



CORONAVIRUS: AN OVERVIEW OF THEIR REPLICATION & PATHOGENESIS

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Coronaviruses are (COV) RNA viruses with club like spikes. Diseases in mammals and birds like enteritis in cows and pig and upper respiratory infection in chicken and lethal human respiratory infection. CORONA belongs to Nidovirales order $\alpha\beta$ delta and gamma COV. It has very large genome for RNA viruses.^[1]

Virion structure

Coronavirus contains four main structural proteins. Spike (S), membrane (M), envelope (E) and nucleocapsid (N) have different functions. M protein is most abundant. E protein is in small quantities, E protein facilitates assembly and release of virus.^[2,3]

N protein is highly phosphorylated and triggers the structural change and is important for enhancing the affinity for viral versus non viral RNA and subsequently packaging the encapsulated genome into viral particles.^[4,5]

A fifth structural protein, the hemagglutinin esterase, is present in the subset of β coronaviruses and haemagglutinin esterase is present in the subset of β coronaviruses and haemagglutinin binds sialic acids on the surface of glycoprotein and contains acetyl esterase activity. These activities are thought to enhance S protein mediated cell entry and virus spread through mucosa. The enhanced murine hepatitis virus of neurovirulence.^[6,7]

The initial attachment of the virion to the host cell is initiated by interaction by S protein and its receptor. The sites of receptor binding domain (RBD) within S-1 region of a coronavirus S protein vary depending on the virus, with some having RBD at N terminus of S1 (MHV) while others (SARS-COV) have RBD at C terminus of S1.^[1]

Many of alpha coronavirus utilize aminopeptidase N (APN) as their receptor, SARS-COV and HCoV-NL63 angiotensin converting enzyme 2 (ACE2) their receptor, MHV enters through CEA CAM 1 and recently identified MERS-COV to dipeptidyl peptidase 4 (DPP4) to gain entry into human cells for a list of COV receptors.

Following receptor binding the virus must next gain access to the host cell cytosol which is roughly accomplished by acid dependent proteolytic cleavage of S proteins by a cathepsin and through various processes there is release of viral genome into the cytoplasm.

There are many theories regarding the coronavirus replication.

Assembly and release

The M protein directs most protein-protein interactions required for the assembly of coronavirus. However, the M protein alone is not sufficient for virion formation; the virus-like proteins (VLP). However, when M protein is expressed with E protein, VLPs are formed to produce coronavirus envelopes. N protein helps enhance the formation of VLPs which enhances viral development.^[8]

Following assembly, the virions are transported to the cell surface in vesicles and released by exocytosis. In severe coronaviruses, the S protein that does not get assembled in various transits to the cell surface where it mediates cell-cell fusion between infected cells and adjacent uninfected cells. This leads to the formation of giant multinucleated cells which allows the virus to spread with an infected organism without being detected or neutralized by virus-specific antibodies.^[1]

Animal coronavirus

Coronavirus cause a large variety of diseases in animals such as pigs, cows, chicken, dogs, cats — a lot of research in the last half of the 20th century which we will not go into detail.

Human coronavirus

Prior to SARS-COV outbreak, coronaviruses were only thought to cause mild and self-limiting respiratory infections in humans. Two of these viruses are α coronavirus and β coronavirus were isolated 50 years

ago.^[9, 10] These viruses are endemic in human population causing 15-30% respiratory infection each year.

These cause more severe infection in neonates and elderly and in individuals with other systemic diseases with lower respiratory infection in these population. α virus infection with HCoV-NL63 is also associated with acute laryngo- tracheitis.^[11] There were genetic variability in different pocket of the population.

SARS- COV group 2b- β coronavirus was identified as the causative agent of severe acute respiratory syndrome (SARS) out break that occurred in 2002-03 in Guangdong province of China, approx. 8098 cases occurred with 774 deaths with mortality rate of 9%. The mortality rate was much higher in elderly or 60 years and even upto 50%. It is widely accepted SARS-COV originated in horse shoe bats, through the outbreak was traced to a hotel in Hong Kong and spread to SE Asia and Toronto, Canada. They were also found to use the same receptor as the human virus, angiotensin converting enzyme 2 (ACE 2) providing further evidence that SARS- COV originated in bats. Only a small number of SARS causes occurred after the outbreak was controlled in June 2003. While the SARS-COV epidemic was controlled in 2003, the virus not since not returned ; a novel human COV emerged in middle east in 2012, the virus named middle east(9) respiratory syndrome COV was found in highly pathogenic respiratory tract infection in Saudi Arabia and other countries in the Middle East^[12]. Based on high mortality rate of > 50%, it was feared that virus would have serious repercussions. However the outbreak did not accelerate in 2013, although sporadic cases continued throughout the rest of the year. In April 2014 a spike of 200 cases occurred with 40 deaths. As of August 27, 2014 there have been a total of 855 cases of MERS –COV with 333 deaths that is case mortality of nearly 40% according to European centre for disease prevention and control.

SARS and MERS outbreak have stimulated research and many anti viral agents such as proteases, polymerases and anti proteinsvaccines. These were also used but they were not very effective. Significant work remains.

