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COVID-19 AND HEMOLYSIS, ELEVATED LIVER ENZYMES AND THROMBOCYTOPENIA (HELLP) SYNDROME - ASSOCIATION OR CAUSATION?

COVID-19 and HELLP syndrome

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Abstract

Pregnant women are among the high-risk population for severe coronavirus disease 2019 (COVID-19) with unfavorable peripartum outcomes and increased incidence of preterm births. Hemolysis, the elevation of liver enzymes, and low platelet count (HELLP) syndrome and severe preeclampsia are among the leading causes of maternal mortality. Evidence supports a higher odd of pre-eclampsia in women with COVID-19, given overlapping pathophysiology. Involvement of angiotensin-converting enzyme 2 receptors by SARS-CoV-2 for the entry to the host cells and its downregulation cause dysregulation of the renin-angiotensin-aldosterone system. The overexpression of Angiotensin II mediated *via* p53 MAPK pathways can cause vasoconstriction and uninhibited platelet aggregation, which may be another common link between COVID-19 and HELLP syndrome. On PubMed search from January 1, 2020, to July 30, 2022, we found 18 studies on of SARS-COV-2 infection with HELLP Syndrome. Most of these studies are case reports or series, did not perform histopathology analysis of the placenta, or measured biomarkers linked to pre-eclampsia/HELLP syndrome. Hence, the relationship between SARS-CoV-2 infection and HELLP syndrome is inconclusive in these studies. We intend to perform a mini-review of the published literature on HELLP syndrome and COVID-19 to test the hypothesis on association *vs* causation, and gaps in the current evidence and propose an area of future research.

Key Words: SARS-CoV-2; HELLP syndrome; Preeclampsia; Hypertension; Pregnancy-induced; Liver dysfunction; Pregnancy-induced

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Core Tip: Observational studies showed an increased prevalence of preeclampsia and hemolysis, elevated liver enzymes and low platelet (HELLP) syndrome in pregnant

women with coronavirus disease 2019 (COVID-19). Despite a possible pathophysiology linkage between COVID-19 and HELLP syndrome, the evidence on temporality to prove a causal association between infection with severe acute respiratory syndrome coronavirus 2 and HELLP syndrome is lacking.

INTRODUCTION

With immense knowledge on the pathogenesis of Coronavirus Disease 2019 (COVID-19), the viral-host immune interaction plays a critical role in multi-system presentation of the disease. Most of the patients, infected with Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), develop a non-severe illness. However, those patients with specific comorbidities are predisposed to advanced stages of severe COVID-19 infection. Some of the prevalently-reported comorbidities are as follows; age above 75 years, male gender, pre-existing cardiovascular disease (CVD), chronic lung, kidney or liver disease, sickle cell disease, diabetes, active cancer, severe obesity and pregnancy[1,2]. The risk factors that aggravate the development of severe COVID-19 among pregnant women include obesity, smoking history, pre-eclampsia and Diabetes Mellitus (DM)[3]. Though pregnancy, per se, does not increase the susceptibility to SARS-CoV-2 infection, pregnant women are highly prone to developing severe illnesses with SARS-CoV-2 infection compared to non-pregnant women. Further, they are also associated with adverse pregnancy and perinatal outcomes[4].

Hemolysis, Elevated Liver enzymes and Low Platelets (HELLP) syndrome is an uncommon yet deadly complication that is associated with severe pre-eclampsia. Early diagnosis and termination of pregnancy only have been proved to be effective in treating HELLP syndrome[5]. A meta-analysis, conducted recently, inferred that COVID-19 infected women recorded high levels of pre-eclampsia and HELLP syndrome odds[6]. However, abnormal liver enzymes, thrombocytopenia and hemolysis are not only associated with HELLP syndrome, but are observed in most of the critically-ill patients, as a component of multi-organ dysfunction. This phenomenon

occurs especially in case of certain infectious diseases and other pregnancy-related liver disorders, for instance, Acute Fatty Liver of Pregnancy (AFLP)[7]. Substantial evidence infers that some of the viral infections, for instance SARS-CoV-2, tend to mimic HELLP syndrome among women during pregnancy[8,9].

