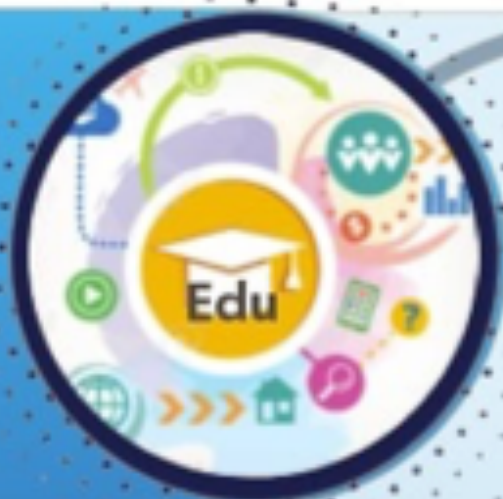


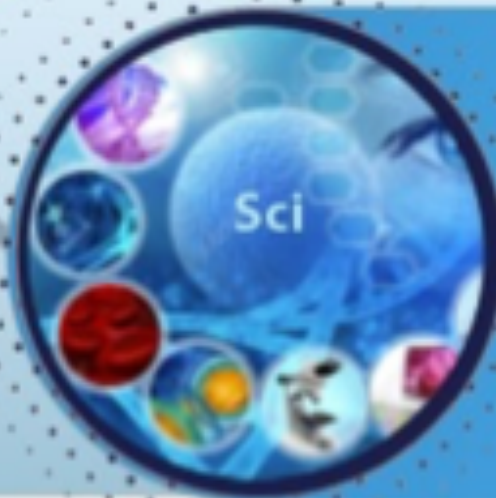


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Features of PDL-1 Expression in Abortive Tissues of Women with Non-Developing Pregnancies Associated with COVID-19 Infection

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ABSTRACT

Background. Coronavirus infection significantly impacts the immune system, contributing to the development of inflammatory reactions that may disrupt normal implantation and embryo development.

Aim. To study the level of Pdl-1 receptor expression in abortive tissues from non-developing pregnancies associated with COVID-19 and to evaluate the correlation between Pdl-1 expression and the clinical course of the infection.

Materials and methods. The study included 60 women with non-developing pregnancies associated with COVID-19 infection. Participants were divided into two groups: Group I consisted of 30 women with pregnancies of 5-7 weeks, terminated by vacuum aspiration; Group II included 30 women with pregnancies of 8-12 weeks, terminated by medical abortion. The control group comprised 30 women who terminated pregnancies of 6-12 weeks by choice.

Results. The results of IHC analysis of abortive tissues in Group I showed focal Pdl-1 receptor expression in decidual tissue in 67% of cases, heterogeneous expression in chorionic villi in 58% of patients, and diffuse expression in syncytiotrophoblast in 54% of cases. In Group II, moderate Pdl-1 receptor expression in decidual tissue was found in 57% of samples. Focal expression in syncytiotrophoblast was observed in 53% of cases, and heterogeneous expression in chorionic villi was detected in 51% of cases. In the control group, Pdl-1 expression was significantly lower compared to both COVID-19 groups, indicating the virus's impact on the immunohistochemical characteristics of the tissues.

Conclusions. The study revealed a high level of Pdl-1 receptor expression in abortive tissues from both groups with non-developing pregnancies associated with COVID-19. This increased expression likely reflects the negative impact of SARS-CoV-2 on fetal tissues, possibly due to the virus entering and damaging these tissues. Additionally, a correlation was identified between the level of Pdl-1 receptor expression and the severity of COVID-19's clinical course. Specifically, a weak positive correlation was found between Pdl-1 expression levels and severe COVID-19 ($r=0.24$) and mild COVID-19 ($r=0.14$). This suggests that the severity of COVID-19 infection may be associated with Pdl-1 receptor expression levels in abortive tissues, potentially indicating a direct link to the immune response.

Key words: COVID-19; non-developing pregnancy; Pdl-1 expression immunohistochemistry abortive tissues

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INTRODUCTION

The COVID-19 pandemic, caused by the SARS-CoV-2 virus, has had a significant impact on healthcare systems and continues to be the subject of intensive research, particularly regarding its effects on various physiological and pathological processes in the human body [1]. SARS-CoV-2 manifests not only as a respiratory pathogen but also as an agent affecting the immune, endocrine, and reproductive systems [2]. In recent years, there has been increasing discussion about its potential effects on reproductive health, particularly on pregnancy progression and outcomes [3].

