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A Review and Possible Treatment Mechanisms against Covid-19

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Abstract

A human coronavirus, which has caused an outbreak of COVID-19, has caused pandemic which commenced in December (2019) from Wuhan (China). This SARS-CoV-2 virus has so far infected more than four million people and killing more than 310,000 up to May 17, 2020. Based on phylogenetic relationship and genomic structure, this virus belongs to genera Beta coronavirus. In a study, Leila M. (2020) observed that Human Beta coronaviruses like SARS, MERS and SARS-CoV-2 have similarities, but also differences in pathogenetic characters influenced by their genomic and phenotypic structure. Till now the disease in laboratories, is being diagnosed by the RT-PCR with RLAMP under the possible better testing kit consideration. As we know only antiviral treatments are can halt the epidemic, but no vaccines have been successfully discovered but in the given paper, the possibilities have been discussed by using the antisense treatment and M protein targeting and old and proposed diagnosis along with reviewing the whole COVID-19 pandemic and its epidemiology.

Keywords

Antisense Treatment, COVID-19, M protein, RLAMP, RT-PCR, SARS-CoV-2

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INTRODUCTION

The outbreak of COVID-19, which commenced in December (2019) has quickly become a boundless and uncommon global challenge [1]. A global health emergency was declared by World Health Organisation (WHO) as Corona Virus disease 2019, which has spread rapidly throughout the world [1, 2, 3]. The median incubation period of the virus is 4–5.2 days [3]. Before the onset of the disease the patients are observed to be infective and even can maintain ability to infect during the recovering phase [4, 5, 6]. According to the report of Cao's the shortest duration observed was 8 days of viral shedding, whereas the longest duration was about 37 days [7]. It has been observed in human CoVs that a seasonal pattern during the incidence of the infectious diseases occur. There are several environmental and physical factors such as temperature and humidity, which play a big role in the outspread and progression of infection of SARS-CoV-2, with the 5 days viability at relative humidity (RH) of 40–50% and temperature from 22 to 25°C. It has been observed that higher temperature and higher Relative Humidity (38°C, and >95% RH) reduce virus viability [8, 9].

The spread of COVID-19, caused by SARS-CoV-2, started in low temperature areas of China, with major outbreaks following in countries like Japan, Iran, USA, Spain, South Korea and Northern Italy. The global travelling brought this disease to spread to higher temperature areas like India, Thailand and Middle East. COVID-19 can easily be spread from person to person [6, 10], which makes a major challenge to control the disease to recognise and

quarantine the potent infection sources as soon as possible [5]. The viral transmission dynamics depends on many physical properties of the virus, indoor and outdoor environment, hygiene, genetic predispositions, population densities and space and maybe the reason for the warmer area spread of disease [11, 12].

THE CAUSE OF COVID-19: CORONA VIRUS

The coronavirus name was taken from Latin word "*corona*", which means crown [13]. This name was given to the virus due to its characteristic appearance observed under electron microscope. Coronaviruses are commonly known as a group of associated viruses that causes disease in aves and mammals. When it comes to humans, respiratory tract infections are commonly generated by these viruses that has mild to lethal range. The mild illness leads to common cold like infections but the lethal are COVID-19, MERS, SARS.

Structure: Coronaviruses are big pleomorphic spherical particles accompanied by bulbous surface projections [14]. The virus particles have the diameter of 120nm. The envelope of the virus consists of a lipid bilayer where the envelope (E), membrane (M) and spike (S) structural proteins are anchored [15]. The envelope of the virus bears club-shaped glycoprotein projections (Figure 1). There are several copies of the nucleo-capsid (N) protein, which inside the envelope forms the nucleo-capsid, possessing the genetic material. The RNA found in the virus as genetic material is Positive-Sense Single strand [16, 17]. The virus is protected from the outside environment of host cells by the lipid bilayer envelope, membrane proteins, and the nucleo-capsid [18].