Hence, the overlapping laboratory features of SARS-CoV-2 infection and HELLP syndrome may increase the possibilities of misdiagnosis than a causal association. The current review discusses about the pathogenetic linkage between COVID-19 and HELLP syndrome, reviews the evidences available on association or causation between the variables and proposes novel suggestions for future research.

Pathogenesis of Pre-eclampsia and HELLP syndrome

Pre-eclampsia is a multi-system disorder characterized by *de novo* hypertension that occurs after 20 wk of gestation. Recently, the International Society for the Study of Hypertension in Pregnancy (ISSHP) provided a new definition for pre-eclampsia as given herewith; new onset of hypertension (systolic >140 mmHg and diastolic >90 mmHg) accompanied by at least one feature as listed below and is developed either at or after 20 wk of gestation: 1) proteinuria, 2) maternal organ dysfunction (like liver, kidney, neurological and haematological) and 3) evidence of uteroplacental dysfunctions like fetal growth restriction or abnormal Doppler waveform findings of uteroplacental blood flow or stillbirth[10].

The exact pathogenesis of pre-eclampsia remains uncertain. However, the termination of pregnancy by removing the placenta seems to be an effective therapeutic measure. This method confirms the importance of placenta in the pathophysiology of pre-eclampsia. Two pathogenic phenotypes are established such as early and late pre-eclampsia. The major cause of early pre-eclampsia is placental in nature whereas the late pre-eclampsia is a result of interactions that occur between placental senescence and other factors such as genetics, obesity and nutrition or environmental factors. The oxidative stress upon syncytiotrophoblast, a cell that covers the placental villi on the maternal side, plays a crucial role by getting released into maternal circulation factors like inflammatory cytokines, cell-free fetal DNA, exosomes, and anti-

angiogenic agents. This results in the endothelial dysfunction and hypertensive syndrome[11].

Oxidative stress occurs as a result of either uteroplacental hypoperfusion from the defective remodelling of uterine spiral arteries (i.e., early pre-eclampsia) or due to a mismatch between supply and demand in maternal perfusion and placental or foetus requirements (i.e., late pre-eclampsia). Placental stress results in the dysfunction of vascular endothelium which in turn releases the placental factors that cause systemic manifestations of pre-eclampsia. The pathways proposed earlier for the above discussed phenomenon include an increased release of pro-inflammatory cytokines, cell-free fetal DNA, p53 Mitogen-Activated Protein Kinase (MAPK), placental apoptotic debris, soluble receptor for Vascular Endothelial Growth Factor (VEGF), and soluble fms like tyrosine kinase (sFlt-1)/Placental Growth Factor (PlGF) ratio[11, 12]. (Figure 1)

The role played by Renin-Angiotensin-Aldosterone System (RAAS) in placenta homeostasis is crucial since it regulates the proliferation of trophoblasts, angiogenesis and blood flow. When RASS is not regulated, it creates an imbalance of vasoactive peptides due to high production of angiotensin II (ATII) and low vasodilatory angiotensin 1-7. ATII is a pro-inflammatory, pro-thrombotic element that induces vascular constriction, endothelial injury and vascular smooth cell proliferation which altogether contribute to pre-eclampsia[13]. ATII actions are mediated through the activation of p53 MAPK pathway. p53 MAPK is a critical component in immune functions as well as stress response pathways. p53 MAPK may also be involved in normal embryonic development through the modulation of embryonic angiogenesis. The upregulated p53 MAPK pathway is linked with increased expression of pro-inflammatory cytokines like Tumour Necrosis Factor (TNF)-α, interleukin (IL)-6 and IL-1β. On the other hand, Angiotensin 1-7 attenuate this inflammation by simulating the Mas receptor. Hence, the dysregulation of RASS and high ATII levels lead to uninhibited feedback loop to p53 MAPK pathway which in turn causes untamed inflammation observed in pre-eclampsia[14,15].

