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Comprehensive Literature Study on SARS-CoV-2 with Key Factor of Infection: Spike Protein & Emerging Role of Cytokines

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ABSTRACT

The coronavirus disease 19 (COVID-19) is a highly transmittable and pathogenic viral infection caused by Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The uncontrollable spread of this viral Infection is due to lack of vaccine and effective therapy against this novel virus. Hence, the situation required an immediate necessity to explore all the possible therapeutic and prophylactic strategies which can prevent further risk of infection and provide effective treatments. The principle factor of this infection is found to be Spike (S) protein; S- protein of coronavirus plays key parts in the induction of neutralizing- antibody and T-cell responses, as well as protective immunity, during infection with SARS-CoV. In this article, I highlight the emerging role of body protein; cytokines i.e. interferons & interleukins to decrease the severity of infection by inhibiting the replication of viruses. Also includes recent advancement in the development of vaccines and therapy based on the severity of infection and also summarizes the history, pathogenicity, sign & symptoms, transmission mode and diagnosis of COVID-19 infection.

HISTORY OF COVID-19:

Coronavirus was first discovered in the 1930s, as acute respiratory infection of domesticated chicken was shown to be caused by infectious bronchitis virus. However; human coronavirus was discovered in 1960s in UK and the United states. [2] First coronavirus (SARS CoV-1) epidemic was identified in China in year 2003 & round about 8000 people were affected (774 death) by this viral infection. Then after 9 years, modified coronavirus (MERS Cov) again appeared in Saudi Arabia with approximately 2500 cases. But current COVID-19 pandemic is one of the most devastating events in recent history. [27] This COVID-19 infectious disease is spreading or infecting the people very rapidly throughout the world; more than 50,40,000 cases has been detected within 5 to 6 months & around 3,30,000 death reported. This severe acute respiratory syndrome corona virus-2, disease first reported in Wuhan (China), named SARS CoV 2.[1]

INTRODUCTION:

SARS CoV-19 is one of the seven types of coronavirus, including the one that cause severe disease MERS and SARS. Coronaviruses are enveloped, non-segmented, positive-sense single-stranded RNA virus genomes in the size ranging from 26 to 32 kilobases, the largest known viral RNA genome. [24] The other coronaviruses cause most of the colds that affect individually during the year but are not serious threat for otherwise healthy people. Coronaviruses (CoVs) are a diverse family of viruses. They cause a variety of diseases in mammals and birds ranging from enteritis in cows and pigs and upper respiratory disease in chickens to potentially lethal human respiratory infections. These viruses infect a variety of human and animal host cells and also carry out their infection and replication. [23]

SYMPTOMS:

Sign & symptoms of COVID-19 may appear 2 to 14 days after exposure i.e. known as incubation period. The severity of symptoms can be from mild to very severe. People who are older or already suffering from chronicle diseases like; cancer, diabetes, hypertension, kidney failure or weak immune system may be at higher risk of infection. Some people also experience worse symptoms such as shortness of breath and pneumonia. The period from onset of COVID-19 symptoms to death; ranges from 6 to 41 days, however, this period is dependent on age & status of patient and their immune system.

