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Research Article

EGFR expression in COVID19 placentas

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Abstract

Introduction: Coronavirus disease 2019 (COVID-19) pandemic is a global health problem that has claimed many lives worldwide. The disease is affecting almost all populations, but it poses a particular risk for groups such as the elderly and those with chronic diseases. Although the number of studies on clinical situations involving pregnant women and their infants infected with COVID-19 is increasing, the factors supporting the negative association between COVID-19 and pregnancy remain unclear. In this study, we aimed to examine the placental histopathologic structure using EGFR (Epidermal Growth Factor) antibody in placentas of COVID-19 pregnant patients.

Materials and methods: In our study, placentas of a total of 40 pregnant women (regardless of age), including 20 pregnant women with C0VID-19 and 20 normotensive pregnant women, were used, and placentas were compared histopathologically and immunohistochemically.

Results: In the hematoxylin and eosin stained sections of the control group placentas, tissue integrity was preserved and cellular structures were normal. In the placentas of Covid -19 group patients, it was observed that degenerations increased and cellular deformations developed. In sections of control group placentas, EGFR expression was negative in decidual cells and in areas with syncytial nodes. EGFR expression was positive in cytotrophoblast cells. In placenta sections of Covid-19 patients, EGFR expression was positive in vascular endothelium and Hofbauer cells.

Conclusion: In our study, it was observed that EGFR expression, an important biomarker in tissue regeneration, was inhibited. We also concluded that Covid-19 caused degeneration and inflammation in placental tissue.

Keywords: Covid-19, Placenta, EGFR, Pregnancy, Histopathology.

INTRODUCTIOUN

The SARS-CoV-2 virus has a positive RNA genome that is linear. It has the ability to continually adapt through random genome mutations as part of natural selection. The mutations that the SARS-CoV-2 virus undergoes may allow the virus to evade the host's immune response, resist antiviral drugs or increase its transmissibility, thus giving the virus a selective advantage.

Since the emergence of the virus, there has been a lack of a consistent scheme for naming variants of concern (VOCs) as well as variants of interest (VOIs) of SARS-CoV-2. Three scientific nomenclature systems, including the Global Initiative on Sharing All Influenza Data (GISAID), Nextstrain and Pango, have been used to identify and track genotypes of SARS-CoV-2 virus [1].

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 coronavirus associated with SARS results in a high rate 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 [2,3]. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is highly contagious and can be transmitted from person to person through multiple transmission routes [4]. There has been a rapid increase in knowledge about the genetic, virologic, epidemiologic 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. There is ongoing debate about whether SARS-CoV-2 can be transmitted in utero from an infected mother to her baby[5,6].

The placenta, which provides the circulation between the mother and the fetus, is an organ that contains maternal and fetal structures that produce hormones necessary for fetal nutrition and the continuation of pregnancy and whose function ends with pregnancy [7]. One of the key growth factors during placental development is epidermal growth factor (EGF) and its receptor (EGFR). EGF is a polypeptide composed of 53 amino acids that has the ability to provoke mitogenic effects on epidermal and mesothelial cells[8]. In this study, we aimed to examine the placental histopathologic structure using EGFR (Epidermal Growth Factor) antibody in placentas of COVID-19 pregnant patients. This study aimed to

structurally investigate the effects of COVID-19 disease on the placenta.

MATERIAL AND METHODS

Procurement of Placentas and Experimental Protocol

In our study, the approval of the Ethics Committee of Dicle University Faculty of Medicine dated 17.03.2022 and numbered 66 was obtained and the study was started. A total of 40 pregnant women (regardless of age), including 20 pregnant women diagnosed with C0VID-19 and 20 normotensive pregnant patients, were used in our study, and patients with chronic and systemic diseases were not included in the study. Patients with symptoms of cough, sore throat, difficulty breathing and PCR test positive for COVID-19 virus were accepted, informed consent form was obtained from the patients and placentas were obtained.

Hematoxylin-Eosin Staining Protocol

Paraffin sections were soaked in Xylol 2 times for 15 minutes, in decreasing degrees of alcohol (100%, 96%, 90%, 70%, 50% ethyl alcohol) for 10 minutes each and then brought to distilled water. Harris Hematoxylin solution was kept for 8 minutes. The sections were kept under running tap water for 5 minutes. The sections removed from tap water were kept in distilled water for a few minutes to rest. For counterstaining, the sections were kept in Eosin solution for 2 minutes. The sections were passed through a series of rising grades of ethyl alcohol for dehydration and finally passed through Xylol for 2x15 minutes for polishing. The sections were covered with entellan and examined under a light microscope using Zeiss Imager A2 light and Zen 3.00 software program.

