What role does the microbiome play in the immune function of the pregnant patient during the COVID-19 pandemic? Can probiotics help?

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ABSTRACT

The COVID-19 pandemic has upended life and has left the world facing an uncertain future. It is thought that a global approach needs to be the focus before life can "return to normal." COVID-19, first identified as a geographically localized viral infection, rapidly spread to become a global pandemic. Eighteen months later, the prediction is that it will recur in waves due to its many mutations. This suggests its recurrence will be similar to the influenza virus. This is of particular concern for the more vulnerable population like pregnant women. In pregnancy the immune response is altered and many pharmaceuticals are contraindicated. Vaccination of pregnant women is still a subject of investigation with trials being carried out in many countries. But in the pregnant population many are vaccine hesitant because of unknown longterm effects for them and for their offspring. Ongoing strategies and alternative methods of preventing the disease need to be investigated. In COVID-19 patients, an alteration of the microbiome composition has been identified, which points towards a related decrease in the integrity of the immune system. Additionally, disease severity has been related to the amount of dysbiosis in the gut flora. Utilizing a therapeutic protocol of prebiotics and probiotics might be a viable alternative in preventing infection or decreasing the risk of severe outcomes when infected with COVID-19.

Key words: Pregnancy, Immune System, COVID-19, SarsCoV-2, Microbiome, Gut flora, Pregnancy Immune system, Th1 Immune system, Th2 Immune system.

Introduction

The repercussions of the COVID-19 pandemic have continued since the winter of 2019-2020. People have been under the strain of lockdowns with public health and governmental restrictions and they long for it to end. Some have begun to rebel and are refusing to comply with govermental restrictions. Others are questioning how effective those restrictions and recommendations have been.

The vaccination program seems to be showing evidence of success. According to the Center for Disease Control (CDC), the COVID-19 vaccines currently authorized in the United States are demonstrating effectiveness against Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2), including asymptomatic infection, symptomatic disease, severe disease, and death. Study findings, along with the early evidence for reduced viral load in vaccinated people who develop COVID-19, suggest that any associated transmission risk is likely to be substantially reduced in vaccinated people. Additionally, available evidence suggests that the COVID-19 vaccines presently authorized in the United States offers protection against known emerging variants as well.[1]

A study done in Israel by Haas et al. has shown, that in all age groups, as vaccine coverage increased, the incidence of SARS-CoV-2 negative outcomes declined.[2] And a NIH pre-print found that the numbers of overall rates of infection with COVID-19 have decreased to 4.6% from the previous 9.0 % without vaccination, over 300 days.[3] This reported progress has raised hopes for a return to normal life. However, critical monitoring and data collection concerning any potential side effects of the vaccination should not be ignored and its health benefit ratio needs to be continuously evaluated.

Based on current available evidence, should vulnerable populations, like pregnant women, be vaccinated? And if not, what other means of protecting them is available to us with a known risk/benefit ratio? Does resistance in the general population to follow recommended protective measures, vaccine hesitancy or outright refusal increase the risk for vulnerable individuals to contract COVID-19? And despite the preliminary reported success of the current vaccines, with new mutations continuing to be reported, is there sufficient protection for our vulnerable populations?

A recent study by Egeren et al.[4] found that the overall size of the pandemic in terms of number of active infections will play a significant role in whether the virus can be brought under control with neutralizing antibody prophylactics or vaccines. The speed at which mutations develop in the population increases substantially as the number of infected individuals increases. This suggests that strategies to prevent stress on the coronavirus (e.g. antiviral prophylactics, high-

efficiency air filtration, masking, ultraviolet air purification) are key to reduce the risk of new mutations.[4]

