

The Evaluation of COVID-19 Effect on Pregnancy Loss-Molecular and Diagnostic Approach: A Narrative Review

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ABSTRACT

The new severe respiratory disease causing virus (SARS-CoV-2) originates from Wuhan, China and has spread around the world. According to studies, the results of the SARS-CoV-2 test have been reported to be positive for a number of pregnant women. However, not much is known about the effect of this virus on pregnancy and the outcome of the baby. The aim of this study was to evaluate the molecular and diagnostic approach in evaluating the effect of Coronavirus disease 2019 (COVID-19) on pregnancy loss.

The entry of COVID-19 virus into the pregnant mother's body through various channels, including the Angiotensin-Converting Enzyme receptor (ACE2), affects the immune and coagulation systems and hormone levels. These changes include increased D-Dimer, platelet and decreased Protein C (PC) levels, increased antithrombin III (AT-III) and elevated levels of proinflammatory cytokines, including IL-6, followed

by disruption of various signaling pathways such as JAK/STAT and PI3K Is. And SMAD factors 1/3; Decreased regulation of the expression of cyclooxygenase 1 (COX1) and prostaglandin E2 (PGE2) and hormones such as progesterone was observed. These changes disrupt decision-making in the endometrium and ultimately lead to serious pregnancy risks, including miscarriage. Due to the important role of signaling pathways in maintaining the body's homeostasis and regulating the body's immune functions, disrupting the pathways through the entry of the virus into the body disrupts the body's homeostasis increases the risk of miscarriage in pregnant women.

Keywords: COVID-19, Pregnancy loss, Pathogenesis, Diagnosis

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INTRODUCTION

Coronavirus disease 2019 (COVID-19) is a virus from the coronavirus family, whose study of clinical effects has become one of the main topics of research in the world scientific community. The pandemic of the virus began in late 2019 in Wuhan, China (Najafi S, *et al.*, 2020). Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-COV-2) is the name now given to this new virus (Ahn DG, *et al.*, 2020). It is named by the International Committee on Taxonomy of Viruses, as well as other virologists based on the similarity of this new virus to SARS-COV, which broke out in 2002 (Li H, *et al.*, 2020).

LITERATURE REVIEW

COVID-19 can infect all age groups; it is easily transmitted through the respiratory tract (Ahn DG, *et al.*, 2020). Clinical signs of the virus include shortness of breath, fever, cough, headache, gastrointestinal and heart problems, blood clotting disorders and other complications (Guo YR, *et al.*, 2020).

Many studies have focused on the effect of this disease on the general population, but not enough research has been done to investigate the effect of this disease on people with certain conditions, including pregnancy conditions, while pregnant women due to physiological changes is considered as in danger group (Juan J, *et al.*, 2020). Including these physiological changes hematological, cardiac, renal, respiratory, metabolic, gastrointestinal and hormonal changes (Pillay SP, *et al.*, 2016). In one case, placental infection was observed with COVID-19, which may cause preeclampsia and worsening the mother's physiological condition, and premature termination of pregnancy or abortion (Lu-Culligan A, *et al.*, 2021). Another study of 116 pregnant women with COVID-19 found clinical findings of preterm delivery and mis-

carriage, but found no significant association between the Infection with the virus and those reported (Yan J, *et al.*, 2020).

In another case, the traces of COVID-19 left in placenta and miscarriage for no reason in the second trimester in a 28-year-old mother can bring us closer to understand the relationship between viral placental infection and miscarriage (Baud D, *et al.*, 2020). Due to the clinical effects of this virus on various organs of the body and to improve the control and management of this pandemic, and increase the knowledge about the pathogenesis of this disease, it is necessary to conduct researches about the effect of virus on different populations in different conditions such as pregnancy (Afshar Y, *et al.*, 2020). Therefore, in the preset study we evaluated the clinical effects of this virus on pregnant women and their specific physiological conditions, as well as the relationship between maternal infection with this virus and abortion.

COVID-19 and coagulation disorder

Coagulation disorders are one of the clinical symptoms in COVID-19 infected individuals, which cause an imbalance in homeostasis. Among the related factors to the coagulation system, D-Dimer has been frequently observed in pregnant women with COVID (Zou Y, *et al.*, 2020). D-Dimer is a protein that has been shown to increase plasma levels as a result of blood clotting, and high levels of D-Dimer can be explained by a local increase in fibrin formation (Wells PS, *et al.*, 2003). Coagulation disorders and increased risk of Disseminated Intravascular Coagulation (DIC) are the results of D-dimer and inflammatory cytokines increment; they can lead to clot formation and deposition of Fibrin in the bloodstream of the placenta, and prevent proper blood supply to placenta and abortion (Kumano O, *et al.*, 2020).

