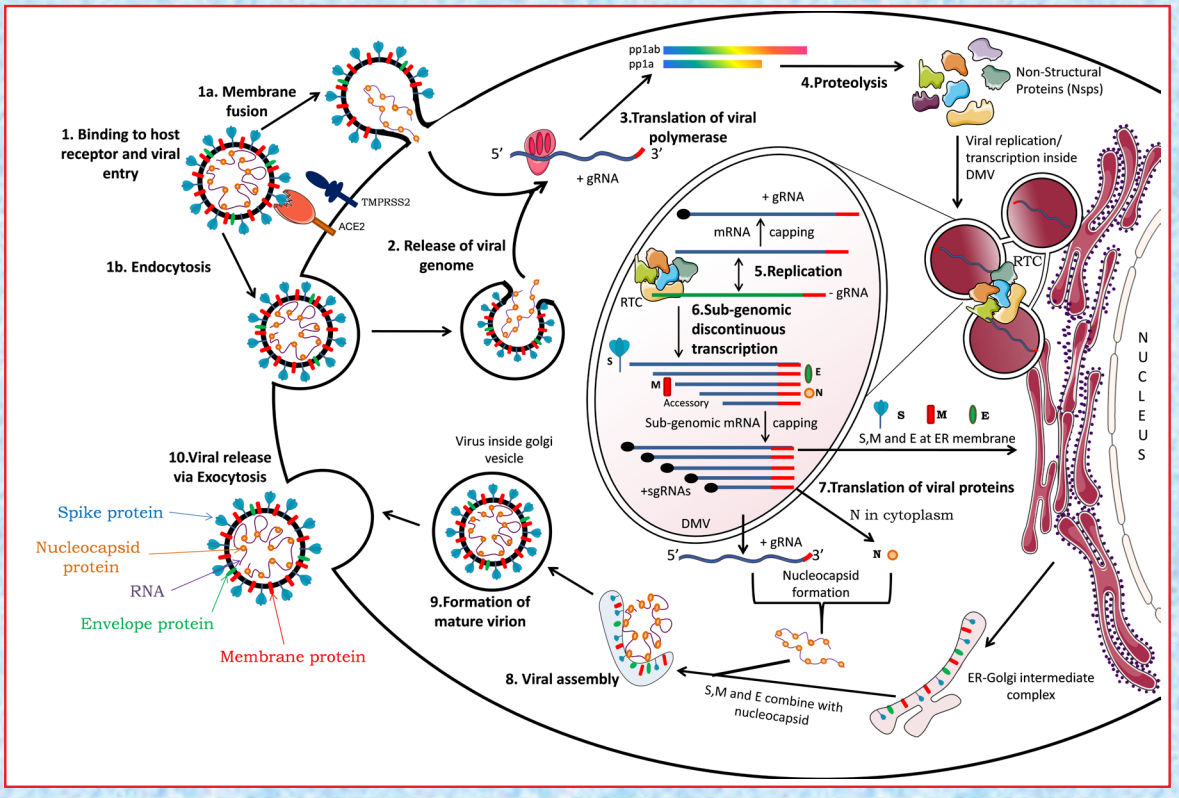
SMC Bulletin

ISSN 2394-5087

A Publication of the Society for Materials Chemistry

Volume 11 No. 02 August 2020



Special Issue on

COVID-19: BASIC ASPECTS AND MANAGMENT

SOCIETY FOR MATERIALS CHEMISTRY

Society for Materials Chemistry

Society for Materials Chemistry was mooted in 2007 with following aims and objectives:

(a) to help the advancement, dissemination and application of the knowledge in the field of materials chemistry,

(b) to promote active interaction among all material scientists, bodies, institutions and industries interested in achieving the advancement, dissemination and application of the knowledge of materials chemistry,

(c) to disseminate information in the field of materials chemistry by publication of bulletins, reports, newsletters, journals.

(d) to provide a common platform to young researchers and active scientists by arranging seminars, lectures, workshops, conferences on current research topics in the area of materials chemistry,

(e) to provide financial and other assistance to needy deserving researchers for participation to present their work in symposia, conference, etc.

(f) to provide an incentive by way of cash awards to researchers for best thesis, best paper published in journal/national/international conferences for the advancement of materials chemistry,

(g) to undertake and execute all other acts as mentioned in the constitution of SMC.

Executive Committee

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Volume 11 **No. 02** **August 2020**

Special Issue on

COVID-19: BASIC ASPECTS AND MANAGMENT

SOCIETY FOR MATERIALS CHEMISTRY

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Vol. 11 **No. 2** **August 2020**

Guest Editors

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Published by

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Front cover shows Schematics of SARS-CoV-2 life cycle. The sequential events, from binding to host cell receptor through the release of next progeny of virus, are represented graphically as steps 1 to 10.

Guest Editorial

Dr. Adish Tyagi

Dr. Sandeep Nigam

We are living in times where a viral disease has broughtnormal life in much of the world to a halt by COVID-19 infection caused by SARS-CoV-2 virus. Public health and healthcare professionals are at the frontline, working tirelessly for last one year to mitigate the spread of this disease and ameliorate the condition of the people. Modified version of the virus in form of it`s new strains are posing greater threat to public health due to enhanced transmissibility or infectivity.

In this special issue of SMC bulletin, we have sought to bring together the expertise of the established groups from different disciplines with an aim to provide overview about SARS-CoV-2 and various therapeutic and vaccines platforms explored to combat it.

The article written by Dr. Gupta's group serves as an excellent introduction about the structural features of this virus. Subsequently the articles from Dr. Chittela and Dr. Hassan groups have addressed a comprehensive overview on diagnostic and therapeutic development for COVID-19. Dr. Kunwar's article has provided the link between selenium deficiency and COVID-19 mortality in their article, while Dr. Vinod has elucidated the role of nanotechnology in vaccine development. Dr. Dhruv Kumar has discussed the role of mutations in SARS-CoV-2 on increased transmissibility. Towards the end, Dr. D'Souza, has penned the brief history of coronaviruses with special emphasis on the SARS-CoV-2 and the biology behind it.

The understanding developed from these articles will be very useful for the materials scientists' fraternity. We believe that material scientists can play a very pivotal role in combating COVID-19 because nanoparticles and viruses operate on the similar length scale, and material science has big role to play in diagnostics, therapeutics and vaccine development.

We are extremely privileged to be the guest-editor for this special issue on 'COVID-19'. We thank all the authors, who are acclaimed experts of their field, for the quality of their contributions.

From the desks of the President and Secretary

Dr. V. K. Jain
President

Dr. R. K. Vatsa
Hon. Secretary

Dear Esteemed SMC Members and colleagues,

Warm greetings from the Executive Council of ***Society for Materials Chemistry*** (SMC),

The Editorial Board of SMC Bulletin has been proactively working to bring out thematic issues on contemporary subjects. Ongoing pandemic COVID-19, caused by SARS-CoV-2, has not only disrupted normal functioning of life but also put immense pressure of health care system. The first confirmed case of COVID-19 was reported from Wuhan city of China on 17th November 2019. World Health Organization (WHO) was formally notified about a cluster of cases of pneumonia in Wuhan city on 31st December 2019. WHO declared ***Covid -19*** outbreak a 'public health emergency of international concern' on 30th January 2020 and pandemic on 11th March 2020. Having realized the impact of pandemic, several countries (*e.g.,* Italy, UK, Spain, France, Germany, India, etc.) imposed nationwide lockdown, while other countries (*e.g.,* China, USA, South Korea, etc.) did not observe nationwide lockdown. During the past 15 months or so more than 17.3 crore people have been infected and more than 37.3 lakhs people have died globally.

Understanding the properties of the virus is the key to disrupting its spread and infection. Chemistry, 'being a central science' can play a key role in addressing different aspects of this viral disease from diagnosis, treatment and protection. To provide an overview about the structural features of this novel coronavirus (SARS-CoV-2), special issue of SMC bulletin was conceptualized covering various diagnostic and therapeutic platforms used to control the menace of this virus. This issue contains seven invited articles which sequentially provide the details about the virus and recent developments in diagnosis, therapeutics and vaccine front to surmount the virus challenge. We believe that this bulletin will be of interest to its audience.

We place on record our sincere appreciation to Dr. Sandeep Nigam and Dr. Adish Tyagi, Guest editors, who have taken keen interest to bring out this special issue in a timely manner. We acknowledge the efforts of all the authors for contributing interesting and informative assays on the topic of great concern. We also thank all the members of SMC for their continued support and cooperation in the growth of the Society for Materials Chemistry.

(Dr. V.K. Jain)
President, SMC

(Dr. R.K. Vatsa)
Hon. Secretary, SMC

CONTENTS

Structural proteins of SARS-CoV-2 and assembly of new virions in the host cell

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Abstract

SARS-CoV-2 are enveloped RNA viruses whose genome encode four structural proteins: *Spike* (S), small *Envelope* protein (E), *Membrane* (M), and *Nucleocapsid* (N). The glycoprotein S, protruding from the surface of the virus, mediates the attachment and fusion of the virion to the host cell. The N protein binds to genomic RNA and forms helical filaments of ribonucleoprotein complex, that is crucial for the confinement of 30 kb long RNA genome within the limited volume of the viral envelope. The M protein, the major constituent of the viral envelope, forms a lattice-like dense matrix within the CoV envelope. Other structural proteins S and E are incorporated into the matrix through their lateral interaction with M protein while the ribonucleoprotein complex is incorporated inside the envelope via interaction of N protein with the cytosolic domain of M protein. These proteins are accessible on the viral surface and thus induce first immune response. In the present article, we have summarized the three-dimensional structure information of SARS-CoV-2 structural proteins and their role in the new virions assembly. Since virus assembly is the most important event in the viral life cycle, it will help in understanding viral biology and could be of value in drug development process.

