COVID - 19

PATHOPHYSIOLOGY OF COVID-19: KNOWN AND UNKNOWN

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Abstract: *The corona virus disease 2019 caused by severe acute respiratory syndrome corona virus-2 starts as a respiratory infection but can progress to multi-organ involvement with some very unique and unusual clinical presentations. This can appear at times puzzling and can account for significant morbidity and mortality. Understanding the pathophysiology of this disease can help reveal the various mechanisms of the progress of the disease and can explain the clinical symptoms and offer hope for prevention and treatment modalities.*

Keywords: *SARS-CoV-2, COVID-19, Pathophysiology, Children.*

The severe acute respiratory syndrome corona virus-2 (SARS-CoV-2) that originated in China towards the end of December 2019 has spread rapidly all over the world and hence labelled as the corona virus disease 2019 (COVID-19) pandemic. This virus belongs to the Corona group, which commonly causes minor upper respiratory tract infections in both children and adults. The SARS-CoV-2 however is a new mutant with specific features which are different from the other Corona viruses. These are:

1. A novel mutation increasing susceptibility (that makes practically every human susceptible).
2. An easy transmissibility (that has caused rapid spread worldwide).
3. An unusual pathophysiology with involvement of many systems of the body beyond the respiratory tract (that has contributed to different clinical presentations and higher morbidity and mortality).

The focus here is to elaborate on the third feature i.e., the unusual pathophysiology. It offers an insight into the mechanisms of damage caused by the virus and the disruption of the host systems, which can explain the clinical features of the disease and may help in finding appropriate treatment modalities. However, since this is a new disease, few well documented studies are available. With the rapid spread and high morbidity and mortality associated with this pandemic, the efforts of researchers and clinicians have naturally been focused on risk stratification, prevention of transmission, treatment methods and of course the race to find a vaccine. It is but natural that there are very few peer reviewed published studies on the pathophysiology or autopsy findings of this novel disease. It is an evolving field and hence including the phrase "Known and Unknown" in the title is justified.

The pathophysiology of the disease has several facets but essentially the disease occurs in three stages (Fig. 1).

Stage I. Entry of the virus and early replication

During the first week, when the virus gets inoculated, it establishes itself inside the host. The route of infection is through the mucosa, usually of the upper respiratory tract. The conjunctiva is also a suspect portal of entry. The virus is believed to gain entry by attachment to a metallo-peptidase named angiotensin-converting enzyme receptor (ACE2 receptor). Studies on the earlier SARS virus had shown that the S1 domain of the spike protein of the virus binds well to the ACE2 receptor.[1] In fact, the SARS-CoV-2 virus has a 10-20 times higher affinity to these receptors compared to the earlier SARS virus. This receptor has therefore been described as the functional receptor for SARS-CoV-2. It is uncertain whether the SARS-CoV-2 virus has any other receptor for entry. CD209L has been proposed as an alternative receptor, but confirmation is lacking.[2]

The importance of this hypothesis is that drugs that alter the receptor to prevent attachment by the virus can be potentially used in the prevention and treatment of COVID-19. Examples of drugs that can change the glycosylation of these receptors and reduce viral entry in vitro are chloroquine and hydroxychloroquine (HCQ) and an initial small uncontrolled study with HCQ had shown clinical promise.[3-5] Although the genes for the ACE2 receptor are present in all human cells, they are expressed