Non-developing pregnancy, or missed abortion, is a condition in which the development of the embryo or fetus ceases at an early stage, resulting in its demise. The causes of non-developing pregnancy are varied and include genetic and infectious factors. One of the key elements involved in the pathogenesis of this condition is an impaired immune response [4]. SARS-CoV-2 can cause significant changes in the immune system, which may lead to adverse outcomes for the developing embryo [5].

Among the various immunological mechanisms involved in the response to infection, the expression of the Pdl-1 receptor (Programmed death-ligand 1) plays a critical role in regulating the immune system and controlling inflammatory reactions. It is known that Pdl-1 participates in mechanisms of immune tolerance, which are especially important during pregnancy to maintain a certain immune balance needed to protect the fetus [6]. However, SARS-CoV-2 infection can disrupt this balance, promoting pathological changes in Pdl-1 expression in embryonic and maternal tissues, which may contribute to the development of non-developing pregnancy [7].

The aim of this study is to investigate the level of Pdl-1 receptor expression in abortive tissues of women with non-developing pregnancies associated with COVID-19. Additionally, this study aims to determine the correlation between Pdl-1 expression levels and the clinical characteristics of COVID-19, which may help to better understand the pathogenetic mechanisms underlying the virus's impact on pregnancy [8].

It is hypothesized that Pdl-1 expression could serve as a marker of the negative impact of the infection on the fetus and could be used to predict complications in early pregnancy [9]. In the context of the COVID-19 pandemic, research on this topic is of paramount importance for the development of more effective diagnostic and therapeutic approaches [10].

The Study aims to investigate the level of Pdl-1 receptor expression in abortive tissues in cases of non-developing pregnancies associated with COVID-19 and to assess the correlation between Pdl-1 expression and the clinical course of the infection.

MATERIALS AND METHODS

This study involved 60 women with non-developing pregnancies associated with COVID-19 infection. Based on the gestational age and the termination method, participants were divided into two groups. Group I consisted of 30 women with pregnancies diagnosed at 5-7 weeks, terminated by vacuum aspiration. This method was chosen for its minimally invasive nature, which is particularly important in early pregnancy. Vacuum aspiration allowed for the collection of high-quality tissue samples for subsequent immunohistochemical (IHC) analysis to identify Pdl-1 receptor expression.

Group II included 30 women with pregnancies at 8-12 weeks, terminated through medical abortion. This method allows controlled tissue evacuation, minimizing trauma to the body and ensuring high-quality abortive material for research. Medical abortion, performed at later stages compared to vacuum aspiration, enabled the collection of comprehensive tissue samples for analysis, including chorionic villi and decidual tissue.

A control group was also formed, consisting of 30 women whose pregnancies were terminated by choice, without COVID-19 infection. The gestational age in the control group varied from 6 to 12 weeks, matching the gestational periods of the main study groups. The control group was essential for comparing results from women with non-developing pregnancies linked to COVID-19 against those from women without any infections, including COVID-19.

Tissue samples from each group were collected for immunohistochemical (IHC) analysis. The IHC method provided a detailed assessment of Pdl-1 receptor expression, a key immune response marker that may indicate pathological changes in tissues due to infection. This method involves the use of antibodies that bind to the Pdl-1 receptor, allowing visualization and quantification of its expression in various tissues, including decidual tissue, chorionic villi, and syncytiotrophoblast. The immunohistochemical analysis was conducted using modern techniques, yielding clear results for Pdl-1 receptor expression. The analysis covered different tissues, including decidual tissue and chorionic villi, to identify potential differences in receptor expression based on the gestational stage and termination method. Tissue samples were treated with Pdl-1 antibodies, enabling visualiza-

tion of receptor expression through high-magnification microscopy.