The association between pre-eclampsia and HELLP syndrome is unclear. According to a few experts, HELLP syndrome is nothing but an extended manifestation of severe pre-eclampsia. However, a few others argue that HELLP syndrome is an independent entity since it exists without the classical features of pre-eclampsia like proteinuria and oedema. A few resemblances exist between the pathogenesis of pre-eclampsia and HELLP syndrome such as endothelial dysfunction, platelet aggregation and consumption, vasospasm, and end-organ ischemia. However, immune dysregulation with maternal immunological intolerance to fetal tissues is considered as a prominent pathway in HELLP syndrome. This immunological maladaptation has been proved in literature *via* the high levels of fetal mRNA and HLA-DR in the blood of women with HELLP syndrome, who was compared with women with pre-eclampsia[16]. One of the recent studies demonstrated that those patients with HELLP syndrome, had a high titer of agonist antibodies to Type I ATII receptor (AT1r-AA), when compared with patients with pre-eclampsia. The agonist antibodies can simulate the ATII effect upon the receptor[17].

Women with HELLP syndrome possess high levels of other types of anti-angiogenesis factors such as endoglin and Fas ligand than the women with pre-eclampsia. These two factors are responsible for vascular endothelial injury and intense inflammation in HELLP syndrome. The role played by p53 MAPK pathway, in the pathogenesis of HELLPsyndrome, is hypothesized to be an angiogenic response for environmental hypoxia. The elevated serum levels of p53 MAPK increase the serum vascular permeability and it has the potential to aggravate edema in different tissues including the brain. A recent study that compared the serum levels of p53 MAPK among patients with HELLP syndrome and pre-eclampsia found that the serum levels were significantly higher in HELLP syndrome patients than their counterpart. The authors also recommended to use serum p53 MAPK in the diagnosis of HELLP syndrome[18]. As per the literature, patients with HELLP syndrome exhibit high serum levels of p53 MAPK and low expression in placental p53 MAPK[18,19]. The future researchers must explore this relationship which may shed more insights about the role played by p53

MAPK in the pathophysiology of HELLP syndrome. Furthermore, the activation of immune complexes, C_{5b-9} complement pathway, anaphylatoxins like C3a and C5a and the release of inflammatory cytokines, TNF-α and active von Willebrand factor from leucocytes, macrophages and platelets also cause endothelial injury. In turn, endothelial injury contributes to multiple activities such as hemolysis, platelet aggregation and consumption (causing thrombocytopenia), intraluminal fibrin deposition, vasospasm and end-organ ischemia (causing hepatitis) that are generally observed in HELLP syndrome[20].

Conventional pre-eclampsia screening includes a periodic assessment and an early detection of hypertension and proteinuria. But, the precision of pre-eclampsia screening has increased tremendously, thanks to the measurement of circulating biomarkers and Doppler assessment of uteroplacental circulation. sFlt-1/PlGF ratio is a potential and a highly-accurate marker that can be used in the prediction of pre-eclampsia and fetal growth restriction[21]. In the prediction of early pre-eclampsia and the complications associated with it, a combination of multiple factors such as demographic risk factors with periodic blood pressure measurement, doppler assessment of uterine artery and the measurements of biomarkers is found to be highly accurate[22].

Pathogenesis of COVID-19

The internalization of SARS-CoV-2, within the host cell, occurs by binding the S-spike protein of the virus with Angiotensin-Converting-Enzyme 2 (ACE2) present on the cell surface and is supplemented by Transmembrane Serine Protease 2 (TMPRSS2) on the host cell. Though ACE2 is found in multiple tissues, it is predominantly expressed in lung and heart tissues. This phenomenon may explain the high incidence of Acute Respiratory Distress Syndrome (ARDS) and myocarditis among patients with COVID-19 and the primary cause behind the high mortality rate. ACE2 is an integral part of RAAS and is directly associated in the conversion of ATII to Angiotensin 1-7. Like SARS-CoV, when SARS-CoV-2 interacts with ACE2 receptor, the receptor gets downregulated, thus potentiating RAAS and ATII. The interaction between SARS-CoV-