As a result, it is important to protect the vulnerable subset of pregnant women and to empower them to protect themselves. Pregnant women have been at greater risk of complications with prior coronaviruses (Severe Acute Respiratory Syndrome (SARS) and Middle Eastern Respiratory Syndrome (MERS)).[5] They were identified as a vulnerable subset in the early stages of the COVID-19 pandemic.[5,6] In general preventative measures and treatment options for women in pregnancy are limited. However, the CDC recommendations published in April 2021 were that pregnant women should also receive the COVID-19 vaccine. New CDC research suggests that Pfizer/BioNTech and Moderna are safe for expectant mothers.[7] The COVID-19 Treatment Guidelines Panel of the NIH recommends that potentially effective treatments for COVID-19 should not be withheld from pregnant women because of theoretical concerns related to the safety of therapeutic agents in pregnancy. However, drugs need to be administered on an individually assessed basis.[8]

Before the pandemic, news of a pregnancy was usually a joyous event, but during this pandemic, joyful anticipation and celebration of new life is overshadowed with worry. Pregnant women agonize over how to protect their growing child and themselves from this disease. There is not enough data available to fully understand the effects of the virus or the vaccine on the pregnant patient and her unborn child. This is complicated by the changes that occur in immune function during pregnancy outlined in this paper. Does the pregnancy shift of the T-Helper (Th) cells reduce immunity to SARS-CoV-2? Would preventative measures be the appropriate path to take? And if yes, which one?

A connection between the gut and the COVID-19 virus has been found in recent studies.[9] There appears to be a deficit of certain strains of beneficial gut bacteria that serve as important immune modulators while other "bad" or pathogenic bacteria overpopulate the gut. This leads to the hypothesis, that when the microbiome's population is less immune enhancing, the individual may be more susceptible to an invading pathogen like the coronavirus. This also leads to the assumption that it might serve a pregnant woman to enhance the stability and healthy diversity of her microbiome to support the immune system. The pregnant patient may benefit from the use of a probiotic. But is this hypotheses supported by evidence?

Impact of COVID-19 on maternal health in pregnancy

According to the CDC, pregnant woman are at increased risk of developing more severe or complex symptoms when contracting SARS-CoV-2 compared to non-pregnant women. In a cohort study the CDC found that pregnant women were at higher risk for hospitalization, mechanical ventilation and ICU admission as well as mortality versus non-pregnant women with SARS-CoV-2.[10,11] Additionally, pregnant women with COVID-19 might have an increased risk for adverse pregnancy outcomes, such as preterm birth.[12]

Results of a large systematic review and meta-analysis involving 1,100 patients from China, North America and Europe show that in the majority of cases the clinical course of infection in pregnant women was not complicated. Most common symptoms were fever and cough followed by anosmia, ageusia, myalgia, fatigue, sore throat, malaise, rigor, headache and poor appetite.[13] The most common laboratory abnormalities were elevated C-reactive protein and reduced lymphocyte count, which are consistent with infected non-pregnant adults from COVID-19.[13] Viral RNA was found to be absent in amniotic fluid, placenta, vaginal secretion and blood, suggesting that intrauterine/intrapartum transmission is unlikely.[14] But it had been found that there was a high frequency of preterm births.[14]

A multi-center retrospective cohort study[15] of facilities in Washington State, with 240 pregnant patients infected, found 10% hospitalizations and 1.25% maternal deaths. They calculated a case fatality rate in pregnant women to be 13.6-fold higher compared to non-pregnant, similarly aged individuals. Interestingly, some of the maternal deaths occurred postpartum, which makes the postpartum period a time of important surveillance. Overall, mild COVID-19 disease occurred in 90.8% of the pregnant patients (including 55 asymptomatic) and 9.2% developed severe and critical disease.[15]

In the same study[15] they also looked at pregnancy outcomes in SARS-CoV-2 infected pregnant women and found two spontaneous abortions in the first trimester. In the second and third trimester, nearly all had live births. Preterm births, however, were significantly higher among women with severe or critical COVID-19 at delivery, than for women who had recovered. Furthermore, neonates born to mothers with severe or critical COVID-19 at the time of delivery, were more likely to be low birth weight (<2500g) as well as more likely to be admitted to the NICU for fetal indications than those born to women with mild or recovered symptoms at the time of delivery. Most common diagnoses of the neonate were respiratory distress, hyperbilirubinemia and possible sepsis.[15] Therefore, the findings suggest that the first trimester and before delivery are times where the pregnant woman needs more protection. And as it will be explained in this paper, this time coincides with the times where the immune system is in a pro-inflammatory state.