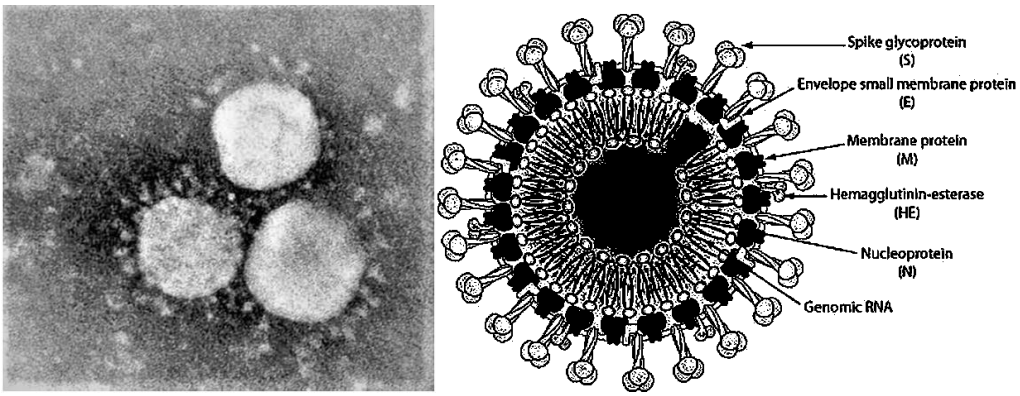


Figure 1 The coronavirus observed under electron microscope (Courtesy S.Sikotra, Leicester Royal Infirmary, Leicester, England) and schematic representation of virus [56]

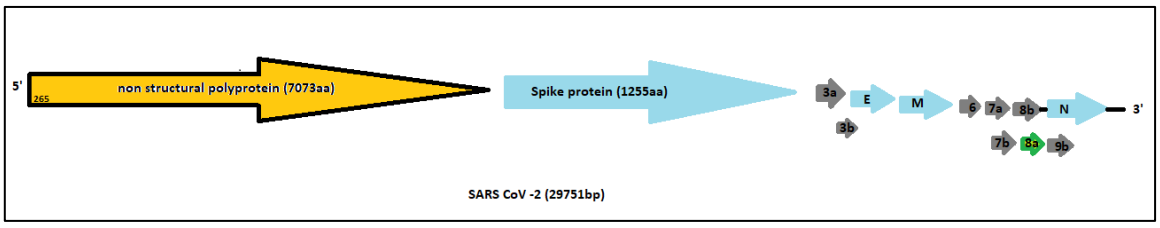


Figure 2 The genomic organisation of SARS Cov-2 [56]

Genome: The genome size of the corona virus is 29,571bp, making it one of the largest among all RNA viruses with varying G+C contents within 32-43%. Coronavirus contains a positive sense, single stranded RNA genome ranging between 26.4-31.7 kilobases in size [19]. The genome comprises of methylated cap at 5'and polyadenylated tail at 3' [17].

All major structural proteins forming genes in all coronaviruses occur in the order as Spike, Envelope, Membrane, and Nucleo-capsid (5' to 3') encoded by Open Reading Frames, 10 and 11 on the genome, on the one by third part, near the 3'end (Figure 2) [20,21]. A typical CoV contains at least six Open Reading Frames in its genome, in which the first two-thirds of the genome is occupied by 1a and 1b, encoding the transcriptase polyprotein which self cleaves to form non-structural proteins [22]. Besides the main structural proteins (Table 1), different Corona Viruses encode special structural and additional proteins which are responsible for several important functions in genome maintenance and virus replication [20].

Protein	Feature and Function
Membrane glycoprotein	crosses the membrane three times, which leaves a short domain outside the virus at NH2-terminal and a long terminus inside it of COOH [55]
Spike protein	main inducer of antibodies for neutralisation and between the proteins for envelope, exists a molecular interaction which determines the composition and formation the viral membrane

Table 1 Feature and Function of coronavirus structural protein

SYMPTOMS	PERCENTAGE OF PATIENTS SHOWING IT
Headache	14%
Sore throat	14%
Muscle pain	15%
Shortness of breath	29%
Phlegm production	33%
Fatigue	38%
Dry cough	67%
Fever	88%

Table 2 Symptoms of SARS CoV-2 and its observation in percentage of population showing it

SYMPTOMS

The infected person has shown a broad range of symptoms which range from mild to severe illness. After subjection to the virus, these symptoms may even appear between 2 to 14 days of it (Table 2). The clinical and demographic factors that promote progression towards a serious form of COVID are the age (more the age more the risk), cardiovascular diseases and malignancy.

In the fullness of time, there are patients who require therapy for renal replacement and dialysis, which affects 5% of ICU patients, arising during the second week of infection [23], which was also observed in the SARS and MERS pandemic [24].

TRANSMISSION

There are different aspects for the COVID-19 transmission (Figure 3) from which the coronavirus can transmit from an individual to other, at a very high rate.