Also, according to studies, antithrombin III (AT-III), which

has a binding site for Thrombin and Heparin, is an important inhibitor of coagulation cascade. AT-III can inhibit serine-activated proteases such as FXa, FIXa, FXIa, and FXIIa (Yang S, *et al.*, 2021). It also potentially prevents the activation of Nuclear Factor- κ B (NF- κ B), which is involved in inflammation (Rezaie AR and Giri H, 2020). When the Corona virus enters the body, ACE-2 glycosylation facilitates the entry of the virus into the body. On the other hand, glycosylation of AT III causes thrombosis. In the virus infected pregnant women, who have recurrent miscarriages, we see an increase in AT-III levels (Ceriello A, *et al.*, 2020).

On the other hand, protein S (PS) and protein C (PC) also have a vitamin K-dependent anticoagulant role and can bind to Activated Protein C (APC) attached to the phospholipid platelet surface and reduce the effect of FXa on FVa in the coagulation cascade. As a result, PS and PC significantly prevent Fibrin from clotting and forming (Medfisch SM, *et al.*, 2020; Amiral J and Seghatchian J, 2019). PS and PC also impair the expression of thrombomodulin, and the endothelial PC receptor on the endothelium. Studies have shown that thrombomodulin has anticoagulant function by binding to Thrombin and activating the PC. Decreased PC levels in people with COVID are associated with an imbalance in thrombin regulation (Okada M, *et al.*, 2020; Tiscia GL, *et al.*, 2020).

COVID-19 entry the body is associated with an increase in interleukin-6 (IL-6) and Tumor necrosis factor α (TNF α), which are linked to insulin resistance, endothelial dysfunction and blood clotting. Insulin resistance is associated with increased plasminogen activator inhibitor-1 (PAI-1) (Yanai H, 2020). Increasing PAI-1 has a significant effect on the fibrinolytic process by increasing platelet count and decreasing Prothrombin activity time and Thromboplastin activation (Bigdeli R, *et al.*, 2018). Therefore, maintaining balance in PAI-1, especially in pregnant women with COVID, is essential for a successful pregnancy. Platelet Lymphocyte Ratio (PLR) and Neutrophil Lymphocyte Ratio (NLR) levels also change in people with COVID. And the increase in PLR and NLR levels with the effect on inflammatory factors and ROS, causes a lack of coagulation factors, followed by bleeding and miscarriage (Biyik I, *et al.*, 2020).

It can now be concluded that the COVID-19 can affects coagulation factors; it increases D-Dimer, platelets and prothrombin time and also decreases PC and vitamin K levels, especially in pregnant women. It would be a risk for mother and fetus, and finally it may lead to abortion.

COVID-19 and immune system disorder

The immune system plays an important role in the body homeostasis; its irregularities can trigger systemic immune responses, following the rapid spread of the virus, which in turn can lead to COVID-19. The spread of viral agents and inflammation can cause tissue damage that result in damaged cells releasing cytokines. Due to the activated signaling cascade, these cytokines affect gene expression. High levels of cytokine in association with variety of infectious conditions are often called cytokine storms, indicating an immune response characterized by the release of interferons, interleukins, tumor necrosis factors, chemokines, and several other mediators (Wong YP, *et al.*, 2021).

In COVID-19 infected pregnant women, pro-inflammatory cytokines including IL-6 increase. This factor causes the adverse consequences of abortion or abnormal fetal development. Modulation of the mother's immune system during pregnancy may affect the response to infections, particularly viruses. In general, the immune system during pregnancy adapts to the growth of semi-allogeneic fetus, resulting in an altered immune response to infection during pregnancy (Giri TK, 2021). In the placental trophoblast cells of women with the abortion history, this response includes increased production of pro-inflammatory cytokines including Interferon gamma ($IFN-\gamma$)/IL-2/IL-6/IL-7/TNF- α /IL-1, and decreased concentrations of anti-inflammatory cytokines (Han H, *et al.*, 2020).

In other words, in the inflammatory and stimulated conditions, IL-1,

TNF- α and IL-6 also increase, followed by an increase in IL-17; and this increase in cytokines activates the NF κ B, MAPK, PI3K and JAK/STAT pathways (Noack M and Miossec P, 2017). By activating these signaling pathways, serum and urinary concentrations of Progesterone-Induced Blocking Factor (PIBF) expressed by lymphocytes during pregnancy are affected and decreased; As a result, by increasing NK cells and producing TNF, NK cells target trophoblast cells and cause spontaneous abortion in pregnancy (Bartho SJ, *et al.*, 2018).