Data analysis was performed to compare Pdl-1 expression levels between the groups. Descriptive statistics methods were used to calculate the mean values and standard deviations of Pdl-1 expression levels. Non-parametric tests, such as the Mann-Whitney U test and the Kruskal-Wallis test, were used to assess the significance of differences between the study groups. Statistical significance was determined at a level of $p < 0.05$. Thus, the research methodology was designed to conduct a detailed study of Pdl-1 expression levels and its association with the clinical characteristics of COVID-19 in women with non-developing pregnancies.

RESULTS

The average age of the patients was 32.4 ± 1.2 years. COVID-19 infection developed at various gestational stages, with 40% of the women contracting the infection between 5-7 weeks and 60% between 8-12 weeks. Based on the severity of the disease, the patients were distributed as follows: 25% had a mild form, 50% had a moderate form, and 25% experienced severe COVID-19. Upon presenting for the study, their main complaints included physical fatigue, difficulty breathing, headaches, high fever, and general weakness—symptoms commonly associated with the progression of COVID-19. The diagnosis of non-developing pregnancy was established through clinical examination and ultrasound (US) imaging. During the ultrasound, an absence of fetal heartbeat and changes in fetal size were noted, which confirmed the non-developing pregnancy. These diagnostic procedures were crucial for high-quality analysis and accurate identification of non-developing pregnancy cases.

The study included 60 women with non-developing pregnancies associated with COVID-19 infection. Among them, 30 women with non-developing pregnancies at 5-7 weeks underwent vacuum aspiration (Group I), while 30 women with non-developing pregnancies at 8-12 weeks underwent medical abortion (Group II). The control group consisted of 30 women with unwanted pregnancies terminated at 6-12 weeks. In the immunohistochemical analysis of abortive material from non-developing pregnancies associated with COVID-19, the level of Pdl-1 receptor expression was examined.

The results of the study indicated a relatively high expression level of Pdl-1 receptors, suggesting that immunohistochemical changes due to the impact of the SARS-CoV-2 virus negatively affected the fetus, as indicated by COVID-19 infection's adverse influence. In

studying Pdl-1 receptor expression in abortive samples from non-developing pregnancies due to COVID-19, the following IHC characteristics were identified: in Group I, focal expression of Pdl-1 receptors in the decidual tissue was observed in 67% of cases in 12 women (40%) whose pregnancies were terminated by vacuum aspiration. Heterogeneous expression of Pdl-1 receptors in chorionic villi was noted in 58% of cases in 11 women (36.7%), while diffuse expression of Pdl-1 receptors in the syncytiotrophoblast of abortive tissue was recorded in 54% of cases in 7 women (23.3%) (Figures 1, 2, 3).

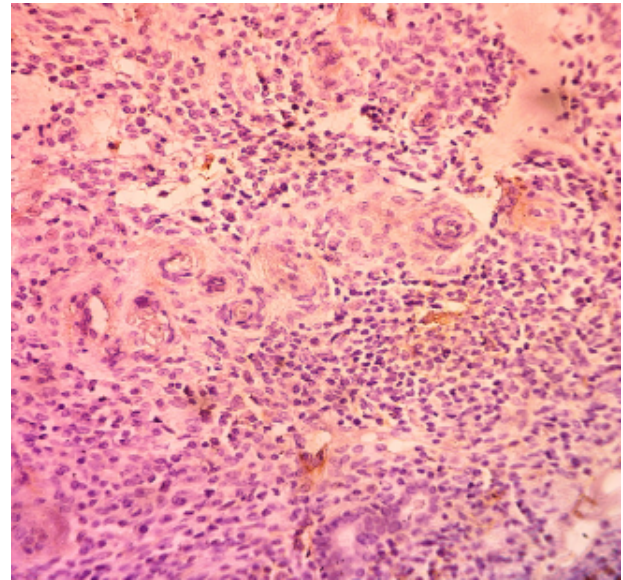


Figure 1. Focal expression of Pdl-1 receptors in decidual tissue. Immunohistochemical staining, $\times 400$ (Patient S., 2022).