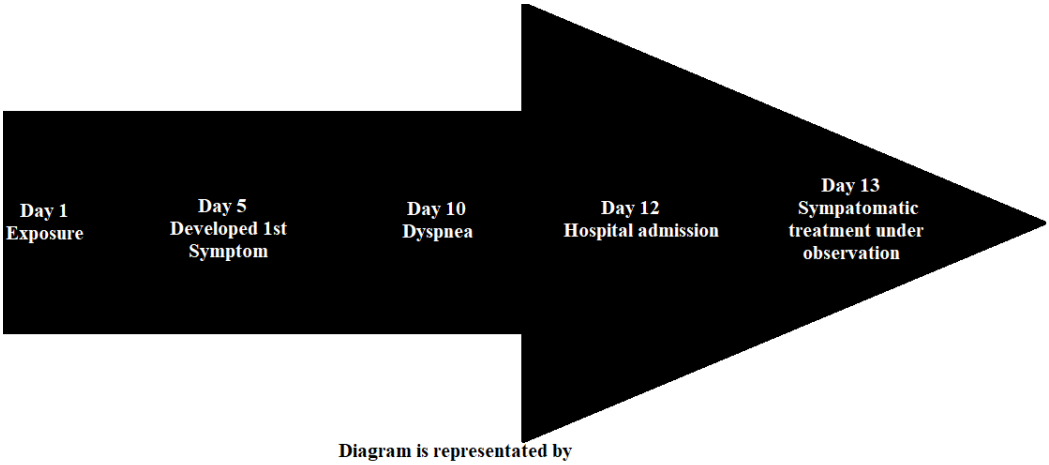


Diagram is representated by
Gayatri Gautam Varma

The common symptoms of SARS CoV-2 includes fever, cough, fatigue, headache, diarrhea, dyspnea, lymphopenia, dry cough, shortness of breath, sputum production and night sweat or chills. [2] Observed timeline for presentation of 1st symptoms to critical situation are as follow;

SURVIVAL TIME FOR CORONA VIRUS:

Survival time of corona virus

On hard & shiny surface	up to 72 hours
On stainless steel & plastic surface	up to 72 hours
On Porous surface (cardboard paper, fabric)	up to 24 hours
On copper surface	up to 4 hours
Airborn droplets	up to 3 hours

Diagram form of the data is created by
Gayatri Gautam Varma

Many research and article has shown that the coronavirus can be inactivated within a minute by disinfecting surface with 62% to 71% alcohol. [2]

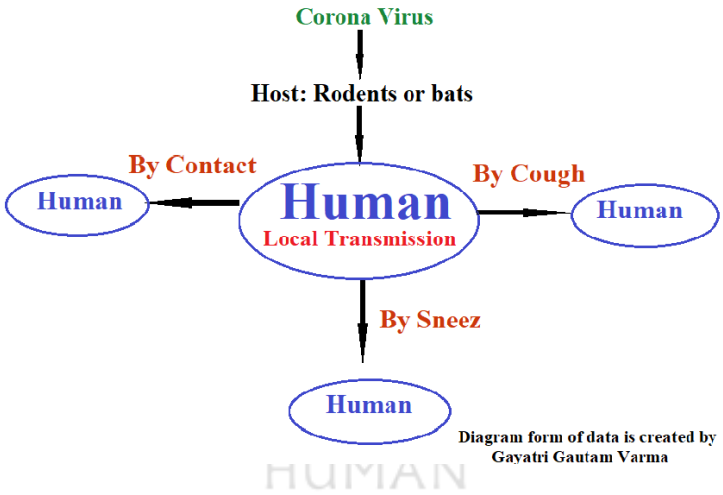
MODES OF TRANSMISSION:

The COVID-19 virus spreads primarily through droplets of saliva or discharge from the nose when an infected person coughs or sneezes. [14] Infection can be transmitted through saliva, sputum, nasopharynx, stool & urine. It can also spread via conjunctiva and vertical; but there is not any strong evidence which proves this. [7] Droplet transmission occurs when a person is

in close contact (within 1 m) with someone who has respiratory symptoms (e.g., coughing or sneezing) and is therefore at risk of having his/her mucosa (mouth and nose) or conjunctiva (eyes) exposed to potentially infective respiratory droplets. Airborne transmission is different from droplet transmission as it refers to the presence of microbes within droplet nuclei. In the context of COVID-19, airborne transmission may be possible in specific circumstances and settings in which procedures or support treatments that generate aerosols are performed.