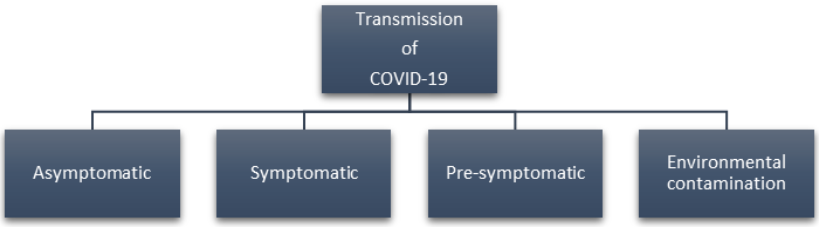


Figure 3 The types of transmission showed by SARS-CoV-2

Symptomatic transmission is when the patient develops signs and symptoms, well matched with COVID-19 infection while they are experiencing symptoms. According to WHO global expert networks, and reports and presentations by Ministries of Health, COVID-19 is majorly transmitted from symptomatic people to the ones who are in close contact through

- respiratory droplets,
- direct contact with infected persons,
- contact with contaminated objects and
- surfaces

The dispersal of the COVID-19 virus is highest in upper respiratory tract in the early course of the infection within the first 3 days from onset of symptoms [6, 25, 26, 27].

Pre-symptomatic transmission is by the period of incubation for COVID-19 that is between exposure to the virus and symptom onset, which is 5 to 6days on average 5-6 days, but could extend up to 14 days but here, some infected people could be contagious. From 1 to 3 days, before they even thrive any of the trait or symptoms, some people can be positive for this disease. Thus, making it possible to transmit the virus before symptom development [28,29].

Asymptomatic transmission is spread by an individual who does not generate any symptoms of the viral infection but is infected by it. It refers to

conveyance of it from an individual, who is devoid of showing symptoms. Several asymptomatic cases have been accounted in some countries, as a primary part of contact detecting efforts [30,31].

The **environmental contamination** also plays a major part in the transference of disease like the faecal shedding, temperature, humidity and time.

EPIDEMIOLOGY

The COVID-19 seasonality and dynamics are not well acknowledged, and the need is to have further studies to identify the conditions, mainly environment, that satisfies as well as prevents its spread. Epidemiological models which incorporates the climate with weather processes along with the human interactions, which help to recognise populations at risk with surveillance improvement with control measures. In high altitude area, the population is generally susceptible, including children, In one of the case studies, residents living in high altitude area were contemplated to be more prone to complex and severe COVID-19 due to more complicated medical as well as social conditions like tuberculosis and hypoxia [32,33].

82,947 cases has been confirmed in China, clinically and in the laboratory, and 4,633 deaths are reported so far while in India the number of confirmed cases is 91,314 and 2,897 death till 17 May, 2020. In one of the case studies [34], the index patient was from Wuhan and had contact with COVID-19 patients before she returned to Yiyang city, whereas the other 4 family members were from Yiyang and had neither left the city, nor have come in any contact with the patient of COVID-19. It is most likely that index patient was the infective source in this family. After further examination of their epidemiological data, it was concluded that the discarding duration of SARS-CoV2 and the span of COVID-19 incubation might be extremely long in this case. The index patient until 28days after returning from Wuhan, did not develop atypical symptoms. The patient was immunocompromised as well due to her long-term use of oral prednisone, explaining why she had a longer duration of viral shedding. So, making it important to recognize potential patients who might have no or only mild symptoms.

In addition to China and India, there are two million confirmed cases in 208 other countries but due to early lockdown in India, a visible difference in patients was accounted as compared to China (Figure 4). The countries with the most cases are USA, Italy, Iran and Spain.

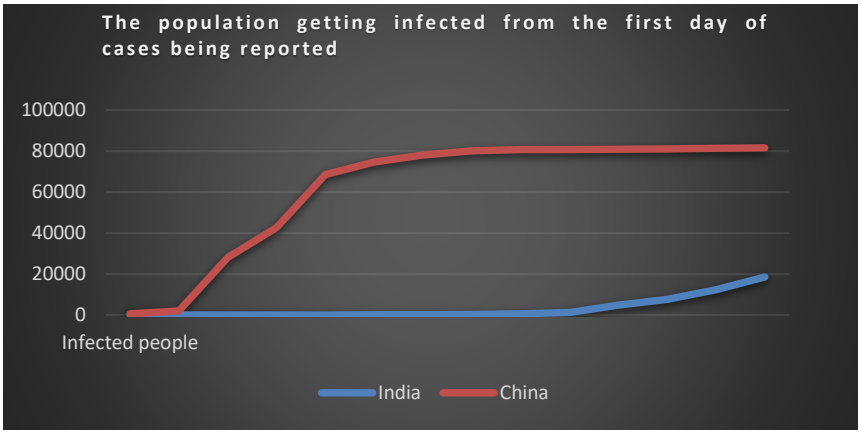


Figure 4 The difference between population getting infected from the first day of cases being reported in China and India due to the early onset of lockdown in India (Courtesy: www.worldometers.info)