Even so, long-term sequelae of a mothers COVID-19 infection at the time of pregnancy or delivery have not been

studied to determine adverse outcomes on a child's health. They might become an important part in neuropsychiatric disease diagnosis, such as autism spectrum disorder[15] as time goes by. Long-term sequelae of viral infections like influenza have been associated with congenital abnormalities, such as cleft palate, neuronal tube and congenital heart defects.[16]

Immune system during pregnancy and with COVID-19 infection

The immune system in pregnancy faces the ultimate challenge. On one hand, the immune system needs to downregulate its activity towards foreign (fetal) tissue. Conversely, it needs to be able to defend against infectious agents. In other words, the immune system adapts to allow growth of the fetus as well as counteracts spontaneous abortion, which in turn, alters the immune response to viral infections.[17] The altered inflammatory response to viruses is mediated in part by a shift in cellular (Th-1) towards humoral (Th-2) immune response.[18] This causes a decrease of circulating natural killer (NK) as well as circulating plasmacytoid dendritic cells.[17]

The balance between the innate and adaptive immunity shifts in favor of the innate mechanisms, particularly in the first trimester. Whether maternal susceptibility to RNA viral infections is due to over- or under-activity of the innate immune system is not yet clear; it is likely that some effector mechanisms are upregulated while others are suppressed.[19]

In addition to this change in immune system function, a hormonal shift occurs and progesterone production is increased.[17] Progesterone is a steroid hormone, which plays an essential role in the establishment and maintenance of pregnancy as well as the onset of parturition.[20] In the myometrium, progesterone hinders a pro-inflammatory cytokine production.[21] Progesterone can decrease local and systemic inflammation, which consequently reduces Th cell function. Th cells play an important role in the adaptive immune system.[22] Therefore, it suggests that a decrease of Th cells causes a downregulated immune response to potential pathogens, like the coronavirus, and a decreased production of neutralizing antibodies to fight off the infection.

Mor and Cardenas[23] found that the immunology of pregnancy is the result of the combination of signals and responses originating from the maternal and fetal-placental immune system. They argue against the notion that the immune system shifts into a state of Th-2 (humoral) immunity or anti-inflammatory state during the entire pregnancy.[23] In fact, they propose that there are three immunological phases, characterized by distinct biological processes, which can be symbolized by how the pregnant woman feels.[23-25] The first and early second trimester, requires a strong pro-inflammatory response[26] in order for the blastocyst to break through the epithelial lining of the uterus to implant. Subsequently, the mother feels unwell and experiences "morning sickness." The second phase happens, when the baby rapidly grows. It is an anti-inflammatory state and the mother feels at her best. Finally, during the third phase containing delivery, the body goes through a renewed inflammation and is in a pro-inflammatory state.[23-26]

These shifts from the pro- to anti- to pro-inflammatory phases, and the respective shifts in the diversity of cytokines, may also be reflected in the sensitivity to infectious disease.[23-26] For example, an exacerbated Th2 immunity shift has been found to induce uncontrolled viral infections, like with the fetal ZIKA virus, which results in microcephaly.[27] This suggests that with the now known overreactive inflammatory response (cytokine storm)[28] in COVID-19, there might be negative pregnancy outcomes as well. Therefore, an adequate balance between Th1 and Th2 immune responses during pregnancy is critical for a successful outcome. Additionally, failure to achieve a proper balance during pregnancy is associated with obstetrical complications,[29] like pregnancy loss[30] and preeclampsia.[31]

In addition to the systemic immune changes, there are anatomical alterations, like the chest shape and elevation of the diaphram, which causes a reduction in total lung capacity and inability to clear secretions.[32] This further puts the pregnant mother at a disadvantage when fighting a respiratory viral infection, like SARS-CoV-2. SARS-CoV-2 can cause lung complications such as pneumonia and, in the most severe cases, acute respiratory distress syndrome (ARDS).[33]