HOW CORONA VIRUS INFECT THE HUMAN:

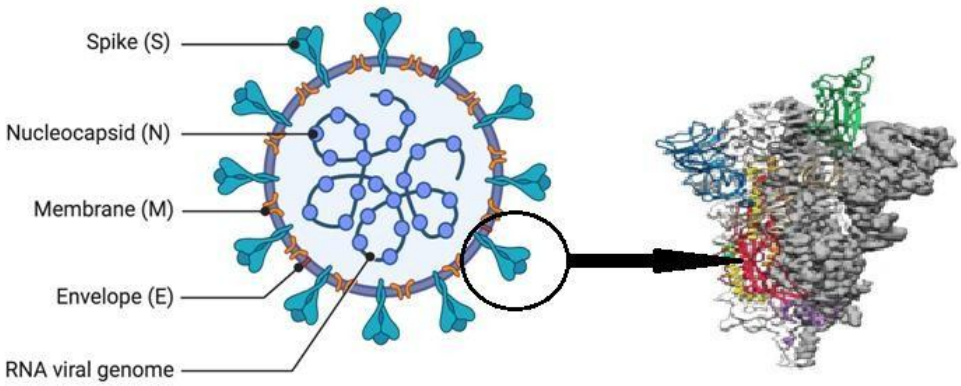
COVID has ability to host inside the bats & rodents. Key source of SARS in humans;



Coronavirus enters in lung alveoli via respiratory tract; inside the alveoli SARS CoV-2 binds to specifically to ACE-2 receptor. [2]

A COMPONENT OF SARS CoV-2: SPIKE PROTEIN

The structure is spherical or pleomorphic in shape containing single stranded RNA which associated with a nucleoprotein within capsid comprised of matrix protein. The envelop bears club shaped glycoprotein projections. [3]



Spike protein in corona virus

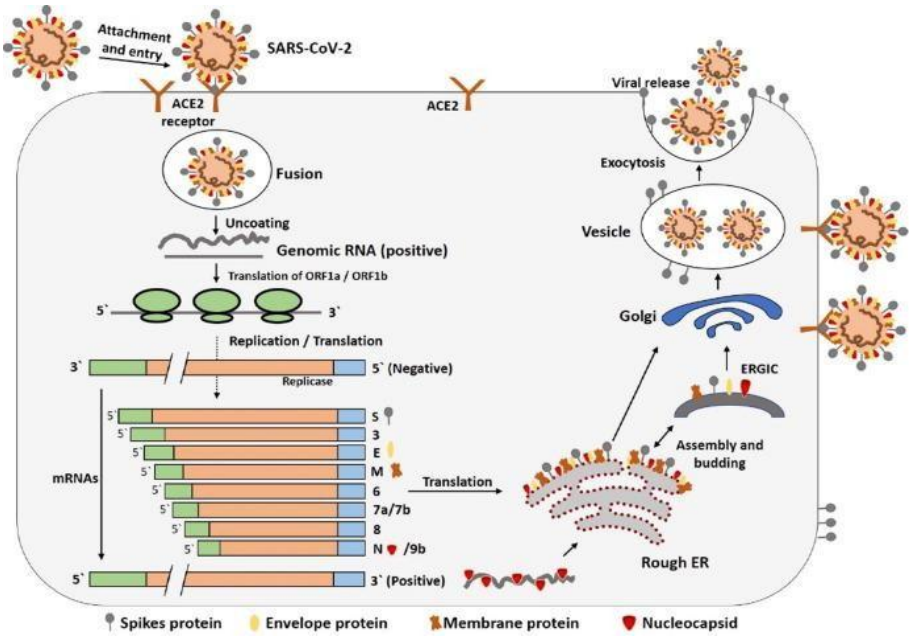
Merged structure is presentaed by Gayatri Gautam Varma

Spike protein is type 1 transmembrane protein; contain about 1,160 amino acid or share 76% amino acid. It has been reported that COVID-19 can infect human respiratory epithelial cells through interaction with humanACE-2receptors (Angiotensin converting enzyme) i.e. spike protein can bind with ACE-2 protein.

[4]The SARS-CoV spike (S) protein is composed of two subunits; the S1 subunit contains a receptor-binding domain that engages with the host cell receptor angiotensin-converting enzyme 2 and the S2subunit mediates fusion between the viral and host cell membranes. The S protein plays key parts in the induction of neutralizing-antibody and T-cell responses, as well as protective immunity, during infection with SARS- CoV.