MECHANISM

Entry: The infection begins with the entry of human coronavirus in the cells, by specific receptors. The receptor consisting of a sialic acid and Aminopeptidase-N have been recognised to play a part for OC43 and 229E respectively. The receptor for the virus is Human angiotensin converting enzyme (hAC2) and human alveolar epithelial type 2 acts as a reservoir for the virus as among all the corona viruses, namely SARS-CoV plus SARS-CoV-2, showed to utilize the hACE2 receptor for cell entry. Atomic details of the bond formed reveals more interactions are present in SARS-CoV-2-CTD/hACE2 than in SARS-RBD/hACE2, like more engaged residues, more H-bonds, more van der Waal contacts, and larger buried surface areas (Figure 5).

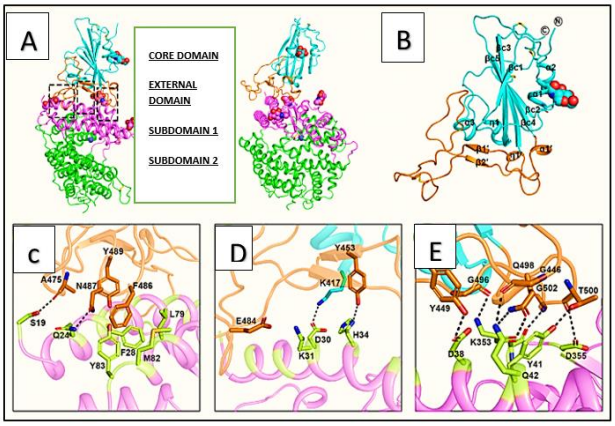


Figure 5 The structure of SARS-CoV-2 complex of CTD bound to hACE2 [57];
(A) represents the complex structure with different colours representing different subdomains;
(B) represents the SARS-CoV-2-CTD secondary structure, arranged according to the location and sequence occurrence in its subdomain;
(C,D,E) represents the contact sites and its residues

When the S glycoprotein anchors to the receptor of cell of the host, a protease is liberated to cleave host cell and activate the receptor-attached spike protein. The proteolysis of the Spike (S) protein, giving rise to subunits (S1, S2) is and additional indispensable condition for CoV infection. This virus, SARS-CoV-2 accommodate one possible cleavage site and could be ably refined into S1 and S2 with 15 amino acids showing more contact with SARS CoV-2 showing large surface area [35]. The Spike protein during its binding to the receptor is cleaved by protease, resulting in S1 and S2 subunit which are responsible for recognition of receptor and membrane fusion respectively. In S1 the CTD is accountable to recognise the RBD, receptor binding domain where CTD 195 residues from T333 to P527 have 1 conserved core domains and the second part has an external subdomain used to recognise subdomain 1 in hACE2 NTD.

The cells, which become infected turns to be vacuolated, form syncytia and show damaged cilia. The manufacture of inflammatory mediators is triggered by the cell damage which increase nasal secretion and local inflammation along with swelling and later severe [36].

Replication: After the virus invade within the host cell, its coat gets ruptured and the genome goes under transcription followed by translation. The coronavirus RNA genome commonly has a 5' methylated cap and 3' polyadenylated tail, which allows the attachment of RNA to the ribosome for translation. 7 mRNAs are produced in which the shortest mRNA codes for nucleoprotein. The initial overlapping ORFs of the genome of the virus is translated by the ribosome of the host and a long polyprotein is formed having its own proteases to cleave it into multiple proteins [17].

A multi-purpose replicase-transcriptase complex (RTC) is formed by a number of non-structural proteins in which RNA dependent RNA polymerase is the main as it directly participates in the replication as well as transcription of RNA from an RNA. The complex main function the viral genome replication. RNA polymerase is straight away involved in the mediation of negative-sense genomic RNA synthesis from the positive-sense RNA followed by the replication of positive-sense genomic RNA from it [17].