Keywords: Coronavirus, SARS-CoV-2, Spike protein, Nucleocapsid, Envelope protein, Membrane protein, Virion's assembly

The current outbreaks of Coronavirus disease 2019 (COVID-19) is one of the worst pandemics in the recorded history of mankind. Till today nearly 150 million people have been infected and nearly 3 million people have died worldwide (WHO report, https://COVID-19.who.int/). This deadly disease is caused by the recently discovered '**S**evere **A**cute **R**espiratory **S**yndrome **Co**rona**v**irus **2** (SARS-CoV-2). SARS-CoV-2 is a member of Coronaviridae family, and *Orthocoronavirinae* subfamily (order Nidovirales), and of *Betacoronavirus* genus (Chen et al, 2020). These are enveloped, roughly spherical displaying a crown-like appearance, and encapsulating within it a single-stranded positive-sense RNA genome (~30kb) with 5′ cap structure and 3′ polyA tail (Cascella et al, 2021). Coronaviruses were initially known to cause disease in animals only, but later human coronaviruses HCoV-229E and HCoV-OC43 were identified causing mild respiratory tract infections (Hamre & Procknow, 1966). Coronaviruses are adapting and evolving at a faster pace and, some of the coronaviruses of *Betacoronavirus* genus, namely the Severe acute respiratory syndrome coronavirus (SARS-CoV), the Middle East respiratory syndrome coronavirus (MERS-CoV), and SARS-CoV-2 have crossed the species barrier in the past two decades and have caused serious illness in humans (Menachery et al, 2017). The SARS-CoV-2 is highly contagious, and spreads rapidly in the human populations. The reproduction number (R_o) for COVID-19 is ~3, much higher than other coronavirus diseases (Wang et al, 2020). At the genome level, SARS-CoV-2 shares ~79% and ~50 % sequence identity with SARS-CoV and MERS-CoV, respectively (**Table-1**). To gain entry into their host cells, viruses require a specific receptor and the SARS-CoV-2 and SARS-CoV both use the human **A**ngiotensin **C**onverting **E**nzyme II (hACE2) receptor to attach and to enter into the host cell. The attachment and fusion to the host cell are mediated by the trimeric spike protein, protruding from the surface of the virus. After entering the cell, the viral genome is released and initiates a sequence of events, temporally and spatially regulated, leading to genome replication, translation and assembly of new virions (**Fig. 1**). The viral genomic RNA is translated by the host ribosome and produces two polyproteins pp1a and pp1ab. These polyproteins are processed/cleaved into several non-structural proteins (nsp1-nsp16) by the two cysteine proteases, nsp3 (Papain like protease, PLpro) and nsp5 (3C like protease, CLpro) (Arya et al, 2021; Chen et al, 2020). These non-structural proteins form the Replication-Transcription-Complex (RTC) and induce the formation of Double-Membrane Vesicle (DMV), the site for replication and transcription of gRNA (Arya et al, 2021; Angelini et al, 2020). The function

Table 1: Sequence comparision of SARS-CoV-2 with other Coronaviruses

	Overall genome	S protein	N protein	E protein	M protein
Bat-CoV RaTG13	96.17	97.71	99.05	100	98.64
SARS-CoV	79.48	77.3	89.74	96	89.59
MERS-CoV	49.56	32.65	46.04	41.33	43.12

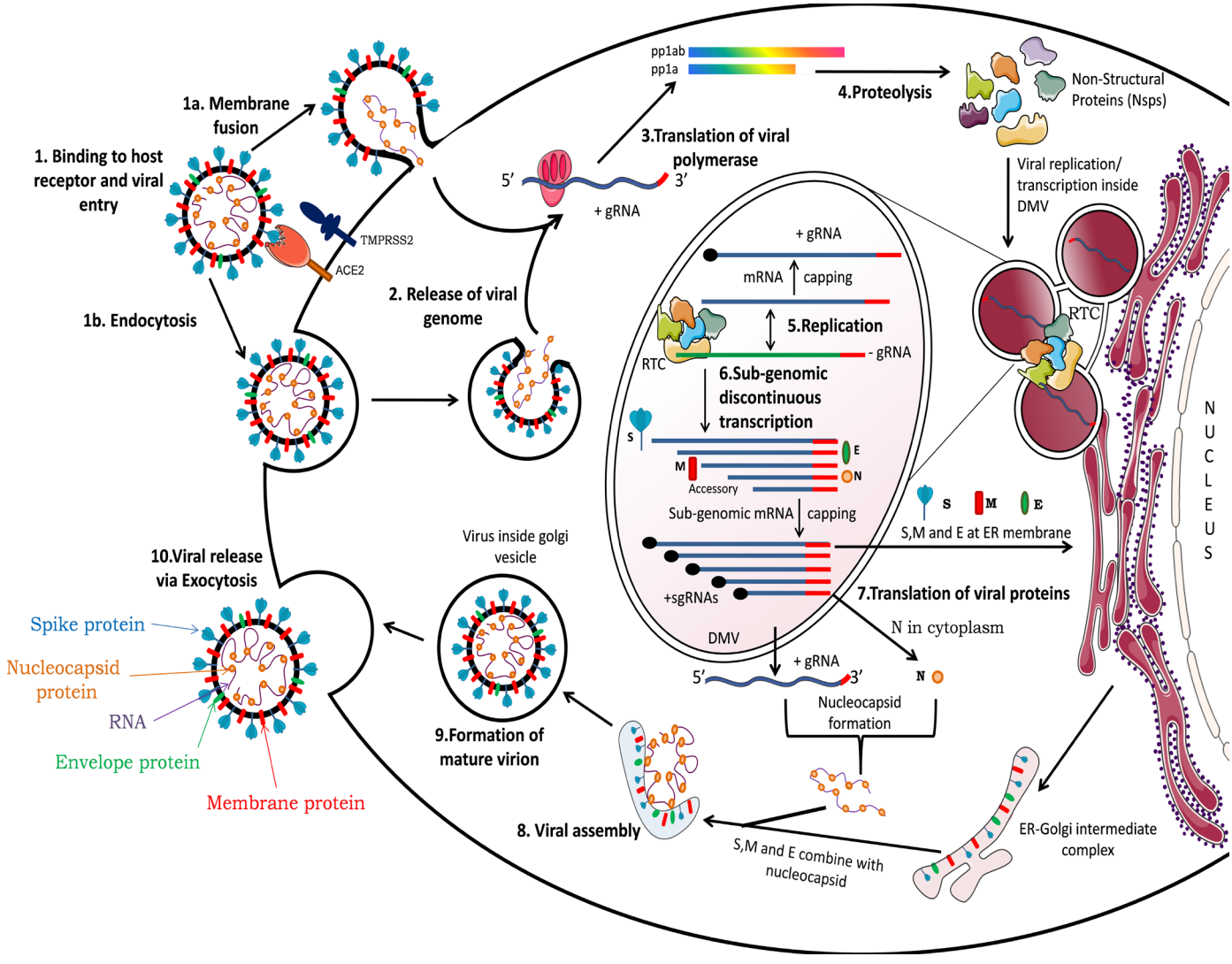

Figure 1. *Schematics of SARS-CoV-2 life cycle. SARS-CoV-2 binds to hACE2 receptor present on the host cell surface through surface S protein and injects its genome (gRNA) into the host cell via endosomes or direct fusion. The viral gRNA is translated by the host ribosomes to polyproteins pp1a and pp1ab which are proteolytically cleaved into non-structural proteins nsp1 - nsp16 by the viral proteases, nsp3 and nsp5. Replication-transcription complex (RTC) is assembled inside the double membrane vesicle (DMV). The RTC either replicates the gRNA or transcribe it to sub-genomic mRNAs. These sub-genomic mRNAs are translated to structural proteins S, M, E, N, and the accessory proteins. The N protein forms the ribonucleoprotein complex with gRNA while the S, M, and E proteins enter the lumens of endoplasmic reticulum (ER) for assembly and budding of new virus particle. The viral progenies are released into the extracellular region through exocytosis. Components of this figure were created using Servier Medical Art templates, (http://smart.servier.com/).*

of individual nsps' has not been discussed in the present article, for which readers are referred to the review article by Arya et al (2021). Other ORFs at the 3' end encode four structural proteins –spike (S), membrane (M), envelope (E), nucleocapsid (N) along with many accessory proteins. The RTC replicates genomic RNA to RNA (-) strand and then using RNA (-) strand as template, it synthesizes either the RNA (+) strand (replication) for new virion assembly or the sub-genomic mRNAs (transcription) for translation of structural and accessory proteins (**Fig. 1**). These structural proteins are involved in assembly and packaging of the new virion particles, and are present in the mature virus and are thus highly immunogenic. In this article we will discuss the entry of virus into the host cell, its packaging and assembly to form new virion particles and transmission to other cells thereof.

Receptor recognition and entry of the virus into the host cell

All the coronaviruses use the spike glycoprotein (S protein), present on the virus surface, to bind to their host cell receptors (Li, 2016). The binding triggers large conformational rearrangements leading to fusion of the viral and host membrane and entry of the viral genome into the host cell. The SARS-CoV-2 S protein is a multi-domain protein of 1273 residues and shares ~77 % and ~32% sequence identity with SARS-CoV and MERS S proteins, respectively (**Table-1**). It mediates receptor-recognition and membrane-fusion, and is therefore a key factor involved in viral pathogenesis and inter-species transmission. The S protein contains three sequence segments; an extracellular region (ectodomain), a transmembrane region and a short intracellular C-tail (**Fig. 2A**). The ectodomain consists of two major functionally distinct subunits S1 and S2; S1 is responsible for receptor recognition while S2 mediates the fusion of the viral and host membranes (Walls et al, 2020). The cryo-EM structure of SARS-CoV-2 S protein complexed with hACE2 (PDB IDs: 6VYB, 6XM3, 7KNE) and the high-resolution crystal structure of the receptor-binding domain of spike protein with hACE2 (PDBID: 6M0J) provide important insights into the virus-host

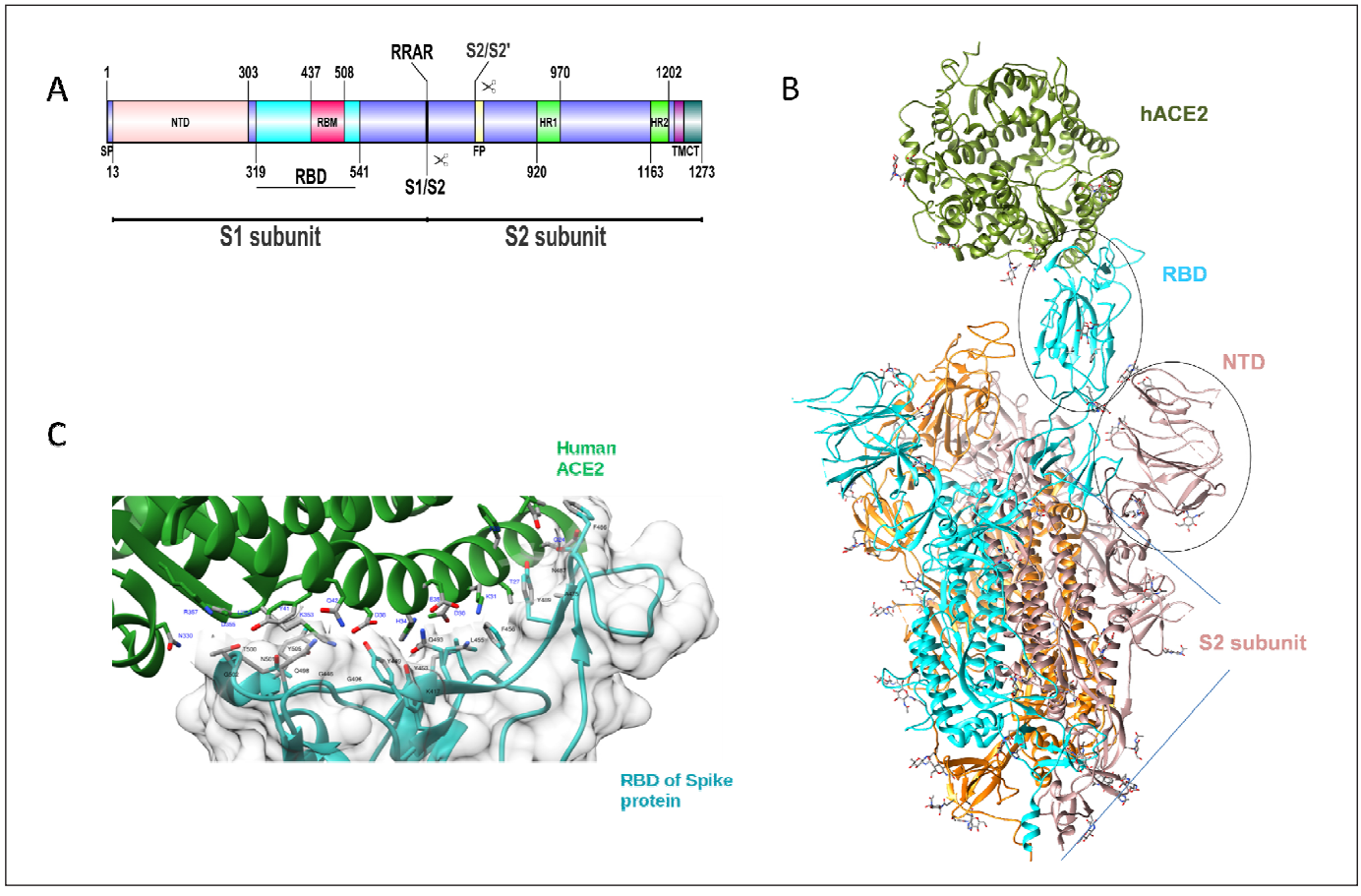

Figure 2. Depiction of sequence features, domain boundaries, and proteolytic cleavage sites of SARS CoV-2 spike protein. B) Trimer of Sars-CoV-2 spike protein complexed with hACE2 (green color) receptor. RBD, NTD and S2 subunit (in prefusion state) are marked here. The S protein is highly glycosylated and sugar moieties are shown as a stick model. RBD of two protomers (gold and purple) are in 'down conformation' while one protomer (Cyan), bound with hACE2 is in 'up conformation'. C) Close view of the interface of the RBD-hACE2 complex (PDB ID 6M0J). The residues within close proximity (<3Å) are labelled and shown here as a stick model.