As the major component for the development of vaccines against SARS, S protein has been shown to induce potent neutralizing antibodies to block virus binding and membrane fusion and/or protective immunity against virus infection. [5] Schematic of coronavirus spike proteins. Spike proteins are representative of those of all groups I to III coronaviruses and of SARS-CoV. The coronavirus spike protein is synthesized as a precursor, cotranslationally glycosylated, and, in some cases, cleaved in the approximate middle into S1 and S2 subunits at a site with dibasic amino acids (BBXBB). S1 forms the external domain containing the receptor binding domain (RBD) at its 5’ end, followed by, in the case of MHV, a hypervariable domain (HVR). A short signal sequence in cleaved from the 5’ end of the mature protein. S2 is the transmembrane subunit containing two heptad repeats (HR1 and HR2) and the transmembrane (TM) domain. [15]

MODEL OF CORONAVIRUS REPLICATION:



After receptor interaction and fusion of viral and plasma membranes, virus-specific RNA and proteins are synthesized, probably entirely in the cytoplasm. Expression of coronaviruses starts with translation of two polyproteins, pp1a and pp1ab, which undergo cotranslational proteolytic processing into the proteins that form the replicase complex. This complex is used to transcribe a 3'-coterminal set of nested subgenomic mRNAs, as well as genomic RNA, that have a common 5' "leader" sequence derived from the 5' end of the genome. Proteins are translated from the 5' end of each mRNA. New virions are assembled by budding into intracellular membranes and released through vesicles by the cell secretory mechanisms. RER, rough endoplasmic reticulum; ER/GIC, endoplasmic reticulum/Golgi intermediate compartment helps them to multiply within the cell. [15][16]

DIAGNOSIS OF SARS COVID-2:

The ground truth test for COVID-19 diagnosis is Reverse Transcription Polymerase Chain Reaction (RT- PCR) with DNA sequence & Identification. However, its result needs several hours to ready. But test based on IgM\ IgG antibody delivers result quickly. [10]

IgM\ IgG test do not detect the SARS CoV-2 presence directly, indeed they detect the serological evidence of recent infections; however, they are nonspecific for COVID-19. This test has 100% sensitivity and 50% specificity. [10]

ASPECTS OF INTERFERONS AND INTERLEUKIN-6 IN DIAGNOSIS & CONTROL OF COVID- 19:

Virally infected cells produce & release small protein called **Interferon** (a type of protein and glycoprotein), which plays a role in immune protection against virus. Interferon prevents replication of viruses, by directly interfering with their ability to replicate with an infected cell. It is a group of signaling protein, made and released by host cell in response to presence of several viruses i.e. interferons causing nearby cell to heighten their anti-viral defenses. Interferon is secreted by eukaryotic cells in response to viral infection. It is first line defense again viral infection but does not protect the virus infected cell that produces it.

When prototypic cell of origin is exposed to virus then type-1 (alpha and beta interferon) & type-2 (gamma interferon) interferon get activated. IFN-alpha detected in serum samples from subjects with viral infection. IFN- alpha, 1st FDA approved biotherapeutic treatment. Purified IFN- alpha preparation as well as IFN- alpha (level) from the stimulated cell can be analyzed by various following methods; (The reference range for total protein is typically 60-80 g\l (6-8 g\dl). However blood contains other proteins like albumin, globulin etc.).

1. Directly determining the mass level

2. Direct protein analysis (ELISA): Sandwich enzyme linked immune sorbent assay has become an invaluable tool for rapid & highly quantitative analysis of cytokines including INF- alpha. May IFN- alpha ELISA kits are developed to detect IFN-alpha2.

3. Biological activation method: Cytopathic protective effect (CPE) assay most widely used. In this method IFN- alpha cells are treated with serial dilution of test samples & know dilution of lab standard cells are then challenged with a single concentration of cytopathic virus. A second incubation step is carried out & completed when cell treated with only virus.