Release: The replicated genomic RNA befits the new genome of virus progeny. E, M and S like structural proteins move into the Golgi apparatus alongside the secretory pathway while the translation of RNA occurs within the endoplasmic reticulum. Most of the interactions between proteins are vital for the congregation of viruses and its attachment to the nucleocapsid is directed by M protein [17].

DIAGNOSIS

During analysis, nasopharyngeal and oropharyngeal swabs kept at 2-8 degrees for less than 4 days with 2-3mL of media is kept and used to take samples from upper and lower respiratory tract, including active virus infection and then diagnosed in RT-PCR which 130 genome sequence known of human SARS-CoV-2.

RT-PCR Assay (Real-time reverse transcription-polymerase chain reaction) is the qualitative detection method and have played a paramount part in clinical diagnosis since the SARS-CoV-2 outbreak However, it is laborious, require specialized instruments, time-consuming and so is not able to

gratify the rapid demands of testing in large scale [37, 38].

RT-LAMP Assay is a POCT i.e., Point of Care Testing, detection method demonstrated to have eminent diagnostic sensitivity as well as specificity among samples with a complete detection within 60 min. It is a cogent tool for SARS-CoV-2 identification in laboratories and in hospitals as no complex equipment is required, but its accuracy will be influenced by the mutations that occurs in the target gene's primers sequence region. It would be useful in observing high-risk groups, suspected patients with their close contacts. According to the color change, the positive results could be easily judged by the naked eye as the change from orange to green, while the negative results remain orange [37, 38].

Serological approach for this SARS coronavirus -2, has been reported to be highly specific (100%) and sensitive [39, 40] making it a reliable source of diagnosis. Although the size of the sample was small, but detailed serological studies indicated that all patients develop high levels of neutralizing antibodies from around the 14th day of the symptom onset [6, 41]. The patients showing mild symptoms only are around 80%, while 30% will not develop any symptoms at all [42, 43]. These features suggest that widespread serological testing could have multiple benefits. Like identifying a number of immune individuals whose serum could be used for convalescent therapy [44,45].

DRAWBACKS OF PROPOSED TREATMENTS

The Type 1 Interferon treatment: When observed in vitro, Type 1 interferons (IFN-1) show a wide range of antiviral activity and are currently under the clinical trial for MERS-CoV treatment. So it could have the potential activity against SARS-CoV-2. In the area of treatment of infections caused by virus, IFN-I are frequently estimated with the combination of other drugs, prior the specific treatments of the disease are evolved due to their undetermined antiviral effects [46, 47, 48]. Recently registered clinical trials evaluate a combination of IFNα2b and lopinavir/ritonavir or a combination of IFNß1b and lopinavir/ritonavir with ribavirin would be administered subcutaneously (NCT04276688) for the COVID-19 treatment. The efficiency could rise, if the integration of IFN-I with ribavirin, lopinavir/ritonavir or remdesivir is used [49]. The Type III IFN might also be applicable to treat COVID-19 [50], because of its protective effects on respiratory stretch. The early stage of infection could be treated by the IFNß1 accounting a safe and easy to upscale treatment against COVID-19 [50,51].

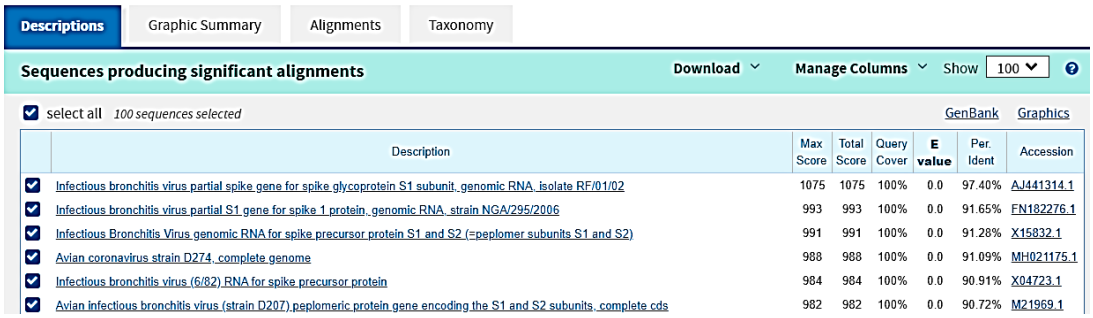
Short-term remdesivir therapy: When in fewer numbers of patients the drug was tested with lack of knowledge of history of patients, enhancement in oxygen-support status was observed in only 68% of patients, and overall mortality was 13% over a median trail of 18 days while it was 22% of the patients hospitalised for COVID-19 under lopinavir–ritonavir medicine [52].