recognition and their interactions (**Fig. 2B, 2C**). Molecular graphics and analyses have been performed with UCSF Chimera (Pettersen et al, 2004).

The S1 subunit has two major structural domains; an N-terminal domain (S-NTD, residue 13-305) and a receptor-binding domain (RBD, residues 319-541). S1 subunit can exist in two conformations; down conformation is receptor inaccessible state while the up conformation is available for receptor binding (Wrapp et al, 2020). A hinge-like motion at the interface of RBD and the rest of the protein, brings the transition from down to up conformation. The SARS-CoV-2 RBD alone is able to bind to hACE2 receptor *in vitro*. The core of RBD is composed of a five-stranded antiparallel β-sheet with short connecting helices and loops. Residues 440-506 form the receptor-binding motif (RBM) and extend out of the main core, forming a concave couch-like structure that provides a large surface area for interaction with the hACE2 receptor. An arc shaped alpha- helix of the peptidase domain of the receptor makes extensive contact with the RBM (Yan et al, 2020). The residues Y449, F456, F486, N487, Y489, Q493, Q498, T500 of S protein at the interface are involved in direct interaction and these interactions are stabilized by 14 hydrogen bonds and one salt bridge (**Fig. 2C**) (Lan et al, 2020, Shang et al, 2020). Receptor recognition by coronaviruses is the determinantal step in viral pathogenesis, thus the RBD of S protein is a potent target for vaccine and drug development (Sternberg & Naujokat, 2020).

The N-terminal domain of the S protein has a galectin fold with a β sandwich core structure consisting of two antiparallel β-sheets. The core is shielded by a ceiling-like structure that helps to invade the host immune system (Li, 2016). The SARS-CoV-2 S-NTD stabilizes the S2 subunit in a constrained prefusion state (Kirchdoerfer et al, 2016). Neutralizing human antibodies have been observed to bind to the S-NTD suggesting that not only RBD but NTD is also a promising target for therapeutic mAbs against COVID-19 (chi et al, 2020). Interestingly, some other coronaviruses like mouse hepatitis virus (MHV) use the S-NTD to bind to their host receptors (Peng et al, 2011).

Coronaviruses exhibit a type-1 membrane fusion mechanism, where the S2 subunit undergoes large conformational transition from prefusion to postfusion states (Wrapp et al, 2020). In the prefusion state, heptad repeats HR1 and HR2 are folded into two separate helices and pack against each other like a 'loaded spring' (Cai et al, 2020). Binding of the RBD to the host receptor triggers the proteolytic cleavage of the S protein into fragments S1 and S2 by the host proteases (serine protease-TMPRSS2 and cysteine proteases-cathepsin B/L). The cleavage relaxes the constrained structure of HR1 and HR2, and both merge to a single elongated helix. Three such elongated helices, from the three subunits of the trimeric S protein, form a very stable core structure. Three hydrophobic fusion peptides (FP) one from each protomer, earlier buried inside, become exposed in the postfusion state and are inserted into the host membrane leading to the fusion of viral and host membrane (Li, 2016; Walls et al, 2017). An intriguing characteristic of the SARS-CoV-2 spike protein is the presence of a basic amino acid sequence RRAR at the S1/S2 interface, creating a specific cleavage site of another host protease i.e furin protease. This site is absent in the S protein of SARS-CoV and other SARS related coronaviruses (Xia et al, 2020). Since S protein needs to be cleaved at the S1/S2 site for membrane fusion and entry to the cell, the presence of an additional cleavage site may provide a gain of function making SARS-CoV-2 more infectious (V'kovski et al, 2020).

The S protein is highly glycosylated that helps in shielding the protein surface from antibody recognition and modulating the host immune response. Glycosylation also plays a role in conformational stability of the protein. The double glycosylation deletion mutations N331Q and N343Q in the RBD domain resulted in nearly complete loss of infectivity (Li et al, 2020). The SARS-CoV-2 S protein is evolving at a faster pace compared to the rest of the viral genome. The recently identified SARS-CoV-2 variant (SARS-CoV-2 VUI) is reported to have 40 - 70 % higher transmissibility (Hu et al, 2021). The variant is defined by the presence of 10-14 mutations with at least three mutations in the spike protein. Out of these mutation N501Y (nick named "Nelly") is altering the key residue in the receptor binding domain of spike protein (Wise, 2020). Though it is less likely to interfere with the present diagnostic methods or vaccine efficacy, it is likely to enhance the binding affinity to hACE2. The other mutation in the RBD, likely to enhance ACE2 binding, is K417T (Faria et al, 2021). Another notable mutation, E484K (nick named "EeeK"), has been characterized as an escape mutation and the monoclonal and serum-derived antibodies were reported to be less effective against this variant (https://covidreference.com/variants). The frequency of another variant, P681H, has also been found to be increasing worldwide, similar to D614G. Analysis of SARS-CoV-2 genomes, isolated from Indian populations, reveal prevalence of the D614G mutation in S protein (Raghav et al, 2021). Li et al (2020) have found that D614G mutation enhances the infectivity of the strain in cell lines experiments. Another double mutant strain has co-existing mutations L452R and E484Q, which are localized in the receptor binding motif of the spike protein. These two mutations are often accompanied by a third mutation, P614R, in the S protein (Cherian et al, 2021).

The formation of virions' progeny

Following the entry of the virus into the host cells, it begins its genome replication and protein production. Once sufficient quantities of genomic RNA and structural proteins have been made, it starts its assembly process into a new virion particle by forming the viral envelope inserted with major structural proteins. The process takes place at the **E**ndoplasmic **R**eticulum-**G**olgi **I**ntermediate **C**ompartment (ERGIC) lumen (Mendonça et al, 2020, Ujike & Taguchi, 2015). Virus-like particles (VLP) models have been studied to understand the role of each structural protein in budding and release of the new virions. These VLP are self-assembled when necessary structural proteins are optimally co-expressed. Morphological and immunological features of VLP resemble the virus, but they lack the genetic material, making them a safe and non-infectious experimental model (Xu et al, 2020). VLP have also been successfully utilized as a vaccine candidate by expressing molecular epitopes on its surface (Ghorbani et al, 2020). It has been observed that presence of M protein along with either E or N protein essential for VLP formation as well as their trafficking and release (Siu et al, 2008). Our present understanding is that M protein, the major constituent of the viral envelope, forms a lattice-like dense matrix within the CoV envelope (de Haan et al, 1998). Other structural proteins S and E are incorporated into the matrix through their lateral interaction with M protein while the ribonucleoprotein complex is incorporated inside the envelope via interaction of N protein with the cytosolic domain of M protein (Siu et al, 2008; Xu et al, 2020). Their detailed role and structural information have been discussed in the following subsections.

Packaging of the viral genome: role of Nucleocapsid protein

The confinement of 30 kb long RNA genome within the limited volume of the viral envelope is a crucial step in the assembly of new SARS-CoV-2. The condensation state results from the binding of gRNA to the Nucleocapsid protein (N protein), where N protein forms helical nucleocapsid filaments that work as a scaffold for RNA. The interaction of N protein with RNA and its higher-order assembly are critical for viral assembly. The recent structures of SARS-CoV-2 N protein and its complex with RNA provide the molecular level details of the assembly (Dinesh et al, 2020; Kang et al, 2020). The SARS-CoV-2 N protein is a 419 residues long protein, with two major ordered structural domains: The N-terminal domain (N-NTD; 46-174), directly binds to RNA and the C-terminal domain (N-CTD; 247-364) is involved in multimerization (**Fig 3A**). These two domains are separated

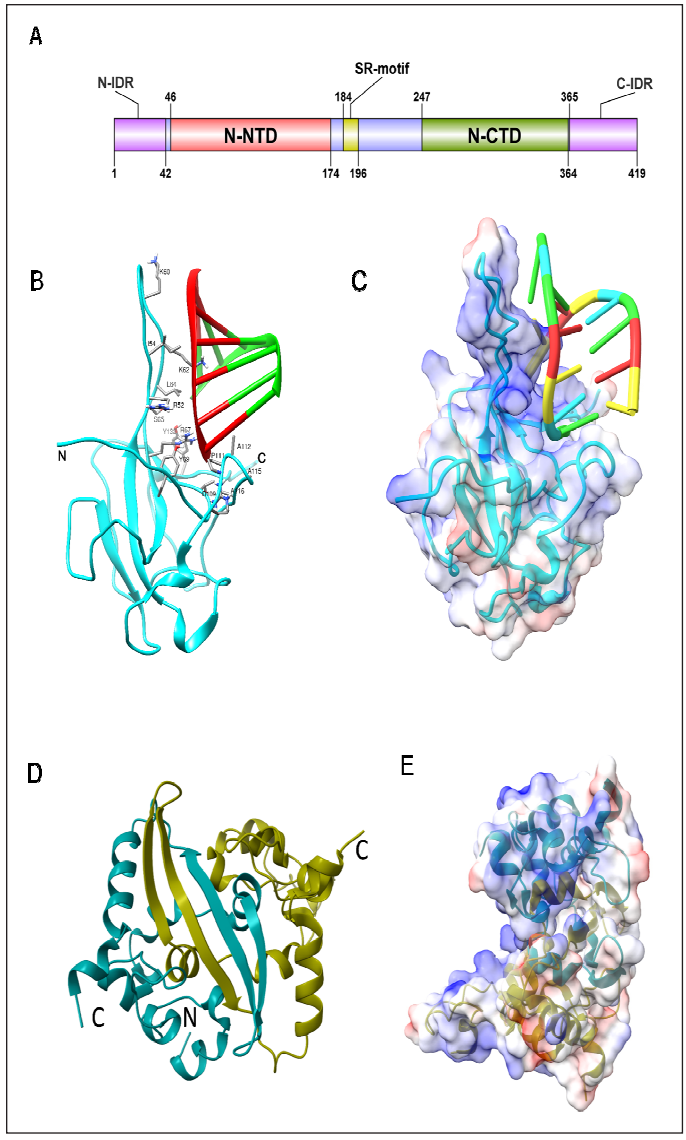

Figure 3. *A) Schematic representations of the domain structure of the Nucleocapsid protein. B) Cartoon representation of N-NTD (Cyan) complexed with dsRNA (Red and green in ladder representation) (PDBID: 7ACS). The residues directly interacting with RNA are labelled and shown here as stick model. C) The electrostatic surface mapping of N-NTD (gradient from Blue (for positive charge) to Red (negative charge). D) The dimer of N-CTD, the dimerization domain (PDB ID 6WZO). E) The electrostatic surface mapping suggests that one side of the dimer is positive charges, and grooved and thus may be interacting with RNA.*

by an intrinsically disordered and highly phosphorylated linker region rich in serine/arginine (184-196, SR motif). The N- and C- terminal ends of the protein (residues 1-42 and 365-419) are disordered (Kang et al, 2020). Overall N-protein shares nearly 90% sequence identity with SARS-CoV N protein. The full-length N protein as well as isolated N-CTD, but not the N-NTD, predominantly form dimers.