In the start of December 2019 a novel corona virus designated SARS covid 2 has caused an international outbreak of respiratory illness caused COVID-19. The full spectrum of COVID-19 illness ranges from mild self limiting respiratory illness to severe respiratory disease and multiple organ failure and death. This started from Wuhan Province of China and has spread exponentially across the globe.^[13] The total number of cases as on April 15, 2020 is nearly 20 lakhs. The total number of deaths reported is 127000. Maximum brunt of morbidity was in China, Italy, USA Spain and UK but also in Germany, France, South Korea, Switzerland and entire Europe and South east Asia including India. The mortality rate was maximum in China but Italy surpassed it with 21000.^[13]

Various drugs like anti-retroviral drugs such as Lopinavir and Ritonavir and anti-malarial like hydroxychloroquine (HCQs) were used with good results in prevention as well as treatments

COV-19 was caused by SARS COV 2. The current mode of transmission is incomplete. Epidemiological investigation in Wuhan at the beginning of outbreak identified initial association with sea food market which most people had visited or worked. Later studies prove that it had more similarities in virusin bats. However as the outbreak progressed person to person transmission the droplets and fomites became the primary mode of transmission.^[14] One study also suggests that virus may be present in faeces.

Collection of samples to test SARS- COV 2 from nasopharyngeal or oropharyngeal swabs is preferred method. Bronchoscopy should normally be avoided as it may infect others.^[15]

SARS COV 2 RNA is detected by polymerase chain reaction. A single positive test should be confirmed by second RT PCR assay targeting in different SAAS- COV 2 gene.^[16]

Covid 19 rapid test qualitatively detects IgG and IgM antibodies to SARS Cov 2 in human whole blood, plasma or serum samples.^[17]

Other investigations are CT chest showing interlobular septal thickening, crazy patterns, air bronchospasm and pleural thickening; lung ultrasound and pulmonary function tests, latter to be avoided due to infection spread.^[18-19]

Transmission

Is by droplet infection when the virus is released by respiratory secretions when and an infected person coughs, sneezes or talks and also by touching infected fomites. that is why one must keep difference of 2 meters from not only infected person, but also from apparently normal looking person as you never know who is under incubation period. and by fomites by touching your nose, mouth, face and eyes. Patients are thought to be most contagious, when they have symptoms.^[20] Some spread might be possible before the symptoms appear.^[21-22] Virus may also spread by faeces and could contaminate toilet bowls or sinks or other fomites.^[23]

Pathological finding

In children there are lymphopenia, leucopenia or leukocytosis(rear) in more than 80% patients and thrombocytopenia which is considered a bad prognostic sign, leucopenia or leukocytosis, raised liver enzymes esp. LDH, muscle enzymes & myoglobin, some critical ill patients have increased troponin, D-dimer but ferritin levels are reduced. Like adults, the children with clinical critical illness may be accompanied by elevated levels of inflammatory factors such as interleukin-6, IL-4 and IL-

10 and tumor necrosis factor(24). In initial stages in children chest X ray is normal, so CT should be done as early as possible.

Inflammatory markers

Serum procalcitonin is often normal at the time of admission, however, increases in the patients who require ICU care. In one study D-dimer & lymphopenia is suggested as poor prognosis.

C- reactive protein is raised in COVID -19 and in patients with severe respiratory illness, if C reactive protein is normal, one must think of an alternative diagnosis.

In **Chinesenew** born, Coronavirus was detected 30 hours after birth. It is unclear how the disease was transmitted in womb or after birth.

Clinical features

Incubation period presented to be 2-14; days most case occurring in 5 days. Spectrum of illness mild 81% patients, severe illness greater than 50% lung involved in 24-48 hours (14%).

Critical disease: Multiple organ failure 5%

Overall case fatality between 2-5%: Age affects mortality middle aged > 30 elderly and following are clinical features.^[25]

Clinical presentation: Fever (85%), fatigue (35%), Dry cough (67%), Myalgia (14.9%), Dyspnoea (18.7%)

Other symptoms can be headache, sore throat, rhinorrhea, GI symptoms. Headache could be due to encephalitis, the virus entering the skull through cribriform plate in nose.

We will not go into details of clinical feature as a lot has been written in the literature already by International pulmonologist consensus over COVID-19.

Children were less affected because of winter vacation, humoral or cellular immune response being less may lead to less symptoms. There are some more theories too, but there are no maternal child infection except duct contact.

COVID-19 exploits ACE -2 receptor to gain entry inside the cells under expression and immaturity of ACE-2 receptor in child might be another reason.

Diagnosis

WHO^[7,22] has divided it into 4 categories

1. Suspected cases
2. Probable cases
3. Confirmed cases
4. Close contacts

Preventive management

The best weapon to keep COVID-19 at bay is reducing the person to person contact and stay at home. For this the government across the globe have locked down and imposed curfews and requested people to stay at home and washing of hands frequently with soap and alcohol based sanitizer and not to touch nose, face or eyes and

not shaking hands. If you get sneezing use handkerchief or even flexed arm to avoid spreading droplet infection.