Around 64% of the patients who undergo the treatment using remdesivir drugs, received interfering ventilation at baseline, including 8% who were receiving ECMO, and in this subgroup 18% was the mortality rate of the people, who were not in intensive care [53] making treatment not so specific in cure data as well as dependent on other factors.

The corticosteroid treatment: The glycemic control during COVID-19 infections is important due to the adverse effect on the pulmonary function and immune response by hyperglycemia. There are many reasons why the patients might have worse glycemic control or be at higher risk than their they were in their pre-Covid-19 state as the corticosteroid therapy raises the level of glucose in 80% of patients which could increase mortality risk in coronavirus infections [54].

FUTURE PROSPECTS OF TREATMENTS

Targeting S1 protein-TMPRSS2 is the serine protease that has been accounted to contribute to priming of the SARS-Coronavirus-2 Spike protein, and its inhibitor has been sanctioned for clinical use, which was able to block entry so could act as a treatment option [35]. The S protein during binding cleaved by protease results in S2 and S1 subunit which are accountable for fusion of membrane and recognition of receptor respectively. In S1 the CTD is responsible to recognise the receptor binding domain (RBD). Where CTD 195 residues from T333 to P527 have 1 conserved core domains and the second part has an external subdomain used to recognise subdomain 1 in hACE2 NTD. So targeting the S1 protein specifically would be able to stop the spread of the virus and S1 show 100% identity with a maximum score of infectious bronchitis virus protein (Figure 6), making it easy to make drug targeting the protein.



Descriptions | Graphic Summary | Alignments | Taxonomy

Sequences producing significant alignments — Download — Manage Columns — Show 100

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Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Infectious bronchitis virus partial spike gene for spike glycoprotein S1 subunit, genomic RNA, isolate RF/01/02	1075	1075	100%	0.0	97.40%	AJ441314.1
Infectious bronchitis virus partial S1 gene for spike 1 protein, genomic RNA, strain NGA/295/2006	993	993	100%	0.0	91.65%	FN182276.1
Infectious Bronchitis Virus genomic RNA for spike precursor protein S1 and S2 (=peplomer subunits S1 and S2)	991	991	100%	0.0	91.28%	X15832.1
Avian coronavirus strain D274, complete genome	988	988	100%	0.0	91.09%	MH021175.1
Infectious bronchitis virus (6/82) RNA for spike precursor protein	984	984	100%	0.0	90.91%	X04723.1
Avian infectious bronchitis virus (strain D207) peplomeric protein gene encoding the S1 and S2 subunits, complete cds	982	982	100%	0.0	90.72%	M21969.1

Figure 6 The protein BLAST results of S1 protein of SARS-CoV-2

Targeting M protein-The membrane (M) glycoprotein crosses the membrane bilayer three times, which leaves a short NH2-terminal domain outside it and inside has a cytoplasmic domain having a long COOH terminus. M without requiring S, also plays a principal part in the intracellular formation of particles of virus. It has also been observed that coronavirus grow and manufacture spikeless, non-infectious forms of virions that contain M protein devoid of S protein, in the presence of tunicamycin [19,55]. So, the M protein should be targeted by the drugs that bind specifically to the protein which would result in the non-formation of progeny virus and stopping the spread of virus inside body and cells.

The antisense treatment-The RNA genome comprises of positive sense ssRNA whose bases has been detected by the genome sequencing and antisense treatment could be conducted by the developing the antisense RNA, which when binds to virus genome makes it double stranded and followed by the disabling it by the use of the site directed mutated strands of RNA provided which would no longer be stable and could not translate any working protein for the spread of the virus. This antisense treated RNA could be developed and enclose in a nanoparticle drug which has a target site of the nuclear and cytoplasmic membrane, thus acting directly on it.

CONCLUSION

The SARS-CoV-2 which has caused pandemic around the world, taking the lives of people in large scale has the genome sequenced, but has been observed to mutate to form new variant so the immediate treatment as well as prevention is very important. Human Beta coronaviruses like SARS, MERS and SARS-CoV-2 have similarities, but also differences in pathogenetic characters influenced by their genomic and phenotypic structure [56]. The virus treatments suggested like the S1 protein, which stop the receptor and viral protein anchoring and targeting M protein means that no intracellular virus formation which prevents the spread of virus in the host. The antisense treatment will not only treat but also would be able to prevent the attack of virus on humans as it would be unable to synthesis any stable and functional protein, resulting in the possibility of successful treatment in the future.

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