Lwt's look at COVID-19 infection in terms of progression stages. There are three chronologic pathological stages, which define severity of the disease:[28]

1) Pulmonary stage with interstitial pneumonia and acute respiratory distress syndrome. In the pulmonary stage the epithelial cells of the upper and lower respiratory tracts are infected. Therefore, the person suffers from cold like symptoms, like a dry cough. The mucus epithelial cells, lining the respiratory and the digestive tracts, contain their very own immune system, the secretory Immunoglobulin A antibody (sIgAa) defense mechanism. A proficient sIgAa immune system neutralizes the coronavirus before the disease enters its second phase and becomes more severe and systemic.[28]

Historically, pneumonia during pregnancy has been associated with increased morbidity and mortality compared with non-pregnant women. Additionally, coexisting maternal disease, like asthma and anemia, increase the risk of contracting pneumonia in pregnancy.[32] And because

COVID-19 is a respiratory disease, it is fair to say that the risk of developing pneumonia with preexisting conditions, like asthma and anemia, is increased when contracting COVID-19 in pregnancy as well. Furthermore, developing pneumonia during pregnancy from COVID-19, is also associated with higher morbidity and mortality compared to non-pregnant women.

2) Proinflammatory stage with an overproduction of pro-inflammatory cytokines, which results in acute lung injury and systemic inflammation (cytokine storm).[28]

3) Prothrombic stage with widespread thrombosis, resulting in multiorgan failure or death respectively.[28]

All these stages suggest that the combination of the coronavirus disease in its second stage with a pregnancy pro-inflammatory phase (according to Mor and Cardenas, at the beginning and end of pregnancy[23]) could lead to a super pro-inflammatory cytokine storm. This means that it is possible, that the various pro-inflammatory cytokines and chemokines already released in abundance by the COVID-19 disease[28] could be facilitated or enhanced by the pro-inflammatory mechanisms of pregnancy. This then could cause a self-elicited and fatal systemic immunological reaction, which adversely affects every key organ in the body and may result in multiorgan faillure.[34] If this premise holds true it would make the first and third immunological phases of pregnancy especially worrisome.

Because the phases of pro-inflammation in pregnancy coincides with a decrease in important immune cells, like lymphocytes, macrophages, Th-18 and NK-cells[36] as well as an increase in pro-inflammatory cytokines,[23] it might explain why the ability of the immune system to fight off an infection greatly decreases.

The second immunological phase, on the other hand, is considered anti-inflammatory. This includes the second trimester to before birth, where the immune response is no longer a predominant endocrine feature.[23] This might explain why negative pregnancy interference and outcomes with a COVID-19 infection[5,6,10-12,15] are seen less in mid-term pregnancy and may therefore reflect the sensitivity to infectious diseases in the other immunological phases of pregnancy.[23] Pregnant women in malaria-endemic regions are more susceptilble to malaria infection during the first half of pregnancy than later on.[37] Lassa fever, caused by infection with an adenovirus, showed a higher rate of case-fatality in prengnant women particularly in the third trimester.[38]

The gut-COVID connection with pregnancy

A paper written by Yeoh et al. found[9] that the microbiome composition was significantly altered in patients with COVID-19 disease compared with non-COVID-19 individuals. Relating this significant finding to pregnancy immunologic changes requires further investigations, such as:

• SARS-CoV-2 pathophysiology can be attributed to aberrant immune responses in clearing the coronavirus.[9] In pregnancy with the aforementioned decreased adaptive immune system or a decrease in numbers of Th cells and antibodies respectively as well as an increase of pro-inflammatory cytokines,[23-25] there could be an even greater aberrant immune response.

