oxygen saturation [13]. In our study, placentas of COVID-19-enfected women showed decidual arteriopathy, pyknotic decidual cell nuclei, fibrinoid accumulation, villous degeneration, vascular dilatation (Figure 1).

SARS-CoV-2 infects target host cells by binding to cell membrane angiotensin converting enzyme II facilitated by Type II transmembrane serine protease (TMPRSS2). Cell types expressing ACE2 in the placenta, syncytiotrophoblasts and cytotrophoblasts in villi, decidual stromal cells, decidual perivascular cells and endothelial and vascular smooth muscle cells. Co-expression of the viral receptors ACE2 and TMPRSS2 in abundance in the human placenta could theoretically increase the susceptibility of the placenta and possibly the fetus to SARS-CoV-2 infection [14].

Ki-67 is a cellular marker for proliferation which functions in interphase and mitotic cells. Measurement of Ki-67 expression level can be used for the prognosis of many tumors [14]. Ki-67 expression index has been used in the normal placenta, hydatidiform moles, and choriocarcinoma. Aberrant placenta formation may be related with abnormal regulation of molecular changes and the proliferation of trophoblast cells so that trophoblasts are able to migrate and invade the uterine wall [15,16]. Kaya et al recorded that Ki-67 expression was increased in villous cytotrophoblasts of preeclamptic placentas relative to controls. They showed an increased number of villous cytotrophoblasts in preeclampsia as compared to in normal pregnancy by analyzing with Ki-67 immunohistochemical staining [17]. Unek et al showed that Ki-67 expression was increased in placental villi of preeclampsia [18]. Another preeclampsia study recorded that the Ki-67 index was high in villous trophoblasts of preeclamptic patients [19]. A study demonstrated that Ki-67 expression was high in trophoblast columns of accrete subtypes [20]. In our study, we found that Ki-67 expression was mostly negative in placentas of women with negative COVID-19 infection. In the placentas of women with positive COVID-19 infection, intense Ki-67 immune reaction was observed in placental villi and Hoffbauer cells (Figure 2 and 3).

COVID-19 caused the pathologies in placenta and elevated the Ki-67 expression. Comparing to COVID-19 negative placentas, positive COVID-19 placentas led to placental injury reflecting abnormalities in placentas with high Ki-67 expression.

5. CONCLUSION

Relative to negative COVID-19 infected patients, COVID-19 infection disrupted the regular histology of placentas, caused the degeneration of placental villi and vascular structures. COVID-19 infection increased the Ki-67 expression, leading to abnormal cellular events.

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Conflict of Interest

The authors declare no conflict of interest and have nothing to disclose.

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