The proliferation, differentiation, and function of NK cells are regulated by the direct action of progesterone and estrogen on intracellular nuclear receptors, or by intermediate cells in the uterus in early pregnancy. In normal pregnancy, lymphocytes are able to produce progesterone and PIBF during pregnancy; they increase fetal immunity, inhibit NK cell activity and prevent the risk of miscarriage by increasing the production of anti-inflammatory cytokines. But when the immune system becomes abnormal due to the virus spread, this ability is lost; NK cells spread is associated with an increase in spontaneous abortions (Widya AC, *et al.*, 2017; Bogdan A, *et al.*, 2017). Meanwhile, IL-10 modulates resistance to inflammatory stimuli by reducing the regulation of pro-inflammatory cytokines expression, such as IL-1A, IL-12, IL-6 and TNF α (Darif D, *et al.*, 2021). In general, with the increase of these inflammatory cytokines, especially IL-6, we see the activation of *STAT3* and *STAT1* in the JAK/STAT pathway and the imbalance created between Treg/Th17, and cellular immunodeficiency; On the other hand, increasing IL-6 disrupts the expression of genes in this pathway (Venkatesan T, *et al.*, 2017).

For example, the Sprouty 4 (*SPRY4*) gene is called *IFN- γ* , which causes the expression of this gene and *STAT1* phosphorylation via the PI3K/AKT pathway. Increased expression of *SPRY4* gene inhibits trophoblast proliferation and increases the apoptosis (Qin S, *et al.*, 2020). Also, the *IRF1* gene, which in collaboration with *STAT1* reduces *miR-103*, leads to repeated spontaneous abortions by increasing the M1 macrophage activity (Zhu X, *et al.*, 2020). On the other hand, *STAT3* factor increment in large quantities, can suppress GP130 through the suppressor of cytokine signaling 3 (*Socs3*), which activates the MAP kinase pathways, PI3 kinase (Liu X, *et al.*, 2019). In these pathways, overexpression of TNF- α and matrix metalloproteinase (MMP)-3 and MMP-9 factors by altering B-catenin/wnt pathway activity, inhibiting the Integrin-Linked Kinase (ILK) and also by acting on caspases and activating caspase3 causes apoptosis of trophoblast cells (Chen Q, *et al.*, 2020).

On the other hand, activation and increase of NF κ B pathway by affecting on hypertension, causes a significant decrease in uterine endometrial immunity and the immunological mechanism of gestational hypertension syndrome. Eventually, these pathways affect estradiol and intracellular hormones and PIBF concentrations, increasing the risk of miscarriage (Arck PC, 2021). On the other hand, it has been shown that inflammation in the dental pulp can also affect the incidence of abortion in patients. It has been shown that inflammation in the dental pulp can stimulate the immune system and produce inflammatory mediators through dysregulation of some miRs (Table 1). Considering that one of the complications of COVID-19 infection is the occurrence of cytokine storm and also since cytokine storm can play a role in the occurrence of abortion due to dental pulp, possibly COVID-19 infection can be caused by dental pulp Abortion in patients (Shen Z, *et al.*, 2020).

On the other hand, this stimulatory effect of IL-6 increases MicroRNA-223-3p (*miR-223-3p*) and *mir-184*. Elevation of *mir-184* by targeting *WIG1* increases apoptosis of trophoblast cells by regulating *FAS* expression. *mir-223-3p* increment also has a regulatory effect on *FOX1* and *APOL1* genes, which are involved in immune cell homeostasis and regulation of inflammation and apoptosis. Finally, they form a complex monitoring network with pro-inflammatory agents and signaling pathways (Zhang Y, *et al.*, 2019; Wu J, *et al.*, 2019).