In Group II, during the study of Pdl-1 receptor expression in abortive tissue samples from women with non-developing pregnancies terminated by medical methods due to COVID-19, the following IHC characteristics were identified: moderate expression of Pdl-1 receptors in decidual tissue was observed in 57% of cases in 14 women (46.7%). Additionally, focal expression of Pdl-1 receptors in the syncytiotrophoblast was noted in 53% of cases in 10 women (33.3%), while heterogeneous expression of Pdl-1 receptors in chorionic villi was recorded in 51% of cases in 6 women (20%) (Figures 4, 5, 6).

Thus, it can be concluded that, regardless of the termination method, samples obtained from abortive material in cases of non-developing pregnancies associated with COVID-19 show high levels of Pdl-1 receptor expression in both groups. The elevated expression of Pdl-1 receptors detected in patients indicates the negative

impact of coronavirus infection on pregnancy, as well as the likelihood that the SARS-CoV-2 virus penetrated and damaged fetal tissues.

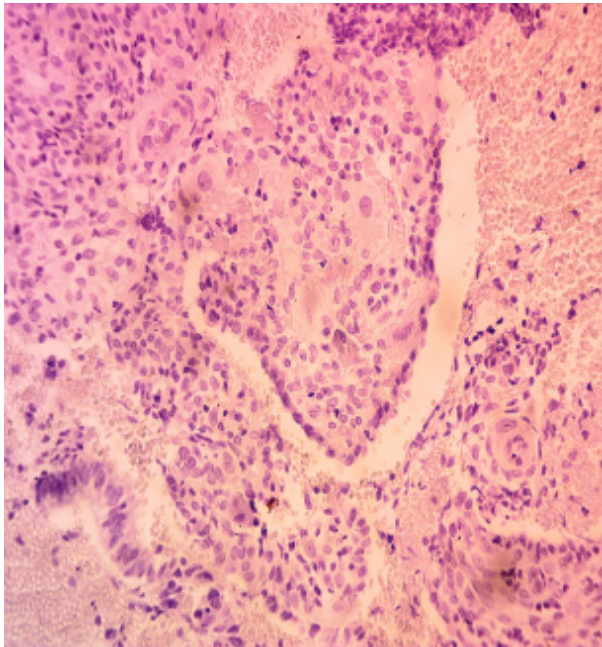


Figure 2. Heterogeneous expression of Pdl-1 receptors in chorionic villi. Immunohistochemical staining, $\times 400$ (Patient D., 2022).

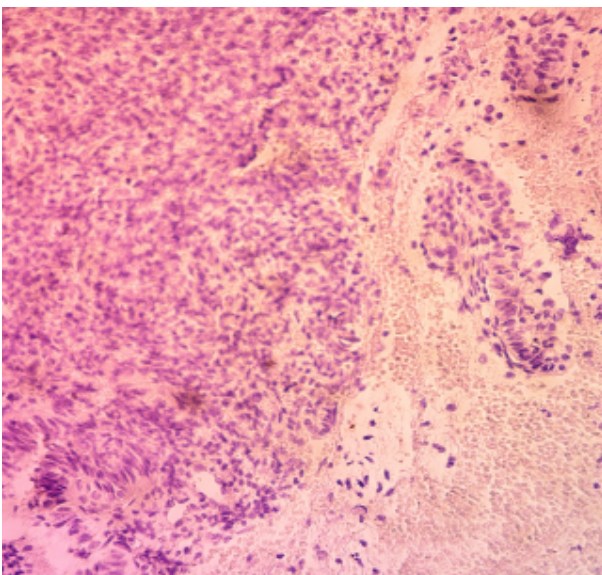


Figure 3. Diffuse expression of Pdl-1 receptors in syncytiotrophoblasts. Immunohistochemical staining, $\times 400$ (Patient D., 2022).