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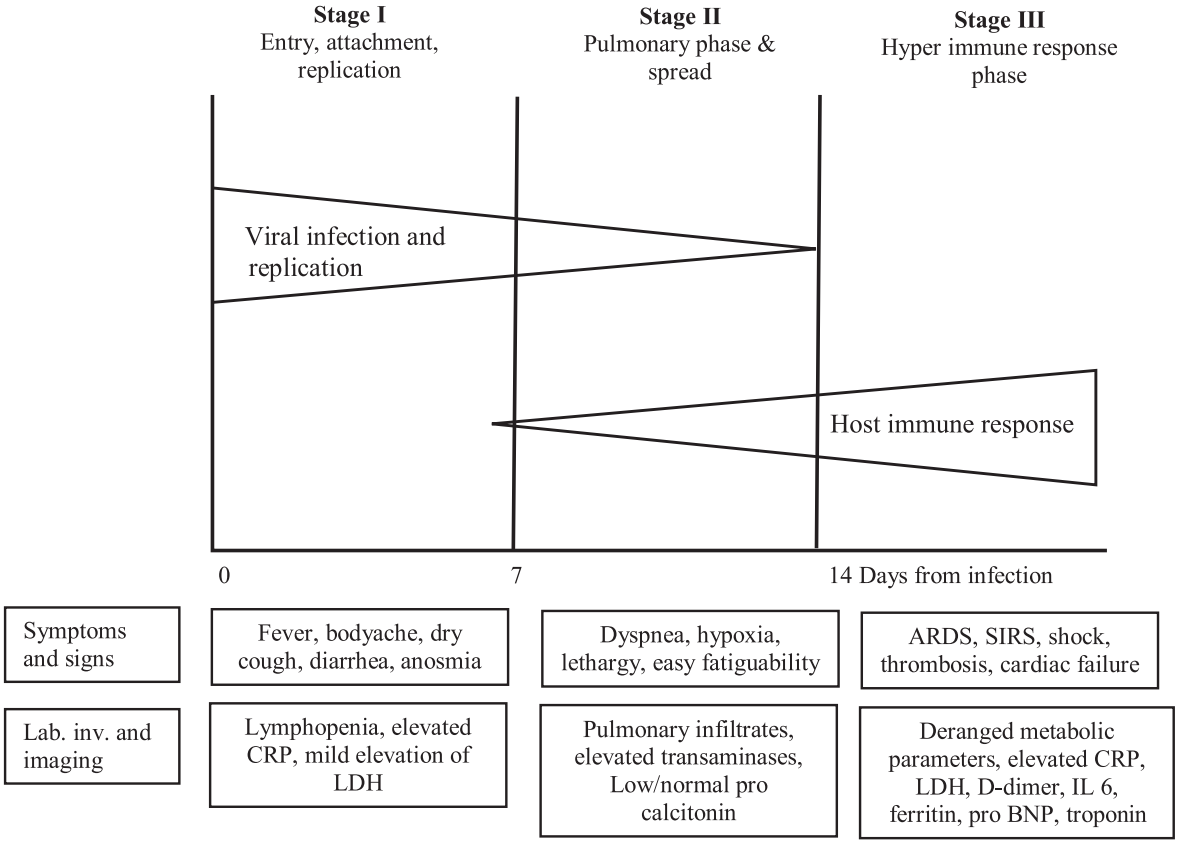

Fig.1. Stages of COVID-19 infection

only in specific tissues of the body and in high amounts in the oral and nasal mucosa and the gastro-intestinal epithelium. This is the reason why the virus is easily able to enter the human host through these routes. Once attached, the virus is able to enter the cells by a process of endocytosis. In vitro electron microscopy studies clearly show hundreds of viral particles clustered around the cilia and in double walled vacuoles within the cytoplasm of epithelial cells derived from the respiratory tract.

Another very remarkable finding is the surface expression of ACE2 protein on lung alveolar epithelial cells and in the endothelial cells of arteries and veins in all organs. This has great relevance to the two important clinical features of the disease i.e. pneumonia and coagulopathy. Lymphoid cells in the lymph nodes, thymus, bone marrow and spleen are surprisingly devoid of ACE2 so there must be an alternative (but yet unknown) mechanism for the hallmark lymphopenia that is seen even in the early stage of the illness.[1] Although the expression of ACE2 metallo-protein in gustatory and olfactory receptor cells has not been specifically studied, it is likely that they are located in the mucosal lining of these organs as they are contiguous. So, they can be invaded by the virus causing the two rather specific symptoms of COVID-19 namely the loss of sensation of smell and taste.