Table 1: Summary of miR's involved in inflammation

miR	Target	Mechanism	Reference
<i>miR-21</i>	KBTBD7	Cause activation NF-kB and lead to inflammation	(Song J, <i>et al.</i> , 2020)
<i>miR-410</i>	MMP-14	Decreased expression <i>miR-410</i> cause increased MMP-14 production and inflammation	(Brodzikowska A, <i>et al.</i> , 2019)
<i>miR-146a</i>	bFGF	Cause production inflammation mediators	(Liu L, <i>et al.</i> , 2016)
<i>miR-Let-7c-5p</i>	DMP1	Down regulation of Let-7c-5p lead to active NF-kB pathway and inflammation	(Yuan H, <i>et al.</i> , 2018)
<i>miR-223-3p</i>	DSPP DMP1	Cause stimulate immune response and inflammation	(Huang X, <i>et al.</i> , 2019)

Note: Where bFGF: Basic Fibroblast Growth Factor; DMP1: Dentin Matrix Protein-1; MMP-14: Matrix Metalloproteinase; DSPP: Dentine sialo-phosphoprotein

It can now be concluded that due to the increase in these inflammatory cytokines in pregnant women undergoing abortion, and given that these inflammatory cytokines including IL-6 also increase in pregnant women with COVID. It can be concluded that these cytokines, by acti-

vating the signaling pathways and the changes mentioned, cause adverse consequences in pregnancy, such as abortion for the mother or abnormal growth and development for the fetus (Table 2).

Table 2: Evaluation of function of some cytokines in pathogenesis of COVID-19

Cytokine	Increase/decrease	Mechanism	Potential therapeutic	Reference
IL-1	Increase	Activation the MAPK and PI3K pathways and proliferative functions, inflammation	Interferon-I- • Inhibition of viral replication, mitigation of host inflammatory response • Slowdown the cell metabolism • Inhibition the production of IL-1b and TNF-α in host	(Ramaiah MJ, 2020; Hiratsuka M, <i>et al.</i> , 2000)
TNF-a	Increase	Activation of the MAPK pathway. inflammation and proliferation of acute phase proteins	Azithromycin- • Inhibition of viral cell penetration • Inhibition of terminal ACE-2 glycosilation • Endosomal pH increase	(Hariharan A, <i>et al.</i> , 2021; Favilli A, <i>et al.</i> , 2020)
IL-5	Decrease	Decreased production of B cell antibodies, activation of macrophages and neutrophils	-	(Bonaventura A, <i>et al.</i> , 2020)
IL-4	Decrease	-	-	(Renu K, <i>et al.</i> , 2020)
TGFB	Decrease	Increased expression of MHC II at the level of monocytes, macrophages and proinflammatory cytokine production	-	(Liu J, <i>et al.</i> , 2020)
IL-10	Decrease	Increased expression of pro-inflammatory macrophage marker/effect on TH1 and increases the production of pro-inflammatory cytokines IL-2 and IFNY and increases inflammation.	-	(Abers MS, <i>et al.</i> , 2021)
IL-8	Increase	With its chemokine activity, it causes the lymphocytes and neutrophils release into the area of inflammation.	Pioglitazon- • Mitigation of host inflammatory response • Inhibition of host IL-8 production	(Tatum D, <i>et al.</i> , 2020; Ota H, <i>et al.</i> , 2008)
Il-6	Increase	Activation of the JAK/STAT pathway and STAT 3 and STAT 1. Increase the trophoblast apoptosis	Tocilizumab- • Mitigation of host inflammatory response • Soluble and membrane-bound IL-6 receptor blockage	(Magro G, 2020; Nakajima K, <i>et al.</i> , 2016)
IL-12	Increase	Activate the JAK/STAT pathway and Stat4 and Stat1, and cause inflammation	Betamethasone- • Mitigation of host inflammatory response • Inhibition of host IL-12 production	(Lusty E, <i>et al.</i> , 2017; Kakoulidis I, <i>et al.</i> , 2020)

Note: MAPK: A Mitogen-Activated Protein Kinase; PI3K: Phosphoinositide 3-Kinase; TNF-a: Tumor Necrosis Factor Alpha, ACE-2: Angiotensin-Converting Enzyme 2; LPS: Lipopolysaccharides; NK cell: Natural Killer cell, TGFB: Transforming Growth Factor Beta; MHC II: Major Histocompatibility Complex Class II; IFNY: Interferon Gamma, jak/stat: The Janus Kinase/Signal Transducer and Activator of Transcription; IL: Interleukin; TH1: T Helper1

COVID-19 and endometrial decidualization

Endometrial decidualization, a vital multicellular process for pregnancy progression, is one of the first changes that the uterus adapts to. The interaction between pregnancy-related hormones and cytokines produced by embryonic and uterine cells has been identified as an essential event for decidualization (Oestreich AK, *et al.*, 2020). In COVID-19 infected pregnant women, due to the lack of coordination and balance between cytokines and hormones, a disorder is observed in the decidualization process (Sills ES and Wood SH, 2020). Endometriosis, one of the most common diseases in pregnant women, has been partly explained by resistance to progesterone and decreased intracellular progesterone receptor expression in the extra uterine endometrium (Naidoo N, *et al.*, 2021). However, overproduction of progesterone can also impair decidualization by inhibiting Leukemia Inhibitory Factor (LIF)/Signal transducer and transcription activator 3 (*STAT3*) (Liang YX, *et al.*, 2018).