Immunohistochemical Staining Protocol

5 µm thick sections were first soaked in xylol for 15 minutes in 2 series. Then, they were soaked in decreasing alcohol series (100%, 90%, 80%, 70%) for 5 minutes each. After soaking in distilled water for 2x15, the sections were subjected to antigen retrieval in EDTA solution in a microwave oven at 90 0 C for 3 minutes. The sections removed from the microwave oven were incubated in Phosphate Buffer Saline (PBS) at room temperature for 20 minutes for resting. Then, before the sections were placed on the immunohistochemistry bar, water prepared in advance was placed on the immunohistochemistry bar to keep the environment moist. The sections were first bordered with a hydrophobic pencil to delineate the tissue area, then hydrogen peroxide blocking solution (2 ml of 30% H2O2 + 18 ml of methanol) was dripped on them immediately after they were placed on the immunohistochemistry bar and kept for 20 minutes. After pouring hydrogen peroxide, the sections were washed in PBS for 2x5 minutes and incubated in Ultra V Block solution (Thermo, cat no: TA-0155-UB, Thermo Fischer, Fremont, CA, USA) for 8 minutes. The blocking solution was then removed and EGFR primary antibody (1:100, Anti-IL-6 Antibody (B-9): sc-7480, Santa Cruz Biotechnology, Inc.) was applied and incubated at 4+ 0 C overnight. The next day, the sections were taken to the stove temperature and allowed to rest for 1 hour. The sections were then washed for 2x5 minutes. After washing, the sections were kept in secondary antibody (Thermo, cat no: TP-015-BN, Fischer, Fremont, CA, USA) for 14 minutes. After removing the secondary antibody, the sections were washed again with PBS for 2x5 minutes. The tissue sections were then incubated with Streptavidin peroxidase (Thermo, cat no: TP-015-BN, Fischer, Fremont, CA, USA) for 15 minutes for enzyme binding. They were then washed with PBS for 2x5 minutes and reacted with DAB chromogen (Thermo, cat no: TA-125-HD). At this stage, the reaction status of the tissue sections was carefully monitored under a microscope. Sections with specific reaction status were removed in PBS. The sections were then counterstained with Mayer's hematoxylin, passed through the increasing alcohol series and xylollen and covered with entellan. The preparations were examined under a light microscope using Zeiss Imager A2 light and Zen 3.00 software program.

RESULTS

Histopathologic Findings

In hematoxylin and eosin stained sections of control group placentas, tissue integrity was preserved. Desidual cells with polygonal appearance were found to have chromatin-rich nuclei. Although vacuole areas were observed in some areas of the placenta structure, tissue integrity was preserved. It was observed that the endothelial cells of the vessels in the areas with chorionic stem villus structures were flat and regular (Figure 1-A). When the placenta structures of Covid -19 group patients were examined, it was found that degenerations were increased. Nuclei of decidual cells were observed to have a picnotic structure. Intense congestion and dilatation were detected in the blood vessels. Hyperplasia in the vascular endothelium was quite prominent (Figure 1-B).