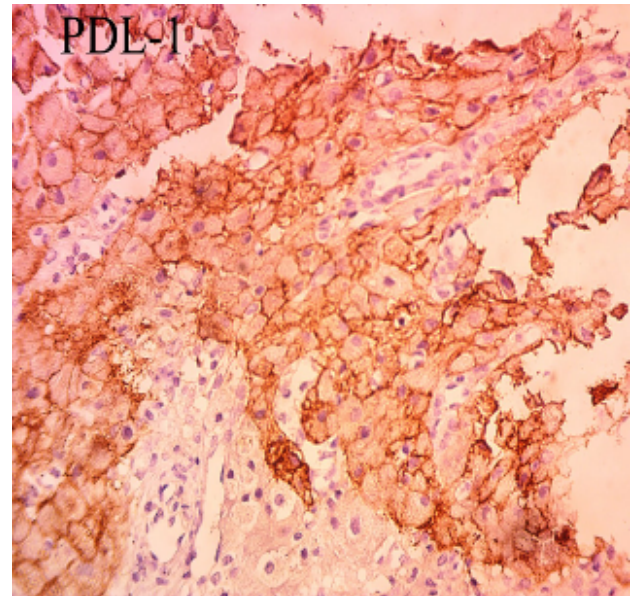


Figure 4. Moderate expression of Pdl-1 receptors in decidual tissue. Immunohistochemical staining, $\times 400$ (Patient G., 2022).

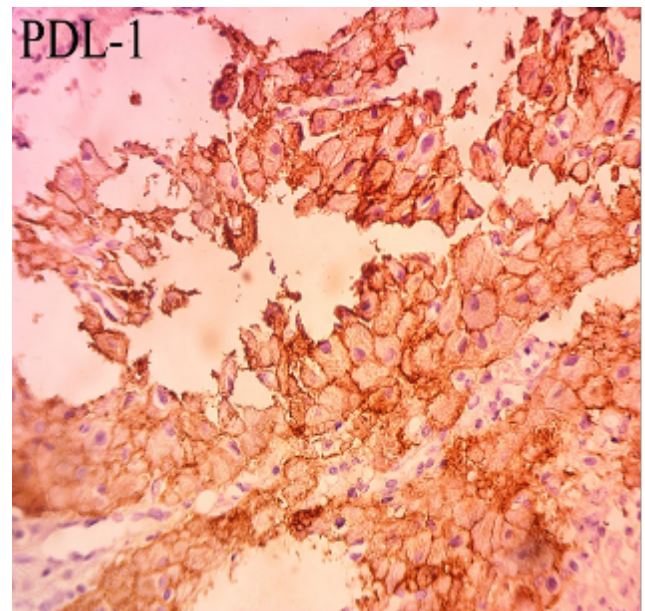


Figure 5. Focal expression of Pdl-1 receptors in syncytiotrophoblast. Immunohistochemical staining, $\times 400$ (Patient M., 2022).

Furthermore, a correlation was found between Pdl-1 receptor expression and the clinical course of coronavirus infection. According to correlation analysis results, Pdl-1 expression is directly associated with the clinical severity of COVID-19. For severe cases, the cor-

relation coefficient was $r=0.24$, indicating a weak positive correlation. For mild cases, the correlation coefficient was $r=0.14$ among the women.

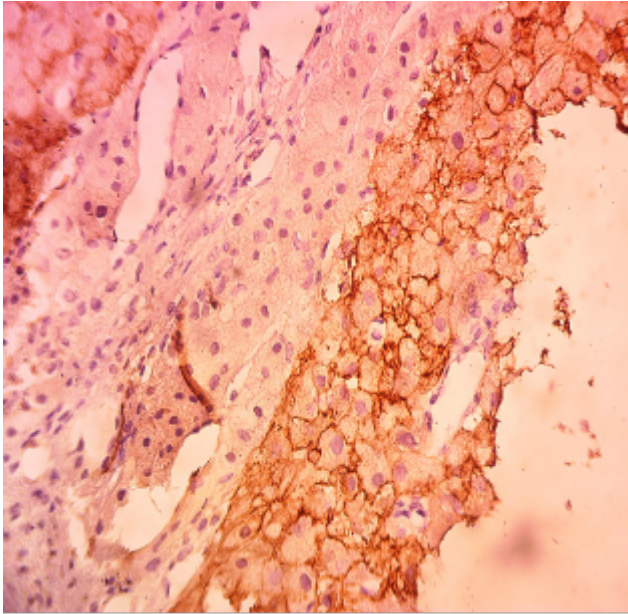


Figure 6. Heterogeneous expression of Pdl-1 receptors in villous chorion. Immunohistochemical staining, $\times 400$ (Patient M., 2022).