As the host's major antiviral molecules, IFNs limit virus spread, and play an immunomodulatory role to promote macrophage phagocytosis of antigens, as well as NK cells restriction of infected target cells and T/B cells. Thus, blocking the production of IFNs has a direct effect on the survival of the virus in the host. [24] The reference range for total protein is typically 60-80 g\l (6-8 g\dl). However, blood contains other proteins like albumin, globulin etc. [24]

Interleukin- 6 (IL-6) is cytokine, signaling molecule stimulate white blood cells to produce antibody. IL- 6 has been implicated in the progression of several viral infections. IL-6 is considered one of the most important cytokines during infection along with IL- 12 (stimulated by pathogen or viruses). There is plentiful evidence supporting a significant role of IL-6 during viral infections. Two different hypotheses may be considered to explain the change in IL-6 production during the immune response to viral infection: (i) the increased ability of some viral strains to overcome the immune response using a variety of evasion strategies and consequently up-regulate the production of IL-6 as a result of increased viral loads, and (ii) polymorphisms in the IL-6 gene promoter stimulating overexpression of IL-6 during the immune response, a fact that has been shown to correlate with HBV progression. [28]

PREVENTION OF VIRUS ENTRY INTO HOST CELLS AND REPLICATION:

PREVENTION OF ATTACHMENT: Since SARS-CoV-2 utilizes the host cell surface receptor uptake. The options to block viral entry include the use of natural neutralizing antibodies from ACE2 to attach itself via its S- protein and gain entry, it is an attractive target for preventing viral convalescent sera and engineered antibodies. The status of ACE2 expression in various tissue following the use of ACE inhibitors and angiotensin receptor blockers, it is difficult to speculate on the relevance of these ACE modulators in COVID-19. **Emodin** (a naturally occurring anthraquinone) and **Promazine** (phenothiazine class of antipsychotics) have been shown to interrupt the binding of S protein with ACE2.[20]

PREVENTION OF FUSION: **Chloroquine**, is well-known for its effective use in the management and prevention of malaria. It evolved as an anti-viral agent by having more than one mechanism in inhibiting the viral life cycle. it increases the pH of acidic vesicles such as endosomes and lysosomes, thereby preventing the viral envelope from uncoating and releasing the RNA into the host cell cytoplasm. It is also known that chloroquine impairs virus replication, assembly and release. More importantly, the chloroquine (chloroquine phosphate or hydroxychloroquine) was also observed to beneficial in the management of COVID-19 patients by reducing deterioration of disease and virus load. [20]

PREVENTION OF VIRAL REPLICATION AND SURVIVAL IN HOST CELLS: **Viral protease inhibitor** (Blocking key proteases such as coronavirus main protease and papain-like protease are considered to be critical in blocking viral life cycle because they are

necessary for the proteolysis of viral poly-protein into functional units). **Viral nucleic acid and protein synthesis inhibitors** (**Remdesivir**, a nucleoside analog that blocks the RNA-dependent RNA polymerase, is showing great promise in the management of COVID-19 patient). [20]

POSSIBLE MEDICATION APPROACH AGAINST COVID-2:

- ACE-2 INHIBITOR: As per the mechanism of infection we know, spike (S) protein of coronavirus binds to ACE-2 receptor and inters in human cell or body. [4]

- ANTIVIRAL DRUGS: Like **Lopinavir**, **Ritonavir** (used in Korea for COVID-19), **Ribavirin;** is a guanosine analog which interfere with RNA & DNA replication of virus. **Remdesivir;** is wide spectrum antiviral drug already used in SARS-1 & MERS coronavirus, decreases viral RNA production. [4]**Lopinavir- Ritonavir** was the most improving administration antiviral combination but this drug combination with antibiotics was not superior to conservative management.[8]

- ANTIMALERIAL DRUGS: **Chloroquine** inhibits pH dependent steps that are essentials for several virus replications and interfere glycosylation of cellular receptor of SARS Cov-2. Chloroquine used with combination with Remdesivir to stop SARS CoV-2 replication. [4] H**ydroxychloroquine** with antibiotics was associated with better clinical outcomes in terms of time to viral clearance. [8] Although antibiotic administration in viral infection may increase the risk of bacterial infection.[9]

- ANTHELMINTICS: According to Australian Research; single dose of **Ivermectin** could block growth of SARS Cov-2 virus in cell culture effectively terminating the entire virus's genetic material during 48 hours of incubation. [4]

- BLOOD PLASMA THERAPY: Isolated blood plasma from recovered patient was also utilized as treatment by injecting plasma to the infected person.[4]