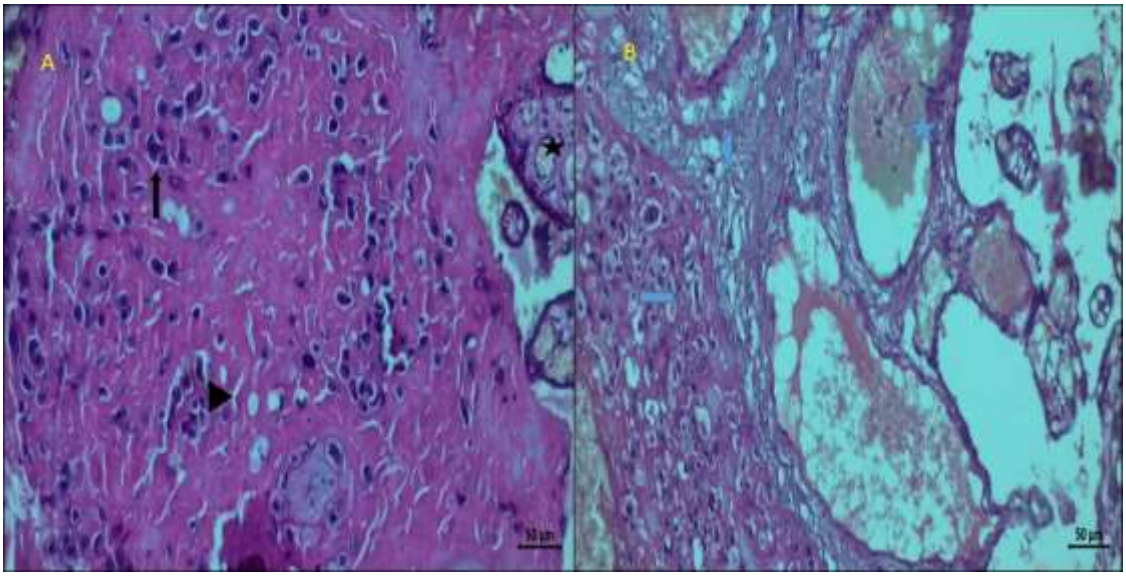

Figure 1-A: control group H&Eosin staining, Figure 1-B: Covid-19 group H&Eosin staining.

Immunohistochemical Findings

When EGFR immunohistochemically stained sections of the control group were examined, EGFR expression was found to be negative in decidual cells. EGFR expression was positive in cytotrophoblast cells. EGFR expression was negative in the areas where syncytial nodes were found (Figure 2-A).

In placenta sections of Covid-19 patients, EGFR expression was prominent in the vascular endothelium. Degenerations were observed in the basement membrane structure of the placenta. In Hofbauer cells, degenerative changes and positive EGFR expression were observed. EGFR expression was positive in syncytial nodes (Figure 2-B).

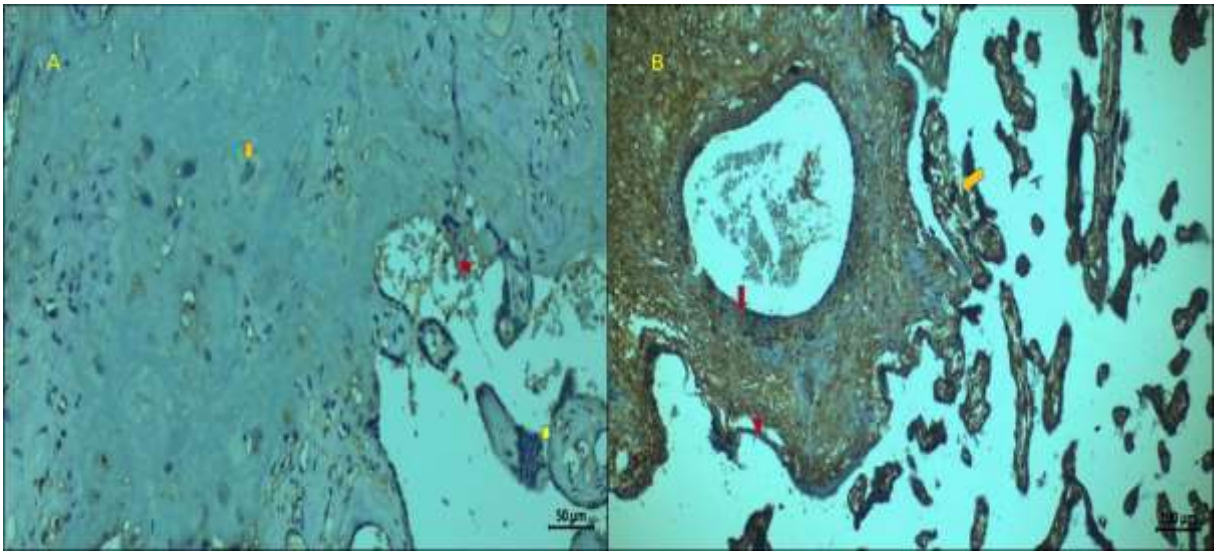

Figure 2-A: control group EGFR Immunohistochemical staining, Figure 2-B: Covid-19 group EGFR Immunohistochemical staining.

DISCUSSION

As of 2019, SAR-CoV-2 with severe infection causes complications in many tissues and organs. In addition to its widespread impact, the coronavirus is known to cause life-threatening respiratory infections and systemic complications in children and adults [9]. Studies have shown that some patients continue to have complaints such as pain after covid-19 infection [10]. As the Covid-19 pandemic continues to spread globally, countless pregnant women are becoming infected, posing a danger to both themselves and their babies. Physiological changes that occur during pregnancy, especially changes in the immune system, predispose to infectious diseases. The effects of COVID-19 on the placenta and fetus, and the potential mechanisms of vertical transmission of the virus between pregnant women and their babies are still under investigation [11].