SARS-CoV-2 N-NTD resembles a right-hand fold (**Fig.**

3B). The protein core is composed of antiparallel β-sheets (resembling a palm) with two loops and a β-hairpin extending out of the core structure like fingers (Kang et al, 2020). The electrostatic surface mapping revealed a basic patch (blue colored) extended between the finger and the palm. Recent NMR studies of the SARS-CoV-2 N-NTD domain, in complex with ssRNA and dsRNA (PDB IDs: 7ACS, 7ACT) found that the residues A50, T57, H59, R92, I94, S105, R107, R149 and Y172 are directly involved in RNA binding. Further, the mutation in conserved arginine residues R92 and R107 abrogates the RNA binding (Dinesh et al, 2020). The basic residues of the palm and finger regions grasp the negatively charged backbone of ssRNA, whereas aromatic residues from the palm region provide additional base stacking interaction (Tan et al, 2006). The residues in this region can be targeted for drug design strategy. NTD and CTD domains are separated by an intrinsically disordered region, rich in serine and arginine (SR region) residues. The region is highly phosphorylated and may provide an additional surface for RNA binding. It might also be involved in interaction with M protein (Siu et al, 2008). The N-CTD forms a compact intertwined dimer and can form extended higher order assembly essential for helical nucleocapsid filaments (Kang et al, 2020). The monomer of N-CTD is composed of five short α-helices, a 3_{10} helix and two β strands. The dimeric interface is large and is composed of four stranded anti-parallel β-sheet and two short helices (two strands and one helix coming from each protomer). The other helices from each protomer are flanked on either side of the interface (**Fig 3D**). The electrostatic mapping shows that one face of the N-CTD dimer is positively charged compared to the opposite face with groove formation; the site has been suggested as an additional site for RNA binding (**Fig 3E**) (Jayaram et al, 2006). Recently, a study based on H-D exchange mass spectrometry has suggested the role of C-tail residues (365-419) in the multimerization of N protein (Ye et al, 2020).

Several crystal structures of CTD are available in the PDB, and in almost all crystal structures, the N-CTD has been trapped as a dimer in the crystalline state. The higher-order assembly of N protein has been investigated by using their crystal contacts (Jayaram et al, 2006; Ye et al, 2020). The N-CTD dimers can pack in two different modes; 'head to tail' packing mode may lead to the elongation of nucleocapsid filaments while 'side by side' packing mode might be responsible for the bending of the long filaments. In this proposed model, the RNA binding region of N-NTD and N-CTD are exposed to the surface, and the flexible linker region may bring these two domains facing each other such that viral RNA is engulfed between these two domains making it inaccessible to RNases (Jayaram et al, 2006).

Other than genome packaging, N protein plays multiple roles in virus pathogenesis and propagation (McBride et al, 2014). The co-localization of N protein with replication complex is essential for the RNA replication (McBride et al, 2014). It associates with ER-Golgi complex, and plays a role in virus budding. Its interaction with M and E proteins increases the yield of VLP (Ujike & Taguchi, 2015). It helps in viral pathogenesis by inhibiting the synthesis of type-1 interferon (IFN) and deregulates the host cell cycle by interfering with cyclin-CDK activity (Zhao et al, 2008). The N protein is produced in high abundance during infection and is highly immunogenic, thus a potent target for vaccine and drug development (Shang et al, 2005; Padron-Regalado, 2020).

Envelope – E protein

Envelope protein is a small (8-12kDa) hydrophobic trans-membrane structural protein involved in assembly, budding and release of viral progeny and activation of host inflammasome (Mandala et al, 2020; Yoshimoto, 2020). The protein is expressed in abundance in the infected cells, however; only a meagre portion is incorporated into the viral envelope during assembly. The majority is localized to ER, Golgi and ERGIC where it participates in assembly, budding and intracellular trafficking (Bhowmik et al. 2020; Nieto-Torres et al. 2011). The E protein has also been suggested to play a crucial role in the scission of the viral particle from the ER membrane during the terminal phase of budding (Vennema et al, 1996). Recombinant CoVs lacking E protein display reduced viral titers, impaired viral maturation and incompetent viral propagation (Bhowmik et al, 2020; Mukherjee et al, 2020).

The E protein is highly conserved among *Betacoronaviruses* and the SARS-CoV 2 E protein shares ~97% sequence similarity with SARS-CoV (Alam et al, 2020; Bianchi et al, 2020). The E protein of SARS-CoV-2 is 75 amino acids long, comprising of 3 domains: 1) short N-terminal hydrophilic domain of about 10 amino acids, 2) hydrophobic transmembrane domain (TMD) spanning 25 amino acids and 3) a long hydrophilic C-terminal domain comprising the majority of the protein (Schoeman & Fielding, 2019). Translated E protein undergoes several post-translational modifications including palmitoylation at cysteine residues and N-linked glycosylation at particular asparagine residues. Palmitoylation aids subcellular trafficking of proteins between membrane compartments whereas glycosylation assist protein folding and trafficking by recruiting host chaperons (Mukherjee et al, 2020; Schoeman & Fielding, 2020).

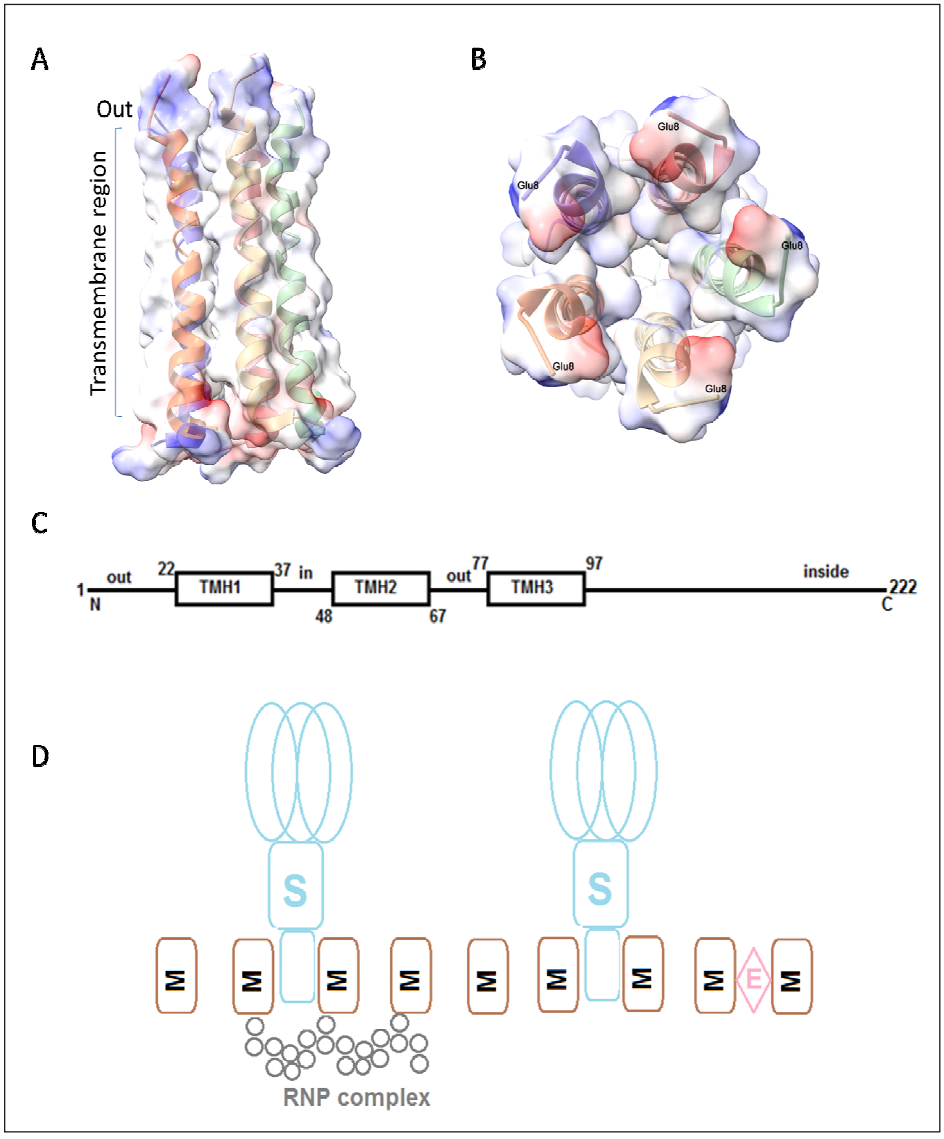

Figure 4. *A) Cartoon representation of one of the NMR models of the transmembrane region (Glu8-Arg38) of SARS-CoV-2 E protein (PDBID 7K3G). B) The cross-sectional view to illustrate the viroporins, formed by pentameric arrangement. C) The sequence feature of SARS-CoV-2 M protein. D) Schematic representation of lattice like matrix model of M protein. The S and E proteins are incorporated into the M matrix through lateral interactions, while ribonucleoprotein (RNP) complex is attached via the interaction of N protein with the endo-domain of M protein.*

The recent NMR structure of the transmembrane region of the E protein displayed a pentameric helix bundle, with pseudo five-fold symmetry, forming a narrow central pore (**Fig 4A, 4B**) (Mandala et al, 2020; Sarkar & Saha, 2020). The cationic hydrophilic channels thus formed in virus-infected cells act as viroporins. These channels permit the transport of positively charged ions (K^+, Na^+ and Ca^{2+}) across the membrane leading to loss of membrane potential and altering permeability properties, thereby activating host inflammasome (DeDiego et al, 2014). The C-terminal tail of SARS-CoV E is predicted to have a β-coil like motif with a conserved proline residue essential for its localization in the ER-Golgi complex (Satarker & Nampoothiri, 2020). A highly conserved CxxC motif present following the TMD is crucial for maintaining the structural topology. A similar CxxC motif present at the C-terminal end of the S protein hints at disulfide bridge formation between S and E proteins as a basis of interaction between these two structural proteins (Schoeman & Fielding, 2019, 2020). The last four amino acids (DLLV) at the C-terminal end in SARS-CoV and SARS-CoV 2 constitute the PDZ (postsynaptic density-95/Disc Large/Zonula occludans-1) binding motif (PBM). This PBM of E protein has been suggested to interact with the PDZ domain of host PALS1 (Proteins Associated with Lin Seven 1) and syntenin proteins, thereby jeopardizing host protein functions and facilitating viral dissemination (Satarker & Nampoothiri, 2020; Toto et al, 2020). This event together with the viroporin activity of the E protein induces a cytokine storm leading to severe complications and Acute Respiratory Distress Syndrome (ARDS) (Hassan et al, 2020; Schoeman & Fielding, 2020).