2 and ACE2, like many other viruses, is associated with upregulation of p53 MAPK through the interaction with ACE2 receptors and its direct activation[15,23]. As discussed earlier, the effect of ATII in heart and lung tissues are mediated by p53 MAPK pathway. The upregulation of p53 MAPK is linked with excessive production of pro-inflammatory cytokines such as IL6, TNF-α and IL-1β. Hence, an unrestrained p53 MAPK results in hyperinflammation, vasoconstriction and thrombosis, a hallmark of COVID-19[15]. (Figure 2)

The role played by virus-host immune interplays is crucial in the pathogenesis of COVID-19. Various pro-inflammatory cytokines like IL-6, IL-10, TNF-α, granulocyte-colony stimulating factors and monocyte chemoattract protein 1 mediate lungs and other systemic manifestations of SARS-CoV-2 infection. Though respiratory system is the primary target site of SARS-CoV-2 infection, COVID-19 can be characterized as a multi-system disease that affects heart, kidneys, brain, liver, gastrointestinal and haematological systems and skin[24]. (Figure 2)

COVID-19 patients generally exhibit different biochemical manifestations of pre-eclampsia and HELLP syndrome such as thrombocytopenia, raised liver enzymes, proteinuria, coagulopathy, acute kidney injury, and increased lactate dehydrogenase[8,25]. Mild thrombocytopenia (the count of platelets stands at 100-150 X 10^9/L) is observed among 20%-36% patients with COVID-19 whereas severe thrombocytopenia (< 50 X 10^9/L) is uncommon[26].

EVIDENCE ON COVID-19 AND HELLP SYNDROME

A total of 11 studies was found by the authors when PubMed database was mined using the following keywords; "COVID-19" OR "SARS-CoV-2" AND "HELLP syndrome" between 01st January 2020 to 30th July 2022. When a broader keyword i.e., "HELLP syndrome" was used within the same period, a total of 361 studies was found. Out of the total studies filtered, 18 studies were finalized and critically analyzed after excluding non-COVID-19 studies and non-English literature (Table)[6, 27-43].

Inference from the evidence

Out of the 18 studies considered for final analysis, 13 were case reports or series in which 23 patients were included[27-34,36,38,40,41,43]. Maternal and fetal mortality rates were 8.6% (2) and 21.7% (5) respectively with the development of severe COVID-19 in three patients. Mendoza M *et al*, authored a case series in which five patients were suspected with pre-eclampsia and HELLP syndrome whereas only one had actual pre-eclampsia features based on the Doppler assessment of uterine artery pulsatility index, sFlt-1/PlGF ratio and lactate dehydrogenase[27]. However, another case report failed to find the elevated sFlt-1/PlGF ratio in a patient who exhibited the biochemical features of HELLP syndrome. The patient was managed conservatively and her biochemical abnormalities were resolved spontaneously while the patient achieved a good perinatal outcome[29]. Most of the studies confirmed the existence of a linkage between HELLP syndrome and COVID-19. However, the inference from individual cases without a case-control remains highly biased. Two retrospective cohort studies, in which women with and without COVID-19 were compared, reported conflicting results on the increased incidence of HELLP syndrome with COVID-19[37,42]. In a population-based study authored by Snelgrove JW *et al*, no increased incidence of pre-eclampsia and HELLP syndrome was observed among women infected with SARS-CoV-2 compared to historical controls[42]. On the other hand, in a large registry developed upon hospitalized women for childbirth in the United States, highly-adjusted odds of pre-eclampsia (1.21, 95%CI 1.11-1.33) and HELLP syndrome (1.96, 95%CI 1.36-2.81) were found in pregnant women with COVID-19 compared to those without COVID-19, during the same duration[37]. A recent meta-analysis, in which 28 studies were included which covered a total of 790954 pregnant women, reported a significantly-high risk of pre-eclampsia (pooled odd ratio (OR) 1.62, 95% confidence interval (CI) 1.45-1.82, p<0.00001, 26 studies) with SARS-CoV-2 infection compared to non-infected individuals[6]. A single study outcomes from Jering KS, *et al*, reported highly-unadjusted odds of HELLP syndrome (2.10, 95%CI 1.48-2.97), in pregnant women with SARS-CoV-2 infection[37].