Studies have reported the detection of SARS-CoV-2 in tissues on the fetal side of the placenta, indicating that the fetus has undergone transplacental infection with SARS-CoV-2. Placentas from infected mothers in which intrauterine transplacental transmission of SARS-CoV-2 occurred show an unusual combination of pathology findings that may represent risk factors for the placenta. SARS-CoV-2 enters human cells via the surface receptor protein Angiotensin Converting Enzyme-2 (ACE2), which is ubiquitous in various human organs, including the placenta [11].

In the literature, there are studies showing histopathologic changes caused by COVID-19 infection on both placenta and umbilical cord [12-13]. Apart from covid-19 infection, diseases such as preeclampsia also cause histologic changes in the placenta [14]. While several routes of human-to-human transmission of SARS-CoV-2 have been identified, vertical transmission of infection from mother to fetus remains a valid and unresolved question. Studies have not tested placental tissue for the presence of the virus, nor have they analyzed the tissue morphologically. Therefore, it is difficult to find comprehensive studies on the interaction of SARS-CoV-2 between complications and the placenta. On the other hand, little is known about the time course of SARS-CoV-2 infection and morphologic changes in the placenta [15]. The literature suggests that SARS in humans is transmitted vertically and fetal vascular malperfusion (FVM) in the form of thrombotic vasculopathy has also been reported. While some previous studies do not support vertical transmission of SARS-CoV-2 in humans, many new studies are emerging suggesting some intrauterine transmission. Therefore, congenital infection caused by SARS-CoV-2 virus is still a controversial issue, as it is limited by the relevant data available to date and the best way to confirm this infection is to identify viral particles in placental tissue [16].

Khong et al. examined the placentas of 20 COVID-19 positive mothers and categorized the lesion using the Amsterdam criteria. In a histopathologic study with Hematoxylin and Eosin (H&E) staining, the most common findings observed were malperfusion of the fetal vasculature and intramural fibrin deposition. In two cases, focal presence of villous stromal-vascular karyorrhexis was observed, and several placentas showed intramural non-occlusive thrombi, meconium, macrophages, MVM lesions and perivillous fibrin deposition. In one case with high-grade infection (pneumonia and acute hypoxia), placental tissue showed features of acute chorioamnionitis and acute funicitis. Chronic villitis was observed in four other cases and obliterative vasculopathy in one case [17].

In a case-control study of placentas from SARS-CoV-2 positive mothers, an increased incidence of fibrin deposition, microcalcifications, syncytial nodes, small fibrotic villi and villous agglutination was observed [17]. In a case series of 5 placentas from COVID-19 positive mothers, histopathologic findings revealed thrombin formation in large blood vessels in all cases, and in one placenta, fibrin was found in the blood vessels of the villous stroma. In another placenta, focal avascular villi were observed[18]. In our study, the

histopathologic changes observed in the placentas were compatible with the histopathologic changes observed in other studies in the literature.

EGFR has important effects on cell division, differentiation, survival, proliferation, growth and migration. In immunohistochemical studies, EGFR was found to have no effect on the development of placental tissue and its expression was very low. EGFR is known to express a specific function in the proliferation and differentiation of astrocytes and survival of postmitotic neurons. In placenta tissues, it is difficult to find the presence of expression of this expression due to degeneration occurring in cells[19]. In our study, significant EGFR expression was observed in the endothelium of the vessels and syncytial nodes of the placentas of pregnant women infected with Covid-19 virus. In addition, EGFR expression was also found to be positive in Hofbauer cells.

CONCLUSION

In conclusion, histopathologic examination of COVID-19 placentas showed that the nuclei of decidual cells were picnotic and there was intense congestion and dilatation of blood vessels. It was observed that EGFR expression, an important biomarker in tissue regeneration, was inhibited. In our study, it was concluded that Covid-19 causes degeneration and inflammation in placental tissue.

REFERENCES

(1) 1- Fox, D. (2020). What you need to know about the novel coronavirus. Nature (Lond.) https://doi.org/10.1038/d41586-020-00209-y

(2) Golden TN, Simmons RA. Maternal and neonatal response to COVID-19. Am J Physiol Endocrinol Metab. 2020;319(2):E315-E319. https://doi.org/10.1152/ajpendo.00287.2020 PMid:32574110 PMCid:PMC7381709

(3) Baergen RN, Heller DS. Placental Pathology in Covid-19 Positive Mothers: Preliminary Findings. Pediatr Dev Pathol. 2020;23(3):177-180. https://doi.org/10.1177/1093526620925569 PMid:32397896 PMCid:PMC7252218