Membrane protein

The SARS-CoV-2 Membrane protein (M) is the most abundant structural protein in the mature virion particle that plays an important role in maintaining virion size and shape (Neuman et al. 2011). It is the monitor protein that interacts with all other structural proteins and ensures their presence in the mature virion particle. The SARS-CoV-2 M protein is a type III transmembrane glycoprotein of 222 amino acid long. It has three major domains: N-terminal ecto-domain, triple-spanning transmembrane region and a long C-terminus intracellular region (**Fig. 4C**). Though the crystal structure of M protein is not yet available, the VLP model-based studies have been useful in predicting the functional role of M protein. For CoV assembly, the structural protein must be retained near the ERGIC instead of being secreted out to the plasma

membrane. The Golgi retention signal of SARS-CoV M protein resides in the first of the three transmembrane helices resulting in its retention and then it recruits other structural proteins in ERGIC (Tseng et al, 2010). The self-association of M protein as well as its interaction with other structural proteins serve as a checkpoint for the assembly of new virions (Liu et al, 2010). The interactions of M with E and N proteins are through their C-terminal endo-domains. Though the co-expression of only the M and the E proteins is sufficient for VLP assembly in vitro, the expression of N protein improves the yield of VLP (Siu et al, 2008). The self-association of M protein results in a lattice like dense matrix, that serves as a scaffold along with E or N protein and incorporate the S protein and RNP into the mature virion (**Fig 4D**). It also excludes other foreign proteins by leaving no vacancy for them. The TM1 and TM2 regions of SARS-CoV M protein are immunogenic and can be a potential immunogen in therapeutic applications (DeDiego et al, 2014).

Conclusion

SARS-CoV-2 are enveloped viruses having four different structural proteins. Two major glycoproteins M and S, and minor E proteins are incorporated into the viral envelope while the N protein encapsidates the viral genome within. All these proteins are accessible on the viral surface and thus induce first immune response. In the present article, we have summarized the mechanistic details of new virions assembly, and three-dimensional structure information of SARS-CoV-2 structural proteins. Since virus assembly is the most important event in the viral life cycle, it will help in understanding viral biology and in drug development process.

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COVID-19 and its diagnostic strategies

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Abstract

Successful management of any pandemic infection relies on effective diagnostic methods, management of infected people and its preventive measures like vaccination etc. Currently, the entire world is under a health emergency like situation due to COVID-19 infection. Initially it was reported in Wuhan, China and in a span of a few months the infection covered most of the globe. Currently, the reported number of infected people is more than 173 million. Scientists, health care professionals and policy makers are thriving to combat this disease in developing different diagnostic methods, therapeutic strategies and vaccine development programs. In this review we focus on the different diagnostic strategies currently being used in COVID-19 detection. Majorly, diagnosis of COVID-19 is on based clinical symptoms, clinical biochemistry parameters and molecular techniques which detect virus directly. Molecular methods are again subdivided into protein based and nucleic acid based methods. We attempted to compile the molecular methods, which will give a snapshot of most of the strategies used for COVID-19 detection.

Introduction:

The severity of **S**evere **A**cute **R**espiratory **S**yndrome **Co**rona**v**irus **2** (**SARS-CoV-2**) causing **co**rona **vi**rus **d**isease 20**19** (**COVID-19**) shook the world, making it a major point of concern for almost all the countries in the world. The first case of SARS-CoV-2 was reported in late 2019 in Wuhan, China [1]. The quick spread of SARS-CoV-2 across the world made it a global health emergency and World Health Organisation (WHO) declared COVID-19 as global pandemic in March 2020 [2]. The number of confirmed cases across the globe has crossed 173 million with the death of more than 3.7 million due to COVID-19 [3]. India alone is contributing around 10% of the confirmed cases [4]. SARS-CoV-2 genetic analyses reveal its bat origin followed by intermittent host passages before entering human. SARS-CoV-2 genome shares 96.3% sequence homology with bat coronavirus (RaTG13) genome isolated in Yunnan, China [5]. Pangolin CoV's Receptor Binding Domain (RBD) of surface glycoprotein (S protein) shares greater homology with RBD of SARS-CoV-2's (86.64%), which is higher than RaTG13 receptor binding homolog domain, indicating intermittent host passages [6]. After its entry into human, the continuous molecular divergence of the SARS-CoV-2 virus makes it difficult to track, hence diagnosis methods need be updated as the virus evolves [7].

Controlling the extent of the infection and management of the infected people depends on the quickness and reliability of the diagnostic and therapeutic methods. Different diagnostic strategies such as clinical symptoms, radiological images and para-clinical findings, for clinical diagnosis of COVID-19 have been purposed and are currently being practiced. The clinical symptoms of COVID-19, such as fever, dry cough, tiredness may help clinicians for primary diagnosis, but the final diagnosis of COVID-19 in patients is confirmed through viral detection methods. The para-clinical diagnostic methods in turn can be broadly categorized in two categories; 1) nucleic acid based and 2) protein based. Many molecular diagnostic tests are currently being used in healthcare setting and others are under rigorous clinical evaluation to develop fast, accurate and cost-effective methods [8]. However, false-positive and false-negative results are the major points of concern for all diagnostic tests and these need to be addressed to effectively control the pandemic spread [9, 10].

Different diagnostic test can provide different information about the patient's current COVID-19 status. Nucleic acid test can provide information regarding ongoing infection, whereas serological tests (antibody tests) can indicate about the exposure and hence immunity against SARS-CoV-2 [11]. These tests vary in cost, turnaround time and accuracy and have different implications towards overall disease management. The present review summarizes different diagnostic methods that are currently employed for diagnosis of COVID-19. The advantages and disadvantages of different methods have been provided to better understand the current clinical diagnostic situation.

Reverse Transcription - Polymerase Chain Reaction (RT-PCR)

RT-PCR is a subset of PCR method where the starting material is RNA instead of DNA [12]. As indicated in figure 1, RT-PCR is a two-step molecular technique where first DNA strand (known as cDNA or complementary DNA as it is the complement of the RNA template) is synthesized on the RNA template by a process called reverse transcription. The second step is the simultaneous amplification and detection of cDNA generated in the previous step, by polymerase chain reaction. The two-step reaction is driven by two driver enzymes, one for each step. Reverse transcriptase synthesizes cDNA on RNA template and DNA polymerase amplifies short regions of the cDNA for downstream detection. A pair of gene/locus specific primers confines the target region to be amplified by the polymerase. A fluorescent probe is required for further detection of the amplified product. The most commonly used probe in RT-PCR based detection is TaqMan hydrolysis probe [13].

SARS-CoV-2 is an RNA virus therefore RT-PCR can be used as a diagnostic assay to evaluate the presence of virus and hence the active infection [14]. This probe comprises of three components; the probe sequence, Reporter and Quencher. The probe sequence is chosen to be from within the region amplified by primer pair; therefore it recognizes the amplified product with a high degree of specificity. One end of the probe sequence is functionalized with a reporter and the other end with a quencher. The signal (generally fluorescence) emitted by the reporter is quenched by 'quencher' due to its physical proximity. DNA polymerase has two activities; 1) polymerase and 2) nuclease. When the polymerase extends the primer anchored on template, it encounters the bound probe and cleaves it using the nuclease activity releasing the reporter. Once separated from the quencher, the reporter can now produce a signal that can be detected. The amplification and probe cleavage cycles repeat multiple times during the PCR reaction, resulting in an increase of the signal which is proportionate to the amplified target.

The diagnostic specificity of the RT-PCR assay for SARS-CoV-2 is provided by the specifically designed amplification primers and probe set for a selected target region. The in-house molecular assays developed by seven different institutes target different genomic region of the SARS-CoV-2 and are displayed on World Health Organisation (WHO) website [15]. Most of the laboratories are using regions mentioned in the documents issued by these seven institutes to develop an RT-PCR based molecular diagnostic test. Assays targeting E gene are used for pan-sarbecovirus detection, whereas assays targeting RdRp and/or N gene of SARS-CoV-2 are utilized as confirmatory assays [15]. In addition, a positive control reaction of known gene is included in the kit to confirm that kit is in good condition. All three reactions are carried out separately in three tubes. However, this method needs more labor, time and consumables and less number of reactions can be performed in a batch. To address these drawbacks, some of the approved RT-PCR kits use multiplex RT-PCR to detect two or more target regions in single tube/reaction.

As of December 2, 2020, the Indian Council of Medical Research (ICMR) evaluated 309 RT-PCR kits, out of which 143 have been approved [16]. Food and Drug Administration (FDA) approved RT-PCR kits can also be used after market approval by Drugs Controller General of India (DCGI). *TaqMan 2019-nCoV Control Kit v1, InnoDetect Covid-19 RT-PCR Kit, GENEASY COVID-19 RT-PCR Kit, Accurex SARS COVID-19 RT-PCR Assay* are some of the RT-PCR based ICMR approved diagnostic kits currently available in the market. RT-PCR based diagnostic kits can provide extremely sensitive and specific (more than 95%) detection of the SARS-CoV-2 RNA. However, they are complex, expensive, and turnaround time is higher. Qualified and trained clinical laboratory personnel and equipped laboratories are required for performing RT-PCR [17].

As per the guidelines issued by FDA for Emergency Use Authorization (EUA), RT-PCR is currently considered as gold standard (reference test) against which a new test can be validated. Yet, some studies raise concerns about both false negative as well as false positive results of RT-PCR diagnostics for COVID-19 [18]. False negatives can have devastating effects by increasing the risk of community transmission, whereas false positives can substantially affect individual's finance and psychology. Spurious RT-PCR results may be caused by inappropriate sampling, long storage degradation, transportation, purification, processing, presence of inhibitors in the reaction, sample cross contamination, carryover PCR product and cross reactions with genetic materials of other pathogens.

The need for improvement in scalability and reduced turnaround time has led to development of Point of Care (POC) tests for the diagnosis of COVID-19 [19]. POC tests can give results within one hour whereas RT-PCR performed in laboratories on an average take 6 hour. *Cepheid Xpert SARS-CoV-2, Mesa BioTechAccula SARS-CoV-2 and Abbott ID NOW COVID-19* are the FDA approved POC tests for COVID-19 diagnosis. In a POC tests all the steps starting from sample preparation (nucleic acid

extraction and amplification) to detection are performed in an automated and integrated system. Automation along with integration reduces handling errors and makes the process time efficient. The single-use disposable cartridges (hold the RT-PCR reagents and carry the RT-PCR process) decrease the chance of carryover and cross contamination [20].