ACE2 receptors are also expressed in the arterial, venous endothelial cells and arterial smooth muscles of organs like heart, liver and brain and these can be affected by the infection.[1] The testis is another particular organ with high levels of ACE2 receptors. They possibly serve as a reservoir for the virus which accounts for the delayed clearance and higher mortality seen in males compared to females. Another reason could be the higher expression of ACE2 receptors in males compared to females.[6] Some studies have also shown that pre-existing heart disease leads to a higher expression of ACE2 receptors on the myocardium which may account for the higher cardiac complications and mortality in this group. This is reflected in higher levels of circulating ACE2 receptors in the male gender especially with pre-existing heart disease like cardiac failure.[7]

In the first stage of the disease, the virus is just getting a foothold and multiplying in the mucosa, near the entry site, which is the incubation period and the infected person remains asymptomatic. This period lasts from 2 to 7 days with a mean of 4-5 days. The symptoms then start and consist of fever (which may be high or even completely absent), constitutional symptoms like headache, body ache, dry cough, throat pain, anosmia, ageusia and diarrhoea. The laboratory findings in this stage (that last about a week)

are lymphopenia, moderately elevated CRP and modest elevation of LDH. However, some individuals and especially children can remain almost totally asymptomatic all through the illness.

Stage II. Spread to lungs and other systems

By the second week, the virus is able to spread to the lungs which has rich expression of ACE2 receptors and hence pneumonia is the most common pathology. The primary involvement is the ACE2 receptor expressing alveolar type II cells, generally at the periphery of the lung.[2] A larger lung alveolar surface area is involved in coronavirus infection than in bronchopneumonia, due to ubiquitous expression of ACE2 on type II pneumocytes.[8] Alveolar type II cells perform many critical functions that include production of pulmonary surfactant, airway epithelial barrier stabilization, immune defence and airway regeneration in response to injury. As the SARS-CoV-2 replicates within these type II cells, the affected cells undergo apoptosis and release a large number of viral particles to infect the neighbouring cells. Spread to surrounding lung is relatively prevented by ciliary activity.

Poor muco-ciliary clearance in the elderly is probably responsible for the higher incidence of pulmonary complications in older population. Chronic damage to the ciliary lining of the respiratory tract by habitual smoking may similarly account for the higher morbidity in smokers. Children normally have a robust muco-ciliary action and are therefore less likely to have COVID pneumonia unless they have pre-existing conditions like cystic fibrosis, bronchiectasis or diabetes with consequent reduced muco-ciliary clearance. This has been validated by clinical data. In a multi-centric cross sectional study in North America on 46 children requiring ICU admission, 83% of them had some co-morbidity or pre-existing chronic illness, generally cardio-pulmonary or diabetes mellitus. Twenty four patients (>50%) had one comorbidity, 8 had two and 9 had three or more concomitant co-morbidities.[9]

With progressive loss of type II alveolar cells, surfactant production is affected and micro-atelectasis occurs causing the streaky shadows seen on chest imaging by radiography or CT and finally a ground glass appearance. The alveolar type II cells are also the precursors of the type I cells that maintain the integrity of the alveolar lining and permit gaseous exchange. Hence the end result when the alveolar type I cells get involved is impaired gaseous exchange leading to hypoxia. The pathological result of SARS and COVID-19 is diffuse alveolar damage with fibrin rich hyaline membranes and a few multinucleated giant cells. Aberrant healing of alveolar lining may lead to scarring and fibrosis that may present as ARDS. This can prolong the need for ventilatory support into the third week overlapping with the hyperimmune stage III of the disease with its attendant complications. Recovery requires a vigorous innate and acquired immune response and epithelial regeneration. This may be defective in the elderly and result in longer duration of sub-optimal lung function with consequent very gradual and incomplete recovery of the lung capacity.

The pneumonia of COVID is thus different in severity and course from the exudative consolidation seen in bacterial infections like pneumococcus. A productive cough therefore is not commonly seen (unless there is a secondary bacterial invasion) and the only symptom may be a progressive shortness of breath with general lethargy and fatigue from the slowly increasing hypoxia.

Another intriguing observation in COVID-19 disease is that the hypoxia is not usually accompanied by air hunger; instead, a paradoxical feeling of calm and well-being may result. This phenomenon has been coined 'silent or happy hypoxia'. The etiology of this observation is not clear at present. Certain structural viral proteins attacking porphyrin moiety of hemoglobin has been postulated as a possible reason, but has not been substantiated by evidence.[10]

Spread of the virus to many other organs like liver, kidney and brain may start in the second week. This is believed to be via the bloodstream and has been documented in Chinese studies.[11] The actual pathophysiology in these organs in the current COVID-19 epidemic has not yet been elucidated as there are no studies on histopathology from these organs. Many of the postulates are based on the studies of the earlier SARS epidemic due to a Corona virus of the same group.