In conclusion, the immunohistochemical analysis of abortive material in cases of non-developing pregnancies associated with COVID-19 revealed high levels of Pdl-1 receptor expression. This high expression of Pdl-1 receptors was observed across both groups — women whose pregnancies were terminated via vacuum aspiration and those terminated via medical abortion. These findings suggest that the SARS-CoV-2 virus not only affected maternal health but also infiltrated fetal tissues, leading to adverse cellular changes. The significant Pdl-1 expression levels in decidual tissue, chorionic villi, and syncytiotrophoblasts indicate an immune response triggered by the viral presence, which may have contributed to the development of non-developing pregnancies. Furthermore, the study identified a direct correlation between Pdl-1 receptor expression and the clinical course of COVID-19. The correlation analysis demonstrated that as the severity of COVID-19 increased, so did the expression levels of Pdl-1 receptors in fetal tissues, suggesting an association between higher viral impact and immune response intensity. For severe COVID-19 cases, the correlation coefficient was $r=0.24$, which, although weak, indicates a consistent positive relationship. This may imply that the more severe the infection, the

stronger the immune response in the maternal-fetal interface, potentially compromising fetal viability.

In women with mild COVID-19 symptoms, the correlation coefficient was $r=0.14$, showing a less pronounced relationship between infection severity and Pdl-1 expression. However, even in mild cases, elevated Pdl-1 levels were noted, suggesting that SARS-CoV-2 infection can induce an immune reaction within the fetal environment regardless of symptom severity in the mother. These findings are crucial, as they shed light on the pathogenic role of SARS-CoV-2 in pregnancy complications, specifically through the activation of immune mechanisms that might disrupt normal pregnancy progression. The elevated Pdl-1 expression likely reflects an attempt at immune regulation in response to viral presence, yet this regulatory process may also impede fetal development. By compromising immune tolerance mechanisms essential to pregnancy, SARS-CoV-2 potentially contributes to non-developing pregnancies.

The study underscores the importance of monitoring and evaluating immune markers such as Pdl-1 in pregnancies affected by COVID-19, as it may serve as a biomarker to predict adverse pregnancy outcomes. These insights could inform targeted approaches to mitigate the virus's impact on pregnancy by developing therapeutic interventions that modulate immune responses without impairing fetal health. Further research is recommended to clarify the mechanisms by which SARS-CoV-2 influences immune responses at the maternal-fetal interface and to explore potential therapeutic strategies aimed at protecting reproductive health in the context of COVID-19.

DISCUSSION

The results of this study provide important insights into the impact of SARS-CoV-2 on pregnancy progression and the pathogenic mechanisms that may contribute to the development of non-developing pregnancies [4]. The observed high level of Pdl-1 receptor expression in the abortive tissues of women with COVID-19-associated non-developing pregnancies highlights the significance of virus-induced changes in the immune system. This high expression level, seen in women undergoing both vacuum aspiration and medical abortion, suggests that SARS-CoV-2 affects not only the maternal system but also penetrates fetal tissues, potentially leading to adverse cellular changes. Immunohistochemical analysis showed elevated levels of Pdl-1 expression in various tissue types, such as decidual tissue, chorionic villi, and syncytiotrophoblasts, indicating activation of the immune response in the presence of

the virus. Pdl-1 is a key marker of immune response that helps maintain immune tolerance during pregnancy. However, the increased expression of Pdl-1 in response to SARS-CoV-2 may reflect an attempt by the body to control inflammatory processes but may also interfere with the normal progression of pregnancy, disrupting immune mechanisms essential for fetal protection [2].

The results of the correlation analysis confirmed a direct link between the level of Pdl-1 expression and the clinical severity of COVID-19. The more severe the infection, the higher the level of Pdl-1 expression in fetal tissues. In severe COVID-19 cases, the correlation coefficient was $r=0.24$, indicating a weak but consistent positive correlation. This finding may suggest that severe COVID-19 induces a stronger immune response at the maternal-fetal interface, which could compromise fetal development. In women with mild COVID-19 symptoms, the correlation coefficient was $r=0.14$, indicating a less pronounced association between infection severity and Pdl-1 expression. However, elevated Pdl-1 levels were noted even in mild cases, suggesting that SARS-CoV-2 can trigger an immune response within fetal tissues regardless of the mother's symptom severity [3].