Pathophysiology linkage between COVID-19 and HELLP syndrome

Recent evidences confirm the worst clinical outcomes for pregnant women with COVID-19 in terms of high incidence of pre-eclampsia, preterm birth and the need for caesarean delivery[44,45].

ACE2 receptors and TMPRSS2, which are required for the entry of SARS-CoV-2 into human cells, are expressed in placental components including villous cytotrophoblasts, syncytiotrophoblasts and extravillous trophoblasts[46]. This makes the placenta, predisposed to SARS-CoV-2 infection. When S-spike protein of SARS-CoV-2 binds with ACE2 receptor, it results in the downregulation of the receptor, dysfunction of RAAS and triggering of local placental inflammation. Further, type I ATII receptor and sFlt-1 are also heavily produced from the infected placenta. The increased serum levels of AT1r-AA, in case of SARS-CoV-2 infection, can be observed in pre-eclampsia and HELLP syndrome too[7].

Some evidence supports the presence of high levels of placental ACE2 in women with COVID-19. This may explain the increased association between pre-eclampsia and preterm birth[47]. Another study showed that ACE2 receptors and the expression of protease are dependent upon each other during gestational age. The increased levels of expression is prevalent during the first trimester compared to the rest of the trimesters in pregnancy[48]. In a molecular linkage study by Beys-da-Silva et al., SARS-CoV-2 infection was found to interact with multiple pathways that are involved in pre-eclampsia and HELLP syndrome pathogenesis like upregulation of sFlt-1 and endoglin, angiogenesis, the balance between vasoconstrictive peptides and nitric oxide modulators, hypoxia and inflammation and prothrombotic-related molecules[49].

There exists a few similarities in the pathophysiology of COVID-19 and HELLP syndrome. The interaction between ATII and p53 MAPK is a plausible linkage among COVID-19, preeclampsia and HELLP[15]. (Figure 3) The upregulation of p53 MAPK pathway is also linked with endothelial injury which in turn causes platelet aggregation and arterial thrombosis. This scenario reveals the systemic manifestations of COVID-19 Like thrombocytopenia and raised liver enzymes[50]. However, it is still unclear whether

the above-discussed biochemical abnormalities are manifestations of COVID-19 or HELLP syndrome. There is a lack of temporal studies in this domain that can establish a causal relationship between COVID-19 and HELLP syndrome. The studies conducted earlier that can prove that exposure occurred before the outcome (HELLP syndrome) establishing the temporality are missing. So, it is crucial to identify the causal association since immediate termination of the pregnancy is the only successful treatment used for HELLP syndrome, a predominant placental pathology, so far. However, an expectant and a watchful continuation of pregnancy with better perinatal outcomes may be considered in selected cases of COVID-19 and HELLP syndrome[29].

Future studies should explore this linkage using the principle of temporality and circulatory biomarkers like serum p53 MAPK, sFlt-1/PIGF ratio and/or doppler assessment of uteroplacental hypoxia to identify any causal association between COVID-19 and HELLP syndrome.

CONCLUSION

There exists an association among SARS-CoV-2 infection during pregnancy, pre-eclampsia and HELLP syndrome. Evidence accepts the plausible overlap in the pathogenesis of COVID-19 and HELLP syndrome through ACE2 receptors and RAAS dysregulation that involve ATII and p53 MAPK pathways. However, no prospective studies are available based on screening biomarkers and temporality to prove the causal relationship in this domain. Future studies should establish a temporal relationship between SARS-CoV-2 infection and the development of HELLP syndrome including circulatory biomarkers and tissue or radiological documentation of uteroplacental insufficiency.

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