In the most comprehensive study of cardiac involvement in patients who died from the earlier SARS epidemic, viral RNA was detectable in a third of post-mortem cardiac tissues and was associated with both decreased ACE2 expression and increased macrophage infiltration.[11,12]

Hepatic involvement is common and leads to elevated liver transaminases and occasional cases present with jaundice. Neurological symptoms, although uncommon can signal invasion of the virus into the CNS and the patient can present with altered sensorium, seizures and neurological deficit. Renal dysfunction is common but this may be as a result of circulatory problems and not due to viral invasion of the kidney. This is buttressed by the fact that RT-PCR studies for SARS-CoV-2 in the urine have

failed to identify the virus.[11] Also, changes in these vital organs would be compounded by problems in blood supply due to cardio-respiratory failure, shock and the vasculopathy / coagulopathy. Autopsy findings have shown ischemia, infarction and effects of shock.

Surprisingly, many children may have little or no respiratory involvement and present with only extra-pulmonary findings. Some have predominant gastro-intestinal symptoms like diarrhea and vomiting, even intussusception and intestinal gangrene and others have only neurological findings like convulsions and altered sensorium without any cough or dyspnea.[12,13] Why this happens only in the pediatric age group baffles many and may be related to the different pattern of distribution or expression of ACE2 receptors.

What is also unclear is the rate of resolution of these pathological changes in various organs and whether there are any residual lesions. This is particularly important in commonly involved organs like the lungs. The reported very gradual recovery of elderly adults with long lasting shortness of breath and dyspnea on minor exertion probably indicate an incomplete recovery of normal gas exchange in the alveoli due to persistent residual fibrosis.

Stage III. Hyperimmune response phase

This stage is not seen in all patients. A majority of patients and an even higher percentage of children seem to recover after stage II or even directly after stage I. This has been the case right from the earliest studies from China. Initial data of 72,314 cases from Wuhan presenting for medical care, showed only 1.3% of them were aged below 20 years and a subsequent report of 171 children younger than 16 years hospitalized in Wuhan province reported that only 3 required ICU care with a single fatality.[13-15] Thus, particularly in pediatric age group, it is only in a minority of cases where there is a progression to this stage with peculiar features and is possibly related to an abnormal or variant host immune response. It is this stage specific to Corona virus that is responsible for the severe morbidity and mortality and hence needs to be addressed. The host immune system comes into play by the end of the second week or so. This is correlated by the presence of IgM antibodies by 5 to 8 days and IgG antibodies in a majority of cases by 10-14 days. Further pathophysiology possibly results from a complex interplay between the direct effects of the virus and the host immune reaction.

A few peculiar clinical situations described so far in the current COVID-19 epidemic are discussed below.

Vasculopathy/Coagulopathy: Virchow's triad delineating the pathophysiology of intravascular thrombosis proposes that it can occur as a result of three factors: a) reduction in blood flow (stasis), b) vascular endothelial injury (leading to triggering of the coagulation cascade) and c) hypercoagulable state due to alterations in the blood constituents.

All three factors are in part responsible for causing the reported complications of thrombosis and embolism in COVID-19. The reduced blood flow is secondary to the cardiac decompensation seen as a part of the systemic inflammation response syndrome (SIRS) or septic shock in severely ill patients. The vascular endothelium is probably damaged directly by the virus via the ACE2 receptors with subsequent triggering of the coagulation cascade. Elevated levels of antiphospholipid antibodies have been found in some patients with COVID-19 in the third week but the correlation with coagulopathy is not clear.[15] The laboratory evidence for the onset of the coagulopathy is the elevation of D-dimer levels which reflects ongoing activation of the hemostatic and thrombolytic system. Deep vein thrombosis in the lower half of the body is the commonest affliction seen followed sometimes by pulmonary embolism. Small pulmonary vessel thrombosis and hemorrhages seen in SARS-CoV-2 reflect pulmonary involvement and possibly add to the deranged respiration and gaseous exchange.[14] Part of the CNS and other organ dysfunction may also be caused by arterial thrombus due to endothelial injury, stasis and hypercoagulable state.