These findings underscore the importance of evaluating immune markers such as Pdl-1 in pregnancies complicated by COVID-19. Elevated Pdl-1 expression could be considered a marker of the negative impact of the infection on the fetus and may potentially be used to predict early pregnancy complications. In the context of the COVID-19 pandemic, studying Pdl-1 expression and understanding its role in pregnancy pathologies is critical for developing more effective diagnostic and therapeutic approaches [2].

Thus, the findings emphasize the need for further research to investigate the mechanisms by which SARS-CoV-2 affects the immune response at the maternal-fetal level and to develop strategies aimed at protecting reproductive health in the context of COVID-19.

CONCLUSION

This study highlights the significant impact of SARS-CoV-2 on pregnancy, particularly in cases of non-developing pregnancies associated with COVID-19. High levels of Pdl-1 receptor expression were observed in the abortive tissues of women with non-developing pregnancies linked to COVID-19, suggesting that the virus triggers substantial immunological changes. The presence of elevated Pdl-1 expression across tissue types, including decidual tissue, chorionic villi, and syncytiotrophoblasts, implies that SARS-CoV-

2 not only affects maternal health but also penetrates fetal tissues, potentially leading to adverse cellular responses that compromise pregnancy progression. The study's immunohistochemical findings suggest that Pdl-1 may serve as a valuable marker for understanding how the immune system responds to SARS-CoV-2 in a pregnancy context. The correlation analysis between Pdl-1 expression levels and the clinical severity of COVID-19 revealed a direct association, with higher Pdl-1 expression observed in cases of severe infection. This association underscores the potential role of an intensified immune response at the maternal-fetal interface, which may contribute to non-developing pregnancies and other complications.

Even in mild COVID-19 cases, elevated Pdl-1 levels were detected, indicating that SARS-CoV-2 infection can induce an immune response within fetal tissues independent of the mother's symptom severity. These findings highlight the importance of assessing immune markers like Pdl-1 in pregnancies affected by COVID-19, as this marker may help predict potential complications early in pregnancy and provide insights into the pathogenic processes involved. Overall, the results underline the urgent need for further research into the immunological effects of SARS-CoV-2 on pregnancy and the mechanisms underlying the virus's influence on immune tolerance at the maternal-fetal level. Developing targeted diagnostic and therapeutic strategies that address these immune disruptions could be critical for improving pregnancy outcomes and protecting reproductive health in the context of the ongoing COVID-19 pandemic.

Ethics approval and consent to participate - All patients gave written informed consent to participate in the study.

Consent for publication - The study is valid, and recognition by the organisation is not required. The author agrees to open the publication.

Availability of data and material - Available

Competing interests - No

Financing - No financial support has been provided for this work

Conflict of interest - The authors declare that there is no conflict of interest.

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COVID-19 INFEKSIYASI BILAN BOG‘LIQ RIVOJLANMAGAN HOMILADORLIK ABORTIV TO‘QIMALARDA PDL-1 EKSPRESSIYASINING XUSUSIYATLARI

Shukurov F.I., Ruzmetova N.F., Ismoilova Sh.I.
Toshkent Tibbiyot Akademiyasi
XULOSA

Dolzarbligi. Koronavirus infeksiyasi immun tizimiga katta ta’sir ko’rsatadi, normal implantatsiya va embrion rivojlanishini buzishi mumkin bo’lgan yallig’lanish reaksiyalarini rivojlantirishga yordam beradi.

Material va usullar. Tadqiqotda COVID-19 infeksiyasi bilan bog‘liq rivojlanmagan homiladorlik tashxisiga ega 60 nafar ayol ishtirok etdi. Qatnashchilar ikki guruhga bo’lindi: I guruh 5-7 haftalik homiladorligi vakuum aspiratsiya usulida to’xtatilgan 30 nafar ayolni, II guruh esa 8-12 haftalik homiladorligi medikamentoz abort bilan to’xtatilgan 30 nafar ayolni o’z ichiga oldi. Nazorat guruhiga esa istalmagan homiladorlik sababli homilasini 6-12 haftalik muddatda to’xtatgan 30 nafar ayol kiritildi.