- POTENTIAL ROLE OF NATURAL PRODUCTS FROM INDIAN TRADITIONAL MEDICINE: Typically, the presence of a variety of phytochemicals such as flavonoids, tannins, triterpenes, phenolic acids, alkaloids, saponins, lignins, proteins and peptides provide a plethora of functions to such natural products and extracts which have been demonstrated to modulate various aspects of viral infection including virus entry, viral gene expression and

replication. Although there is no direct evidence of the effect of such extracts etc.; on the SARS-CoV-2, common natural products such as **curcumin** and **terpenoid s**can inhibit the CoV family member SARS while ***Withania somnifera*** (Ashwagandha) have been demonstrated to inhibit other RNA virus. [20][21][22] And partially purified compound of MeOH extracts of ***C. quadrangularis*** was screened for antiviral activity against HSV type 1 and 2 viruses & found to be effective. [26]

Some drugs which are approved by FDA in COVID-19 pandemic; Baricitinib, Lopinavir, Ritonavir, Darvnavir, Ivermectin. [4]

Drugs which are under investigation (for the treatment of COVID-19); Favipiravir, Cepharanthie, Selamectin, Mefloquine.HCl. [4]

Under clinical trials; Remdesivir[4]

Chloroquine; Drug and derivative of chloroquine is **not approved by FDA**, only for emergency situation (under protocol). [4]

EFFECT OF HIGH & LOW DOSES OF CHLOROQUINE AND ITS DERIVATIVE IN COVID -19 THERAPIES:

Allocated to receive high dosage chloroquine is 600 mg dose twice daily for 10 days and 450 mg dose twice on day 1 & once daily for 4 days. High dose of chloroquine i.e. 12 gm given for 10 days concurrently with azithromycin & oseltamivir was not sufficiently safe, especially treatment on older patient with previous cardiac or chronic disease. [6]

ROLE OF VITAMINS & MINERALS IN COVID-19:

Vitamin C reduces lung inflammation and increases inflammatory response. For calcium and phosphate metabolism **Vitamin D** is responsible, also increases innate immunity. **Zinc** has antiviral effect against number of viruses with innate immunity booster. **Quercetin** is a potent antioxidant found in grains, vegetable, fruits, onion, apple etc. [13]

FACT OF BCG VACCINATION IN COVID-19:

One reason could be national policies regarding childhood BCG vaccination with fewer confirmed cases & a lower death toll reported in countries with Vs without universal BCG vaccine coverage.[11]

In 1939, Fernandez was demonstrate the induction of a positive mistuda reaction. BCG vaccine is a marker for improve cell-mediated immunity against leprosy apart from tuberculosis. A recent systematic review of randomized controlled trials on the effectiveness of the BCG against Buruli ulcer revealed 50% efficacy. BCG vaccine has been proposed to activate the reprogramming of immune cells & alter cell metabolism. This includes accelerating the process of glycolysis as well. On the basis of evidence of antiviral attribute, substantiations have outlined the preventive role of BCG vaccination amongst children & elderly. [12]

CONCLUSION:

It can be concluded by reviewing various publications and research articles, COVID-19 disease is universal pandemic which is still uncontrollable. If we are using different approaches for effective treatment then it may develop resistance against that all medicines and the situation will become out of control. To overcome this, need personnel clinical therapy to individual patient as per their symptoms and condition; this can avoid chances of drug resistance by virus at some extent. Multiple therapy or combination drug therapy should be decided in such a way that they produce minimum adverse effect.

As we know, in our body; cytokines i.e. interferons produces by the infected cell (viral infected or pathogen infected) which behave as signaling protein and minimizing the risk of further exposure of virus to uninfected cells. Therefore it is very necessary to keep such chemical messenger in active state after any viral or pathogen infection. For this, needs red blood cell (for interferon production) and white blood cells (for interleukin production) level in normal range to produce such chemicals after first exposure & thus reduces the severity. Because interleukins are responsible for the production of antibodies to fight against pathogens. Overall, self isolation & immunity play the principle role against COVID-19 infection as there is not any specific & proper treatment developed yet.

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