(4) Özdemir, Ö., & Ayşegül, P. Diagnosis, treatment and prevention of Covid-19 infection in children. Journal of Biotechnology and Strategic Health Research, 2020;4:14-21. https://doi.org/10.34084/bshr.711208

(5) Dong L, Tian J, He S, et al. Possible Vertical Transmission of SARS-CoV-2 From an Infected Mother to Her Newborn [published online ahead of print, 2020 Mar 26]. JAMA. 2020;323(18):1846-1848. https://doi.org/10.1001/jama.2020.4621 PMid:32215581 PMCid:PMC7099527

(6) Shanes ED, Mithal LB, Otero S, Azad HA, Miller ES, Goldstein JA. Placental Pathology in COVID-19. Am J Clin Pathol. 2020;154(1):23-32. https://doi.org/10.1093/ajcp/aqaa089 PMid:32441303 PMCid:PMC7279066

(7) Handwerger, S. and M. Freemark, The roles of placental growth hormone and placental lactogen in the regulation of human fetal growth and development. J Pediatr Endocrinol Metab, 2000;13(4):343-56. https://doi.org/10.1515/JPEM.2000.13.4.343 PMid:10776988

(8) Gauster M, Moser G, Orendi K, Huppertz B. Factors involved in regulating trophoblast fusion: potential role in the development of preeclampsia. Placenta 2009;30: 49-54 https://doi.org/10.1016/j.placenta.2008.10.011 PMid:19027159

(9) Zhu N, Zhang D, Wang W, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med. 2020;382(8):727-33. https://doi.org/10.1056/NEJMoa2001017 PMid:31978945 PMCid:PMC7092803

(10) Taş, A., & Baloğlu, M. Post-COVID syndrome and pain perception in outpatients with COVID-19. European Review for Medical & Pharmacological Sciences, 2023;27(14).

(11) WHO Coronavirus (COVID-19) Dashboard [Internet]. [cited 2022 May 6]. Available from: https://covid19.who.int

(12) Tas F., Yılmaz M., Erdemci, F., Asır, F., & Deveci, E. Examining the long-term effects of COVID-19 on the umbilical cord. Journal of Health Sciences and Medicine, 2022;5(5):1371-1377. https://doi.org/10.32322/jhsm.1138691

(13) Taş, F., Erdemci, F., Yilmaz, M., Aşir, F., Özüdoğru, O., & Deveci, E. Acute and long-term pathological effects of COVID-19 on the placenta during second trimester and labor. Revista de Investigaciones Universidad del Quindío, 2023;35(1):228-235. https://doi.org/10.33975/riuq.vol35n1.1154

(14) Ekinci, A., Aktas, A., Dönder, A., Ekinci, C., Turgut, A., Deveci, S., ... & DEVECI, E. Expression of endothelin-1 and Ki-67 in normotensive and severe preeclamptic placentas. Int. j. morphol, 2018;36(1):109-112. https://doi.org/10.4067/S0717-95022018000100109

(15) Robbins JR, Bakardjiev AI. Pathogens and the placental fortress. Curr Opin Microbiol. 2012;15(1):36-43. https://doi.org/10.1016/j.mib.2011.11.006 PMid:22169833 PMCid:PMC3265690

(16) Jing Y, Run-Qian L, Hao-Ran W, et al. Potential influence of COVID-19/ACE2 on the female reproductive system. Mol Hum Reprod. 2020;26(6):367-73. https://doi.org/10.1093/molehr/gaaa030 PMid:32365180 PMCid:PMC7239105

(17) Khong TY, Mooney EE, Ariel I, et al. Sampling and Definitions of Placental Lesions: Amsterdam Placental Workshop Group Consensus Statement. Arch Pathol Lab Med. 2016;140(7):698-713. https://doi.org/10.5858/arpa.2015-0225-CC PMid:27223167

(18) Linehan L, O'Donoghue K, Dineen S,et al. SARSCoV-2 placentitis: An uncommon complication of maternal COVID-19. Placenta. 2021;104:261-6. https://doi.org/10.1016/j.placenta.2021.01.012 PMid:33465727 PMCid:PMC7832228

(19) Cooper AJ, Sequist LV, Lin JJ. Third-generation EGFR and ALK inhibitors: mechanisms of resistance and management [published correction appears in Nat Rev Clin Oncol. 2022 Nov;19(11):744]. Nat Rev Clin Oncol. 2022;19(8):499-514. https://doi.org/10.1038/s41571-022-00639-9 PMid:35534623 PMCid:PMC9621058