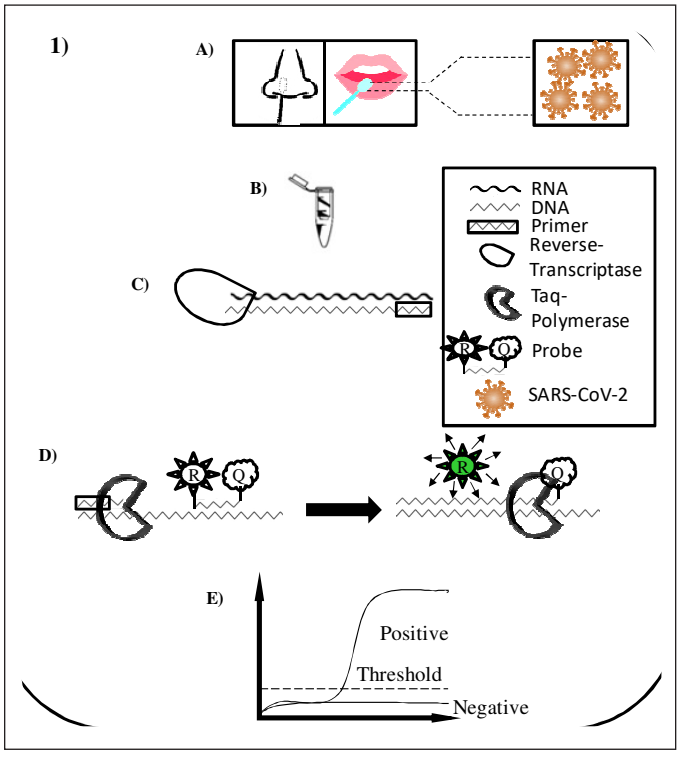

Figure 1: RT-PCR test for COVID-19 detection

A) Obtain nasopharyngeal and oropharyngeal swabs from the individual

B) RNA extraction from the virus

C) Synthesis of complementary DNA (cDNA) using extracted RNA by reverse transcriptase

D) Taq Polymerase extends the primer bound on the target and while extension cleaves the probe hybridized onto the target strand resulting in reporter release which inturn causes increase of fluorescence

E) The fluorescence is proportional to the viral load. When the fluorescence level crosses threshold value, the test is considered as positive.

Micro-Nuclear Magnetic Resonance (µNMR)

The T2 Magnetic Resonance (T2MR) assay is another cartridge-based platform that combines the nuclear magnetic resonance and PCR molecular assay [21]. After reverse transcription and DNA amplification, the amplicon is hybridized with target specific super paramagnetic nanoparticles bound probes that are then detected by T2MR. *T2SARS-CoV-2™ Panel* is the only FDA approved µNMR based COVID-19 detection kit. The limit of detection (LoD) for T2MR assay is 2copies/µ. Sensitivity of the test is 95% and specificity is 100%.

Digital Droplet Polymerase Chain Reaction (ddPCR)

ddPCR is a modification of conventional PCR that involves partitioning of the PCR mix into thousands of nanolitre sized droplets using water-oil emulsion technique (figure 2) [22]. Since, each droplet consists of template, fluorescence-quencher probes, primers, and a PCR master mix, therefore separate polymerase chain reactions can take place in each one. The presence or absence of fluorescence signal in each droplet can be checked after PCR amplification. Sample partitioning leads to increased precision and provides absolute quantification. *Bio-Rad SARS-CoV-2 ddPCR Kit* is the only FDA approved ddPCR based COVID-19 detection kit. The automated droplet generator partitions reaction mixture into thousands of nanoliter-sized droplets in the form of water-in-oil emulsion. In each droplet, reverse transcription followed by target amplification and probe hydrolysis take place. The increase in fluorescence intensity is measured and analysed by a custom designed software. This kit targets two region of N gene of SARS-CoV-2 (N1 and N2). The limit of detection (LoD) for Bio-Rad ddPCR kit is 1.5copies/µ. Both the positive percent agreement (PPA) and negative percent agreement (NPA) was 94.9%.

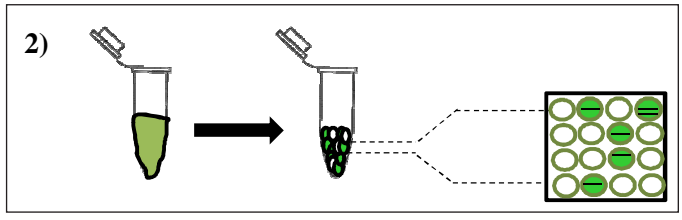

Figure 2: Droplet digital PCR: *Patient sample containing target viral molecules is diluted to single molecule per droplet and partitioned into millions of reaction droplets using water-oil emulsion technique. After multiple PCR cycles, each droplet will produce positive and negative reactions depending on the sample distribution that can be detected for quantitation of viral copies.*

Reverse Transcription loop-mediated isothermal amplification (RT-LAMP)

Reverse Transcription loop-mediated isothermal amplification is single temperature nucleic acid amplification and detection method therefore no thermal cycler is required (figure 3) [23]. Set of 4 (or 6) specifically

designed primers (two inner and two outer primers), and *Bst* DNA polymerase with strand displacement activity amplifies the sample at about 60 to 65 °C [24]. The inner primers are designed in such a way that they can form loop at the ends. Both forward inner primer (FIP) and backward inner primer (BIP) consists of three regions, two of them are complementary to each other and third loop region separates the complementary sequence. The innermost complementary sequence is also complement to the target sequence. The forward outer primer (FO) and backward outer primer (BO) are complementary to the target regions outside the binding sequence of inner primers. Reverse transcriptase utilizes one of the LAMP inner primers for the synthesis of cDNA. Once the reverse transcription is completed, the amplification starts with the FIP binding with the target DNA and its extension to form double-stranded DNA. The FO primer binds to the complementary region outside of the FIP binding sequence and *Bst* polymerase initiates extension. The extending strand displaces the FIP synthesised complementary strand. The complementary regions of FIP can now anneal forming a single loop structure at one end. This strand can now be targeted by BIP and DNA extension takes place which is followed by strand displacement by BO binding and extension. This results in the formation of a single stranded DNA with loop structures on both ends, resembling a dumbbell-like DNA structure. This structure can serve as a template for further amplification using the same inner primers or using another set of loop primers (loop primers are used to improve specificity) [23]. As the *Bst* polymerase used in LAMP has both polymerase as well as strand displacement activity, DNA amplification can take place at a single temperature [24].

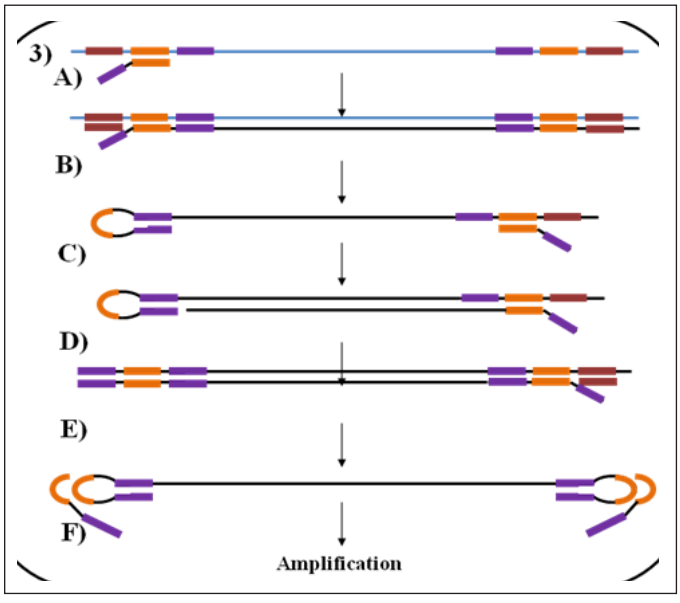

Figure 3: RT-LAMP is an isothermal amplification (at ~60℃) of the target region utilizing polymerase and strand displacement activity of DNA polymerase

AQ-TOP™ COVID-19 Rapid Detection Kit PLUS developed by SEASUN BIOMATERIALS is RT-LAMP based FDA approved COVID-19 detection kit. It uses probes targeting ORF1ab and N gene for detection of SARS-CoV-2 RNA. To detect amplification, forster resonance energy transfer (FRET) probes are used. As amplification takes place, incorporation of FRET probes will result in fluorescence change that can be monitored by a fluorescence reader. The LoD for *AQ-TOP™ COVID-19 Rapid Detection Kit PLUS* is 150copies/ul. Sensitivity of the test is 100% and specificity is 100%. The iAMP COVID-19 Detection Kit is another real-time reverse transcription isothermal amplification test based on a proprietary isothermal amplification technology termed OMEGA amplification.

A) Reverse transcription of extracted viral RNA using Backward Inner Primer (BIP) [Left Purple and Yellow box]

B) The synthesized cDNA is then displaced, and thus released, by reverse transcriptase while extending the backward outer primer (BOP) [Left Brown box]. Resulting in the formation of cDNA with loop on one end

C) Forward inner primer (FIP) [Right Purple and Yellow box] binds to the displaced cDNA

D) DNA polymerase extends FIP

E) Forward outer primer (FOP) [Left Brown box] binds the cDNA and DNA polymerase displaces the previously synthesized DNA forming loops at both ends

F) The dumbbell shaped DNA structure can now replicate using interplay of self-priming and inner primers

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) method

CRISPR-Cas technology is an RNA-guided endonuclease based nucleic acid editing tool. Depending upon application different CRISPR-Cas systems can be employed. For CRISPR-based diagnostic techniques a special "collateral cleavage activity" of certain caspases like Cas12a or Cas13 nucleases has been used. Once the CRISPR-RNA targeted cleavage takes place, it activates the collateral cleavage or trans-cleavage activity of Cas12a/Cas13 nuclease, cleaving all nearby ssDNA/RNA molecules non-specifically [25]. The labeled non-specific ssDNA/RNA after cleavage by Cas12a/Cas13 can give signal that can be detected. *Sherlock™ CRISPR SARS-CoV-2*

kit, SARS-COV-2 RNA DETECTR ASSAY and *SARS-CoV-2 DETECTR™ Reagent Kit* are the two FDA approved CRISPR based SARS-CoV-2 detection. These kits perform RT-LAMP method for reverse transcription and isothermal amplification of the SARS-CoV-2 RNA extracted from the clinical samples. The amplified product is detected by cleavage of reporter molecule by Cas12 enzyme directed by CRISPR guide RNA (gRNA).

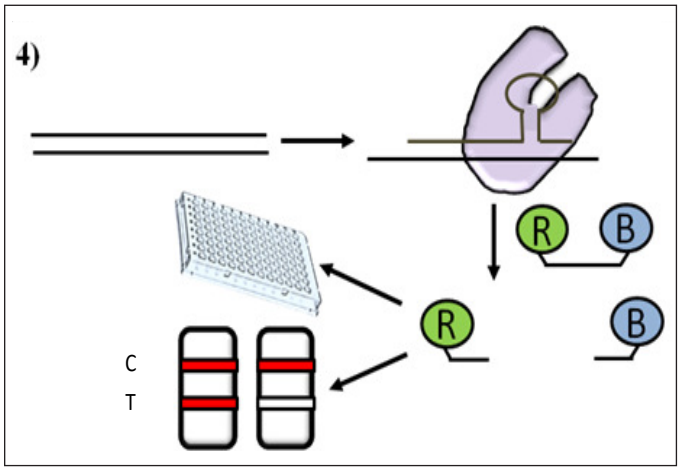

Figure 4: CRISPR based target detection: Target DNA is recognized by CRISPR-Cas 12 enzyme along with guide-RNA leading to collateral cleavage of the reporter that can be detected using plate-based fluorescence assay or lateral flow strip detection. R: Reporter, B: Blocker, C: Control, T: Test.