• The detection of a viral load in fecal samples and the altered gut microbiota composition in COVID-19 infected people has been shown to correlate with disease severity. Additionally, disease severity correlates with the magnitude of plasma concentrations of several inflammatory cytokines, chemokines and blood markers such as C-reactive protein, lactate dehydrogenase, aspartate aminotransferase and gamma-glutamyltransferase.[5,9] This suggests that the depletion of immunomodulatory gut microorganisms and increase of pro-inflammatory messenger substances contribute to severe COVID-19 disease.[9]

In pregnancy (at conception, early second trimester and shortly before delivery) the immune system shifts towards a pro-inflammatory immunity.[23-25] This normal pro-inflammatory state coupled with severe dysbiosis of the microbiome composition[9] could potentially result in a rapid progression of a coronavirus infection to severe COVID-19 disease.

• Patients with COVID-19 were depleted in gut bacteria with known immunmodulatory potential, such as Faecalibacterium (F) prausnitzii, Eubacterium (E) rectale and several bifidobacterial species.[9] Replacing deficient gut bacteria with a probiotic supplement could be a viable and safe treatment option as a preventative measure or for mitigation of severe disease progression[9] in the pregnant patient.

• The dysbiotic gut microbiota composition in patients with COVID-19 persisted after clearance of the virus. This could explain persistent symptoms and/or multisystem inflammatory syndrome, that occurs in some patients after they tested negative to the coronavirus.[9] Recovered patients have experienced persistent symptoms such as fatigue, dyspnea and joint pains, some over 80 days after initial onset of symptoms.[39-41]

This makes postnatal care for mothers who recovered from COVID-19 infection very important.[15] Therefore it could be beneficial to take probiotic supplements for a minimum of three months after the corona test has been negative to rebalance the dysbiotic microbiome[9] and counteract long

COVID disease.[39-41]

Microbiome compositional differences were found in the gut microbiota of COVID-19 patients compared with healthy individuals. The main differences in species in COVID-19 patients was found to be an enrichment of Ruminococcus (R) gnavus, R. torques as well as Bacteroides dorei and depletion of Bifidobacterium (B) adolescentis, F. prausnitzii and E. rectale.[9] All of these depleted species play a positive immunomodulatory role in the human GI tract.[42-44] For example F.prausnitzii has been shown to cause secretion of the anti-inflammatory cytokine Interleukin (IL)-10.[45] Therefore, it appears, that the compositional differences, increase or decrease, of certain species in the gut microbiota of a COVID-19 patient, seem to reduce the functionality of the immune response to the disease.[9]

When identifying microbial species associated with disease severity, F. prausnitzii and B. bifidum were found to be decreased more in severe disease. This finding suggests that the amount of depletion correlates with an increased disease severity.[9] Furthermore, gut microbiota of recovered patients were still depleted in some species, which support immune system function, including E. rectale, R. bromii, F. prausnitzii and B. longum.[9]

Based on the observation that the gut microbiota is altered in patients with COVID-19, Yeoh et al. hypothesized that these compositional changes play a role in exacerbating the disease by contributing to dysregulation of the immune response.[9] In this study they were looking at the non-pregnant person. When adding the pregnancy altered immune response into consideration, an increased risk for complications or death with COVID-19 in the pregnant women becomes probable. And therefore, gut health could play an important role in prevention and/or inhibition of severe COVID-19 disease,[9] especially in pregnancy.

Potential beneficial role of probiotics on the outcome of COVID-19 in pregnancy

The microbiome interacts with the host and performs many viable functions. It plays a major role in immunity, which is especially important when the immune system is challenged with a new virus. There are several axes known, such as the gut/lung axis or gut/brain axis. For example, through the mesenteric lymphatic system, intact beneficial gut bacteria and their metabolites enter the systemic circulation and exert positive influence on the pulmonary immune response (gut/lung axis). Through this pathway intestinal metabolites such as short chain fatty acids (SCFA) or anti-inflammatory cytokines (eg., IL-10) influence the immune status of the gut and other distant organs.[46] SCFA derived from gut therefore suppress lung inflammation.[47,48] And in the case of an ongoing airway infection, Th-cells that produce regulatory cytokines travel from the gut through the lymphatic system to the respiratory system, providing support for the immune response via stimulation of an anti-inflammatory action.[46]