Natijalar. I guruhdagi abortiv to’qimalarning IGX tahlili natijalariga ko’ra, detsidual to’qimada Pdl-1 retseptorining fokal ekspressiyasi 67% hollarda, xorion vorsinalarida geterogen ekspressiya 58% bemorlarda va sinsitiotrofoblastda diffuz ekspressiya 54% hollarda qayd etildi. II guruhda detsidual to’qimada Pdl-1 retseptorining o’rtacha ekspressiyasi 57% namunada aniqlandi. Sinsitiotrofoblastda fokal ekspressiya 53% hollarda, xorion vorsinalarida geterogen ekspressiya 51% hollarda qayd etildi.

Xulosa. Tadqiqotda COVID-19 bilan bog‘liq rivojlanmagan homiladorlikdagi har ikki guruhda Pdl-1 retseptorining yuqori darajadagi ekspressiyasi aniqlandi. Bu yuqori ekspressiya, SARS-CoV-2 virusining homila to’qimalariga kirib, ularni zararlash natijasida kelib chiqqan. Bundan tashqari, Pdl-1 retseptorining ekspressiya darajasi bilan COVID-19 kasalligi klinik kechishi o’rtasida korrelyatsiya aniqlandi. Jumladan, Pdl-1 ekspressiyasi darajasi bilan COVID-19ning og’ir ($r=0,24$) va yengil ($r=0,14$) kechishi o’rtasida kuchsiz to’g’ri korrelyatsiya mavjudligi aniqlandi. Bu COVID-19 infeksiyasining og’irligi va abortiv to’qimalardagi Pdl-1 retseptorining ekspressiya darajasi o’rtasida bog‘liqlik borligini ko’rsatib, immun javobiga bevosita bog‘liqligidan dalolat beradi.

Kalit so’zlar: COVID-19; rivojlanmagan homiladorlik; Pdl-1 ekspressiyasi; immunogistoximiya abortiv to’qimalar

ОСОБЕННОСТИ ЭКСПРЕССИИ PDL-1 В АБОРТИВНЫХ ТКАНЯХ У ЖЕНЩИН С НЕРАЗВИВАЮЩЕЙСЯ БЕРЕМЕННОСТЬЮ, АССОЦИИРОВАННОЙ С ИНФЕКЦИЕЙ COVID-19

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Ташкентская медицинская академия
РЕЗЮМЕ

Актуальность. Коронавирусная инфекция оказывает значительное влияние на иммунную систему, способствуя развитию воспалительных реакций, которые могут нарушать нормальную имплантацию и развитие эмбриона.

Материал и методы. В исследование было включено 60 женщин с неразвивающейся беременностью, связанной с инфекцией COVID-19. Участницы были разделены на две группы: группа I состояла из 30 женщин с беременностью 5-7 недель, прерванной методом вакуум-аспирации; группа II включала 30 женщин с беременностью 8-12 недель, прерванной медикаментозным абортom. Контрольную группу составили 30 женщин, прервавших беременность на сроке 6-12 недель по желанию.

Результаты. Результаты иммуногистохимического исследования (ИГХ) abortивных тканей I группы показали, что фокальная экспрессия рецептора Pdl-1 в децидуальной ткани была выявлена у 67% случаев, гетерогенная экспрессия в хорионических ворсинах — у 58% пациенток, а диффузная экспрессия в синцитиотрофобласте наблюдалась в 54% случаев. Во II группе была установлена умеренная экспрессия рецептора Pdl-1 в децидуальной ткани, зафиксированная в 57% образцов.

Заключение. Исследование показало высокий уровень экспрессии рецептора Pdl-1 в abortивных тканях обеих групп с неразвивающейся беременностью, связанной с COVID-19. Это повышенное выражение, вероятно, отражает негативное влияние SARS-CoV-2 на ткани плода, возможно, из-за проникновения вируса в ткани и их повреждения. В частности, была выявлена слабая прямая корреляция между уровнями экспрессии Pdl-1 и тяжелым течением COVID-19 ($r=0,24$) и легким течением ($r=0,14$).

Ключевые слова: COVID-19; неразвивающаяся беременность; Pdl-1 экспрессия; иммуногистохимия; abortивные ткани