Intravascular clots in COVID-19 can essentially occur in any vessel, arterial or venous. Sudden cardiac death seen in some adults who appeared to be recovering from respiratory failure with decreasing oxygen requirement could be due to acute myocardial infarction occurring from thrombosis in the cardiac circulation. Involvement of these parts of the circulation has been reported to happen more commonly in adults than in children. In contrast, in children, vasculitis/coagulopathy changes have been described more often in the peripheral circulation like the tips of the toes and fingers. Lesions such as purpura, skin necrosis, subcutaneous hematoma and local infarction causing chilblain like lesions on the tips of the fingers and toes (called "COVID toes") have been described, initially from Europe but later on elsewhere too.[16] Clinical importance of this pathophysiology is the possible role of anticoagulants like low molecular weight heparin in preventing or treating these complications.

Cytokine storm

It is well known that sepsis syndrome complicating any infection, bacterial or viral is a complex interplay of

pathogenic effects of the infective agent as well as the host response. It is no different for COVID-19. While the clinical course of the disease in children may be mild, the immune response starting towards the end of the second week can contribute to peculiar clinical presentations and contribute to mortality. It is quite likely that the resulting organ dysfunction is mediated by excessive release of a number of cytokines and these are involved in the pathophysiology of the acute respiratory distress syndrome (ARDS) and the sepsis syndrome described earlier.

But additionally, a specific multisystem inflammatory syndrome has been described to occur in children with COVID-19.[17-19] IL-2, IL-6, IL-7, IL-10, granulocytic colony stimulating factor (GCSF) and TNF-α are some of the cytokines with high levels recorded in these cases.[20] But one of the key cytokines in this process is IL-6 and some studies have shown positive correlation of this agent with disease severity.[21] The multisystem inflammatory syndrome presents with features similar to Kawasaki disease with constitutional features, limbus sparing conjunctivitis, cracked lips, even skin rashes and brawny edema of peripheries and multi-organ dysfunction including fluid refractory shock. Hypoalbuminemia and pleural / pericardial effusions have been described in some cases. Laboratory evidence of high acute phase reactant levels (ESR, pro-calcitonin, CRP), IL-6 and ferritin (moderately elevated) is always present in these cases and markers of cardiac damage like troponin and N-terminal pro B type natriuretic peptide (NT-pro-BNP) may also be elevated. Late thrombocytosis, the hallmark of Kawasaki disease is however absent. In such a situation, anti-inflammatory agents like methylprednisolone, intravenous immunoglobulin and monoclonal antibodies like Tocilizumab which is specific antagonist for IL-6 have been used with some clinical success.[21]

Several explanations have been proposed for the relatively lower morbidity and mortality observed in children as compared to adults with COVID-19. These include the following:

i) The first possibility is that the expression level of ACE2 may differ and ACE2 expression may be lower in pediatric population.

ii) The second possibility is that children have a qualitatively different response to the SARS-CoV-2. Severe COVID-19 infection in adults is characterized by a massive proinflammatory response or cytokine storm that results in ARDS and multi-organ dysfunction (MODS). Ageing is associated with increasing proinflammatory cytokines that govern neutrophil functions and have been correlated with a severe illness.

iii) The third possibility is that the simultaneous presence of other viruses in the mucosa of lungs and airways in young children compete with SARS-CoV-2 virus and limit its growth. But we do not have studies to prove this right now.

Rather a combination of these factors may cause less severe COVID-19 in children.[21]

The pathophysiological mechanisms underlying SARS-CoV-2 infection is an unfolding story and the last words on the clinical features, pathophysiology and its implications for treatment of COVID-19 are yet to be written. Hence it would be prudent to stay abreast of the burgeoning evidence emerging in this long drawn pandemic.

Points to Remember

- ***The pathophysiology of SARS-CoV-2 infection appears to be unique with involvement of many systems of the body beyond the respiratory tract.***
- ***The disease progresses through three stages - virus entry and replication, spread to lungs and other organs followed by a hyperimmune response.***
- ***Only a minority of children progress to the hyperimmune response stage.***
- ***Vasculopathy / Coagulopathy is responsible for the complications of thrombosis and embolism in COVID-19.***
- ***Difference in expression level of ACE2 and qualitative response to SARS-CoV-2 can explain the clinical differences observed in children.***
- ***Multisystem inflammatory syndrome due to cytokine storm may present with features of Kawasaki disease.***

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