Non-COVID-19 individual stool samples were collected before COVID-19 disease.[49] Their microbiome was compared with individuals who had COVID-19 disease between February and May 2020. In the case of COVID-19 infection, the microbiome composition was altered. And Yeoh et al. (2021) found that the microbiome of patients with COVID-19 was immunologically impaired.[9] They concluded that without the appropriate bacteria, communication between the gut and the lung is decreased, which in turn causes an increase in inflammatory activity in the lungs.[9]

Additionally, in the setting of COVID-19, it is important to consider that antibiotics and antivirals are often administered resulting in further gut microbiota dysbiosis.[46] Dysbiosis triggers an increase in pro-inflammatory cytokines. This results in further disruption of the intestinal microbiome and results in damage to the mucous membrane barrier. A damaged barrier or increased permeability of the intestinal wall may lead to or enhance an already existing low-grade systemic inflammation. This causes further dysregulation of the human immune system and impairs the individual's ability to fight a viral infection. This has been called by the authors, Santacroce et al. (2021), as the «immunity dysregulation dysbiosis cycle» (IDDC).[46]

Evidence has demonstrated significant effects of probiotics in strengthening and modulating the immune system against disease.[50-53] Lactobacillus spp. and Bifidobacterium spp. are the main conventional probiotics that are available for use to balance or diversify the intestinal ecosystem in the fight against infections. Probiotics, such as Lactobacillus and Bifidobacteria can lead through an antiviral action to a balanced intestinal microbiome and thus contribute to an anti-inflammatory effect, which could potentially prevent an infection or even super-infection of COVID-19.[47]

Summary

In summary, research has shown, that not only the viral infection of COVID-19, but also the host immune response defines disease evolution. A depleted gut microbiota taxa may play a role in overaggressive inflammation and disease outcome.[9] Gut bacteria such as B. adolescentis, F. prausnitzii and E. rectale have been linked to reducing host inflammatory response in other inflammatory related diseases.[9,42-44] And because they are available as an oral probiotic supplement, it might be beneficial to take a multispecies probiotic supplement before, during and for at least 3 months after pregnancy, with or without COVID-19 infection.

Early therapeutic approaches might be key in battling COVID-19 disease. Pharmaceutical administration in pregnancy is very limited, especially when drugs are still in the investigatory stage and whose mechanisms may be very aggressive with deleterious side effects for the patient. Data has yet to be accumulated and published on the effects of some of the current pharmaceutical therapeutics employed for COVID-19 on the pregnant patient and the fetus. Vaccination in pregnant women has been recommended by the CDC.[6] Pregnant women may be hesitant to receive it because of unknown longterm outcomes for them and their offspring.

This paper was written to provide a possible additional alternative. It has been demonstrated that probiotics can effectively counteract some immune dysregulations caused by viral infections.[54] Therefore, it may be a viable therapeutic approach to target first and second-stage pathogenesis of COVID-19 with probiotics. Potentially hindering the coronavirus from progressing to the third and final stage of the disease or decrease an exaggerated inflammatory response by counterproducing anti-inflammatory cytokines and chemokines as well as SCFA's.[50-53] This is especially important at the beginning and end of pregnancy (pro-inflammatory phases).[23]

Probiotics have been used in pregnancy and are proven to be safe by the FDA.[55] They have been shown to protect against infectious disease by immunomodulatory regulation and antiviral activity.[54] Therefore it's recommendation for use as an additional strategy against COVID-19 infection or it's progression and ensuing complications is warranted. The metabolic actions of the beneficial gut bacteria in producing SCFAs and other anti-inflammatory properties reinforce the modified immune system of pregnancy. They also regulate the naturally occurring immune system shift and counteract dysbiosis in the case of COVID-19 disease.

Conclusion

The challenging circumstances in combatting Covid-19 during pregnancy requires exceptional immune support. There is mounting evidence that probiotics modulate gut health. They are safe and effective in improving immunity and fighting viral activity. Disease severity has been moderated with a healthy microbiota, which could lessen the course of disease severity during pregnancy. This approach might be considered by clinicians caring for these patients.

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