Whom to Isolate

Any person positive in laboratory tested for COVID 19 & to be guaranteed least 14 days.

Any person or health worker knowing fever and respiratory illness or has a close contact with COVID-19 positive patient.

Asymptomatic persons who have come in contact with COVID – 19 patients to be quarantined at home for 14 days.

Adequate ventilation

Don't use other person's mobile

Give food to quarantined person in disposable plastic cutlery.

Treatment Modalities in Nutshell

Mild disease/ asymptomatic cases: only isolation for 14 days.

Severe disease (14%): Respiratory rate >30/ minute, SPO₂< 93%, PaO₂< 300, lung infiltrates > 50% within 24- 48 hours.

Critically ill (5%): Respiratory failure, septic shock and MODs, needing ventilator.^[1]

There is no drug of choice only fluids, maintain oxygen saturation > 90 %, empirical antibiotics/ antiviral oseltamivir; some noted doctors have used retroviral anti HIV drugs like Lopinavir & Ritonavir in Jaipur India (unpublished data through interview on media) and WHO has maintained these agents can be used.

No antiviral drug has been proved to be effective for COVID- 19 in humans.

Chloroquine/ Hydroxychloroquine

Chloroquine/ Hydroxychloroquine are being used with good results.

- The proposed mechanism is that it hampers the low pH dependent steps of viral replication.
- As prophylaxis has been suggested as hydroxychloroquine 400 mg OD for two days in a week, after getting G6PD done, which is a must for males, females have a very very rare chance due to G-6-PD being X linked and too rare for reaction.

Various other drugs like ascorbic acid have been suggested but of little value. Tocilizumab & Interferon beta β, can be considered, the latter in combination with Lopinavir/ Ritonavir and hydroxychloroquine of late plasma from recovered patients is also giving good results.

Some people have used plasma from recovered patients with good results

Role of BCG: A recent study from Houston, Texas has analyzed on data from 178 countries, that countries that do not have a BCG vaccination policy saw ten times greater incidence of mortality from COVID -19, than those who had it. If it is true ultimately, we think BCG vaccine should be repeated at adolescence.

The good role of BCG could only be compared if large study in hotspots of Coronavirus in countries like USA, Italy or Spain are conducted between non resident Indians who have been vaccinated by BCG at birth and other local residents of these countries who have not been vaccinated, regarding the morbidity and mortality. Let us wait for such study.

Outcome factors

- 10-20% of patients are admitted to ICU
- 3-10% require intubation
- 2-5% die

Risk factors

- Older age group
- Male sex
- Medical comorbidities (recently Corona viruses has been seen to affect heart in the shape of arrhythmias and symptoms like myocardial infarction, while their angiocardigraphy was normal. Which was called pseudo myocardial infarction which was due to low oxygen supply due to COVID-19
- Chronic pulmonary diseases
- Cardiovascular disease
- Immunocompromised patients
- Chronic kidney disease
- Diabetes

CONCLUSION

The author's analysis

COV-19 was first reported in China in December 2019 and it is likely to stay till a vaccine without any side effects is prepared which is being tried in many countries like China & USA. The authors feel that the process of disposing of bodies in coffins incemeteries is not an ideal process. Usually it takes 10-12 years for the bodies to decompose, depending upon the wood used for coffins, but it may take even upto 50 years or so for the tons to decompose by which time the mice, rats and other rodents may create burrows and reach upto the cadaver remains and if by chance the same infected rodent is eaten by human as is the habit in some of countries, the old or new virus or bacteria may spread through any of the organs including faeces and hence an outbreak of pandemic usually after many decades. In future chemical warfare too could be spread directly by leaking the virus from laboratories, which should ideally not to be done. No antiviral drug has been proven to be effective in humans or are of doubtful value.

As you can see in leptospirosis and ornithosis caused by mice urine or bird droppings on eatables or fomites going to humans, which are really difficult to diagnose and treat.

Some more important points about the virus which have come up recently are as follows:

As the article was ready to be send but I could not contact my typist due curfews and lockdowns I chanced upon a newspaper of north India The Tribune dates 30-April-2020 quoting Sri Lanka having amended a law to make cremation compulsory for those dying from novel coronavirus to prevent any potential threat despite strong opposition from Muslim community. The quarantine and prevention of Diseases ordinance (Chapter 222) has been amended by a gazette issued by the Health Minister on April 11,2020.^[25]

- That the virus multiplies in 2-3 days.
- It can remain in the air for 24-72 hours eg. If someone has sneezed and you pass that place, droplet infection in the air can infect you.
- Don't touch couriers etc for nearly 72 hours, on sterile surfaces it lasts longer.
- The virus can get into your body through mouth, nose and eyes.
- Lymphopenia is very important and significant in this infection.
- The virus acts at the alveolar levels in lungs and hence little delivery of oxygen to blood however there are other way to bypass the lungs.
- The patient may be totally asymptomatic and yet have the virus and spread to others
- Even the recovered patient sheds the virus for 8 days.

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