Nucleic acid Sequencing Based detection of COVID-19:

Nanopore Sequencing

Nanopore-based sequencing technology uses a biological protein nanopore embedded in a membrane surrounded by electrolyte solution. Voltage is applied across the membrane setting the ions into motion under the influence of electric field. When a charged DNA molecule passes through nanopore it occupies space and restricts the flow of ions across the pore, resulting in drop of ionic current. Different sequences generate unique electrical signals that can be detected for deduction of the DNA sequence [26]. *Clear Dx SARS-CoV-2 Test* is nanopore (Oxford Nanopore GridION Sequencer) sequencing based *in vitro* diagnostic test for the qualitative detection of SARS-CoV-2 RNA. cDNA is synthesized using reverse transcription of extracted SARS-CoV-2 RNA. This synthesized cDNA is used for sequential multiplex PCR amplification with two set of barcoded primers. The dual-barcoded amplicons are then used for sequencing on Oxford Nanopore GridION Sequencer.

Illumina Sequencing

Illumina Sequencing, sequencing by synthesis (SBS) technology uses four fluorescently-labeled reversible terminator nucleotides for sequencing. In every cycle, a single labeled deoxy-nucleoside triphosphate (dNTP) is added to the growing nucleic acid chain. The dNTP incorporation results in specific fluorescent signal that can be imaged to identify the base and subsequent enzymatic cleavage of the reversible terminator for next labeled nucleotide to be incorporated [27]. The process is repeated to complete the sequencing of the reads. *Helix COVID-19 NGS Test, Illumina COVID Seq, UCLA Swab Seq COVID-19 Diagnostic Platform* are Illumina sequencing based COVID-19 detection methods approved by FDA. Similar to nanopore sequencing the pooling of barcoded amplified PCR products is done prior to sequencing reaction. The LoD of sequencing based COVID-19 detection kits are in the range of 1 genome equivalent (G.E.) per µl.

Protein based COVID-19 detection methods

1. Virus detection from the sample:

The virus particles as whole can be detected using immunoassay-based biosensors. The viral genome encodes 4 structural proteins namely; S (surface), N (Nucleocapsid), E (Envelope) and M (Matrix). The spike protein is a transmembrane protein and it can elicit an immune response in the host. The protein also shows amino acid sequence diversity among various coronaviruses such that S protein from a SARS-CoV-2 virus can be differentiated from S protein of SARS-CoV or MERS-CoV. In this method, antibodies raised against the viral S protein were used to specifically recognize and detect the SARS-CoV-2 virus particles. The FET (field-effect transistor) based biosensor was functionalized with the aforementioned antibody. Anti- Spike protein antibody was immobilized on the graphene surface using the probe linker, 1-pyrenebutyric acid N-hydroxysuccinimide ester (PBASE). Electrical signal in response to binding of viral spike protein to the immobilized antibody results in the successful detection of the whole virus. The ability of the FET based biosensor to detect virus from spike protein (in Phosphate buffered saline), cultured virus particles as well as nasopharyngeal swabs stored in UTM (universal transport medium) has been successfully demonstrated. Clinical translation of this technique is yet to be done. Though the limit of detection of the FET based biosensor (~250 copies/ mL) is higher than the in use molecular diagnostic tests (~50-100 copies/ mL), the group has suggested further improvements in the detection limit of biosensor is possible [28].

This assay would have the advantage of being rapid, specific and non-invasive. But the use of antibodies also introduces the risk of false positives due to cross reaction with proteins similar to the target protein. It remains to be seen how the sensitivity of this method can be increased with improvements in the design of the biosensor.

2. Antibody testing:

Antibodies are the body's defense players that work towards elimination of infection. Antibody testing is also known as serology testing and the tests look for the presence of specific antibodies generated in a person's body in response to COVID-19 infection. The presence of antibody indicates that the person has contracted COVID-19 or may have recovered from the disease. The human body can produce five isotypes of antibody in general (IgG, IgA, IgE, IgM, and IgD) and the stimulus decides which isotype will be produced. Various studies have indicated that upon contracting the COVID-19 disease, an individual may produce IgM, IgA and IgG antibodies. Serology tests generally are qualitative tests and they detect the presence of anti-SARS-CoV-2 specific antibodies in the sample (either single or all isotypes).

Antibody or serology testing is helpful for epidemiological study purposes. The cost of testing is lower than generally used molecular biology-based tests. Maximum variants of the test do not require sophisticated instruments or skilled labor. The results are obtained as visual end product which is easy to read. But these tests cannot be used for the diagnosis or detection of COVID-19 disease in individuals. The results for these tests can be obtained rapidly, making it easier to test large populations at the same time. It cannot detect infection in the early stages because antibody production has been shown to begin typically after a few days post appearance of symptoms and even a few weeks in some cases. After the onset of the disease, some individuals might have low antibody titer, increasing the chances of false negative results. Thus, the absence of antibodies does not indicate absence of COVID-19 infection. The antibody titer increases rapidly after 7 days PSO (past symptom onset) and reaches a maximum between 22-35 days PSO in case of IgM and between 17-35 days in case of IgG [29]. Hence, during the 1st week of infection chances of not detecting the infection (false negatives) are high. In addition, antibodies can cross react and detect similar antigens. Thus, antibodies produced against viral proteins that are similar to SARS-CoV-2 proteins can show cross reactivity with SARS-CoV-2 antigens. These can result in false positive results. Serology testing can only be used to study the disease surveillance and epidemiological research, etc.[30]. These tests are qualitative and do not indicate the severity of disease.

Certain quantitative antibody tests have also been introduced in the market that reveal the antibody titers (of anti-S protein antibodies) in a COVID-19 recovered or vaccinated individual. Example: COVIPROTECT by Metropolis India. Though the result interpretation involves seeing if the titer in a person's blood is higher or lower than the arbitrary cut-off and no additional information is provided.

Enzyme Linked Immuno Sorbent Assay (ELISA) based serology test:

ELISA based assays are available to detect the presence of anti-SARS-CoV-2 antibodies from the serum sample. Commercially available antigens (SARS-CoV-2 proteins that elicit immune response in host) like the nucleo capsid protein (N), receptor binding domain (RBD) and Spike protein (S) are used as bait proteins due to their high antigenicity. The bait protein is immobilized on the surface of the ELISA plate. If the serum sample contains anti-SARS-CoV-2 IgG antibodies, these antibodies will interact with the immobilized antigen. This is followed by sandwiching these bound antibodies with anti-human IgG tracer antibody conjugated to HRP (horse radish peroxidase) enzyme. Thus, an immunocomplex of antigen, anti-SARS-CoV-2 IgG antibody and tracer antibody is formed [31]. In case of a COVID-19 positive individual's sample; a colored or luminescent or fluorescent end-product would be obtained depending on the substrate used. When luminescent or fluorescent substrate is used, the assay is called **CLIA** (chemiluminescence immunoassay) and **ELFA** (Enzyme linked fluorescent assay) respectively. Chromogenic HRP substrate or colloidal gold-based assay detection gives colored end product.

In IgM antibody detection ELISA kits, anti-human IgM tracer antibodies are bound to the wells of the plate. If the serum contains anti-SARS-CoV-2 IgM antibodies, they will bind to the tracer antibodies. To this, HRP tagged SARS-CoV-2 antigen is added. Thus, an immunocomplex of tracer antibody, target human IgM antibody and HRP labeled antigen would be formed. This complex can be detected using either chemi-luminescent or colorimetric reaction after adding the substrate [32]. **MCLIA** (Magnetic chemi-luminescence immuno assay), **MESIA** (Magnetic force-assisted electrochemical immuno assay) and **ECLIA** (Electrochemical chemi-luminiscence immuno assay) have also been developed for anti-SARS-CoV-2 antibody detection. CLIA, ELFA, MCLIA, ECLIA and MESIA are all immuno assays detecting the presence of anti-SARS-CoV-2

by using ELISA principle but differ in detection methods. Kits are available for detection of IgG or IgM, as well as both. Different kits utilize different samples such as serum, whole blood or a finger prick sample.

Lateral flow immunoassay:

Immuno-chromatography is a type of lateral flow immunoassay. The test utilizes the separation achieved in chromatographic techniques and the specific interaction of antigen- antibody to detect the presence of antibody of interest in the sample. Whole blood or serum is taken and the components of the sample are separated through the medium using capillary force. The kit has tests in the form of cassettes (strips) where each cassette is a dry medium which has a control and test line. The control line is coated with anti-mouse antibody and the test line is coated with coronavirus 'N' protein. The strip has a release pad section which has colloidal gold labeled anti-human IgG and anti-human IgM antibodies. When sample is introduced, anti-SARS-CoV-2 antibodies (human IgG and human IgM), if present, interact with the colloidal gold labeled tracer antibodies. These immunocomplexes traverse through the strip and first come in contact with the test line. The IgG and IgM antibodies bind to the viral protein and give a colored end result. When the sample travels further up the strip, the free tracer antibodies interact with the anti-mouse antibody giving a colored reaction. The control line indicates that the kit is working properly. The reaction for each antibody is on separate strips [33]. These tests give results in about 8-10 minutes. *Voxpress COVID-19 RDT Kit, Makesure COVID - 19 Rapid test, ACCUCARE IgM/IgG Lateral Flow Assay kits* are currently in use.

Microarray based antibody detection assays:

Multiple proteins from the SARS-CoV-2 virus can act as an antigen and trigger an immune response in the host. Antibody tests based on ELISA and LFA utilize a single antigen to bait the target antibodies. In microarray-based assays, a panel of antigens from the virus is used as probes. The antigens are printed on functionalized glass and they can bind to anti-SARS-CoV-2 antibodies produced in the host. This allows for high throughput screening of samples for different anti-SARS-CoV-2 antibodies in a single attempt [34]. Since the assays are miniaturized, they are resource efficient. Generally, microarray-based assays are automated and this reduces the risk of manual error. But the requirement of sophisticated instrument restricts the usage of these microarray-based assays. *MosaiQ*® COVID-19 Antibody Magazine, *Maverick™ SARS-CoV-2 Multi-Antigen Serology Panel v201030ART-01* are currently developed for COVID-19 detection.

Neutralization assays:

Antibody efficacy can be determined using virus neutralization assays. Neutralizing antibodies block viral infection and assays to check for neutralizing antibodies are necessary for true epidemiological studies as well as for vaccine evaluation, etc. In these assays, virus particles are neutralized by antibodies that may be present in the serum of an infected individual. If neutralized, these viruses will fail to multiply and cause infection in cell lines (ex: Vero E6 cells). Active SARS-CoV-2 particles will be present in absence of antibodies and these active viruses will increase in number. Plaque reduction neutralization test is generally used for testing the serological neutralization of viruses. Muruato *et al,* 2020 [35] cloned a reporter gene, mNeonGreen into the viral genome such that when viral proteins are synthesized, the reporter mNeonGreen would also be synthesized. The viral load in infected cell lines would correspond to the fluorescence intensity of mNeonGreen in the cells. The use of viral particles requires the use of BSL3 (Bio Safety Level 3) facility which restricts the use of this technique in a general diagnostic set up.