Heart- and neural crest derivatives-expressed transcript 2 (*HAND2*), which is a major factor in the transcription of progesterin-induced human endometrial stromal cells, also play a key role in activation and surviving the Uterine Natural Killer (UNK) by regulating IL-15. *HAND2* balances the production of Vascular Endothelial Growth Factor (VEGF) and Placental Growth Factor (PIGF), resulting in a balance in Endometrial Stromal Cells (ESCs) (Murata H, *et al.*, 2020). COVID-19 infected pregnant women are at the risk of miscarriage, due to decreased level of progesterin production (Cui L, *et al.*, 2021).

RESULTS AND DISCUSSION

Studies have shown that ACE2, known as a receptor for coronavirus entry, is essential for decidualization of endometrial stromal cells (Albini A, *et al.*, 2020). ACE2 is increased during primary Human Endometrial Stromal Cell (HESC). Due to the increase in ACE2 in various tissues, such as ovaries and uterus, there is a potential risk to the reproductive system and pregnancy (Wang N, *et al.*, 2021). In addition to ACE2, other factors affecting human endometrial stromal cells include prolactin (*PRL*) and insulin-like growth factor binding protein-1 (*IGFBP1*), which is produced by decidual cells. *PRL* and *IGFBP1* are two known factors for decidual cell maturation and proteoglycan decorin (*DCN*) (Buzio OL, *et al.*, 2006). *DCN* inhibits human trophoblast regeneration, migration, invasion, and differentiation needed to regenerate uterine arteries during normal pregnancy. According to studies, some cytokines increment such as IL-11, is associated with change in the regulation of expression and an increase in *IGFBP1* and *PRL*. This increase is also achieved through the entry of virus to the pregnant women, followed by the activation of other cytokines and changes in the regulation of their expression. As a result, there would be the miscarriage risk during pregnancy (Eyal O, *et al.*, 2007).

Morphogenetic protein-2 (*bmp2*) has also been shown to increase the production of pro-inflammatory cytokines. It also reduces the regulation of cyclooxygenase 1 (*cox1*) expression, followed by a decrease in prostaglandin E2 (*PGE2*), which is disrupted by the signaling pathway *smad1/smad5/(Alk3)* activin receptor-like kinase 3 in ESCs. An increase in the amount of pro-inflammatory cytokines without any controlling effect increases the risk of miscarriage for COVID-19 infected pregnant women (Zhang Y, *et al.*, 2020; Arikawa T, *et al.*, 2004).

According to studies, Sirtuin 1 (*SIRT1*) can also be introduced as an effective factor in regulating homeostasis and ESC decidualization (Li J, *et al.*, 2021). *SIRT1* through regulating the expression of superoxide dismutase2 (*SOD2*) and The nuclear factor erythroid 2-related factor 2 (*NRF2*) as well as by deacetylation Forkhead Box O1 (*FOXO1*), makes adjustments homeostasis Reactive Oxygen Species (ROS) and NAD^+ and enhanced protection Equals oxidative stress. Decreased *SIRT1* followed by decreased *FOXO1* deacetylation and imbalances in ROS and NAD^+ observed in patients with COVID cause Recurrent Implantation Failure (RIF) (Madkour MI, *et al.*,

2019; Yang Y, *et al.*, 2014). *FOXO1* is also considered as an ESC decidualization marker, which acts as a transcriptional regulator of *PRL* and Insulin (Yoshino O, *et al.*, 2003).

Studies have also shown that the effect of Norepinephrine (NEP) on decidualization can be investigated. NEP prevents endometrial decidualization by activating the PKC signaling pathway via a β -adrenergic receptor regulation (Wang J, *et al.*, 2021).

Given the changes mentioned, and the imbalance between hormones, cytokines and factors involved in pregnancy in COVID-19 infected pregnant women, we can conclude that the process of endometrial decidualization is disrupted and subsequently abortion may happen.

CONCLUSION

Pregnant women are in a state of suppressed immune system due to physiological changes; they are considered as COVID high-risk group, due to susceptibility to infections and mechanical functions. Immune system suppression disrupts the pregnancy process by affecting the profiles of cytokines and various coagulation systems and hormones; these disorders are associated with the risk of miscarriage. We hope this review be useful for pregnancy and neonatal services, seeking to respond to COVID-19.

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