Western blotting:

Western blotting is a technique that is used to detect the presence of protein of interest from a mixture using specific antibodies that bind to it. The use of western blotting to detect anti-SARS-CoV-2 antibodies has been suggested as a confirmatory test after obtaining ELISA tests. In this, the viral proteins separated by electrophoresis are blotted onto a suitable substrate (a membrane) and would be probed by patient's serum and a positive result would be obtained as a colored / chemiluminescent end product [36]. No commercial kits utilizing this method are present in the market.

3. Antigen testing:

Antigen is a molecule that is foreign to a host and triggers an immune response in the host. In case of viral particles, it can be one of the protein components like the spike/nucleocapsid/envelope protein, etc. The presence of these antigens in the sample taken from the respiratory tract of a person can be used as a rapid detection test of COVID-19. Samples showing presence of the antigen indicate the status of an ongoing viral infection. Antigen detection-based tests are relatively easy to use and do not require skilled labor as well as advanced equipment. These tests are rapid and the results are typically obtained within an hour of sampling. Viral antigens like the S and N protein have been produced in large scales using recombinant technology and these proteins have been used to generate

monoclonal antibodies (Monoclonal antibodies are all identical and can only recognize a single target that is called an epitope). ELISA tests utilizing these monoclonal antibodies, can specifically detect the viral antigens from patient samples with high sensitivity. Antigen detection by ELISA utilizes multiwell plates coated with anti–N protein antibodies as capture antibodies. If the sample contains virus, the N protein will bind to the immobilized antibodies and they will be immobilized as well. To this, HRP-conjugated anti-N protein antibodies are added which lead to the formation of antibody-antigen-antibody sandwich. Upon addition of substrate, colored product will be obtained that can be monitored spectrophotometrically [37]. *Care Start™ COVID-19 Antigen* is one such kit developed on this methodology.

Similar to serology tests, lateral flow assays for antigen testing is also available. In these tests, capture antibody coated strips are used. Nasal swab samples are taken and the sample is suspended in extraction buffer. Due to capillary force, sample in extraction buffer will move upwards and come into contact with the immobilized antibodies. If the target antigen is present, it will bind to the antibody and a colored end product will be obtained indicating a positive result for the presence of SARS-CoV-2. The results of these LFA antigen tests are time sensitive and need to be read in the time frame specified [38, 39]. Currently, *STANDARD Q COVID-19 Ag, BIOCARD Pro COVID-19 Rapid Ag test* kits have been developed for detecting the COVID-19 antigens.

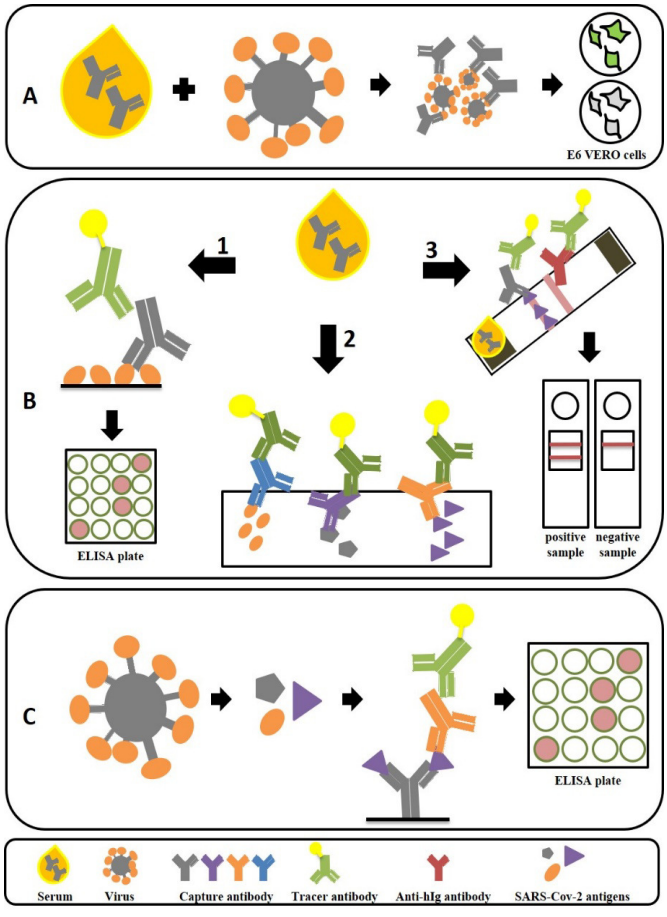

Figure 5: Schematic representation of all the protein-based detection methods

Mahari *et al*, 2020 [40] have developed an ultra sensitive and rapid portable COVID-19 detection test that can detect the SARS-CoV-2 spike protein in saliva samples. It is a biosensor-based assay where anti-spike protein antibodies are the biological receptors to which the viral protein binds leading to generation of electrical signal. The anti-spike protein antibodies are immobilized on screen printed carbon electrode (SPCE). The resulting electrical signal is amplified, processed and converted from analogue to digital. The reading is then displayed on the device screen and corresponds to the titer of virus in sample. The test has successfully detected saliva spiked with small amounts of viral protein and has to be validated with actual patient sample. The biosensor can detect the target protein in within 30 seconds.

A. Neutralization assay: A fluorescent reporter gene is cloned in the viral genome such that expression of viral genes would result in the synthesis of fluorescent protein in the infected mammalian cells (E6 VERO cells). Viral particles get neutralized by anti-SARS-CoV-2 antibodies from the serum. The neutralized viruses cannot infect E6 VERO cells and there will be no fluorescence in the cells. If the virus is not neutralized, the cells express viral proteins and hence fluorescence will be observed.

B. Antibodies from patient sample can be detected using following methods:

1. **ELISA**: SARS-CoV-2 antigenic proteins are coated in the well of an ELISA plate. Serum sample is added to this and if antibodies are present, they will specifically bind to the immobilized protein. Enzyme conjugated tracer antibody binds to the capture antibody and gives a colored product after reacting with substrate. Negative reactions are indicated by absence of color.

2. **Microarray based methods**: A microarray of antigens is synthesized by immobilizing multiple antigens from SARS-CoV-2 virus to the glass surface. To this, serum is added and antibodies from the serum bind to their respective antigens on the microarray. Tracer antibody binds to the capture antibody and an immuno-complex is formed which can be detected by multiple methods giving a qualitative result.

3. **Lateral Flow Assay**: In this assay, antigens are immobilized on test line of the nitrocellulose membrane and anti-human IgG/IgM antibodies are on control line immobilized. Sample moves up the membrane because of capillary action and if serum has anti-SARS-CoV-2 antibodies, they will bind to antigens on the test line. Upon reaching the control line, capture antibody will bind to tracer antibody and show a positive reaction. Thus, if anti-SARS-CoV-2 antibodies are present, both (test and control) lines would show up and if the antibodies are absent, only control line will show color.

C. **Rapid antigen test using ELISA technique can detect viral antigens.** Anti-SARS-CoV-2 antibodies are coated into wells of an ELISA plate. Sample is added and if antigens are present, they will bind to the antibodies. Another capture antibody is added which binds to other epitopes of antigen. Upon addition of tracer antibody and substrate, colored reaction will be seen in positive samples and no color will develop in case of negative ones.

4. Interleukin-6 detection test (to assess the patient condition):

In addition to the Covid-19 diagnosis, this ELISA based test allows for the assessment of the disease severity in the patient. Interleukin-6 is a cytokine (molecules that modulate immune response in a host) and it has been shown to be a serological marker of SARS-CoV-2. The IL-6 cytokine level was much elevated in lethal cases of COVID-19 than in survivors. IL-6 is one of the many proinflammatory cytokines and its high serum concentration can contribute to the cytokine storm in a patient. It can be associated with the appearance of symptoms such as pulmonary edema, lung failure, etc. Hence, IL-6 levels can be determined as an additive test to assess patient condition. Sandwich ELISA is utilized in these assays. Anti-IL-6 antibodies are coated onto the plate. IL-6 from the sample will bind to these antibodies. To this, alkaline phosphatase conjugated anti-IL-6 antibodies are added. Hence, an immunocomplex of antibody-antigen-antibody would be formed. Upon addition of chemiluminescent substrate, light is generated and IL-6 level quantification can be done by measuring the light intensity using a luminometer [41]. IL-6 levels can be determined to assess the severity of COVID-19 disease symptoms and to assist the clinicians in deciding the course of therapeutic intervention [42]. However, COVID-19 detection has to be confirmed by other molecular tests.

Conclusion:

Due to the COVID-19 pandemic, people across the globe experienced health, social, economic disturbances. Continuous efforts have been made to cope with all the damages. The main aspect of these efforts is containing the infection and management of infected persons. Clinical / Pathological, biochemical and molecular diagnostic methods play an important role in this process. Effective diagnostic methods have been developed and deployed while novel methods continue to be developed. Experience gained in COVID-19 pandemic in terms of diagnostics, patient management, vaccine development and massive vaccination process will help global communities in better management of pathogenic disease outbreaks in future.

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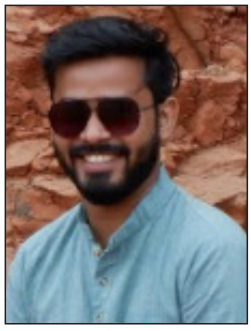	*Dhruv Das is a Scientific Officer(C) at Bhabha Atomic Research Centre (BARC, Mumbai) with threeyears of experience in Molecular Diagnostics. He holds a Bachelor of Science degree in Biochemistry from the University of Delhi. Subsequently he moved to All India Institute of Medical Sciences (AIIMS), New Delhi to pursue his post-graduation degree in Biochemistry and completed the same in 2017. After completion of Masters, he immediately joined BARC Training School and became part of 61st Batch of Bioscience group. On completion of training period, he was recruited to Applied Biology Section, Molecular Biology Division, BARC and started his career in molecular diagnostics. Currently, he is enrolled in PhD program conducted by Homi Bhabha National Institute (HBNI). His research interest includes development of cancer biomarkers for early disease diagnosis and development of inexpensive methods for molecular diagnostic testing.*
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Soft nanotechnolgoy in mitigating Covid-19 pandemic

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Abstract

Covid-19 is a challenging human infectious disease, that requires urgent intervention for its mitigation. Close examination of the structure of these viruses indicate its strong analogy to other self assembled structures of lipids, proteins and RNA. The fundamental understanding of such self assembled systems and its interaction with cells or artificial bilayer membranes can be extended to the design of various drug delivery systems for therapeutic intervention. There have been efforts to develop various nano-engineered delivery systems that could specifically cater to mitigate the Covid-19 pandemic situation. This article highlights some of the recent developments in this direction and strategies for developing effective therapeutic platforms against this pandemic situation

Introduction

The outbreak of viral infectious disease (caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARSCOV-2)) worldwide which originated from China in the late 2019 was officially termed as pandemic by World Health Organization (WHO) on 11th March, 2020 [1, 2]. This disease has been termed as black swan event and almost correlated to the economic downfall like in WWII as it has its pernicious effect on the overall healthcare system globally along with restlessness in every aspect of human life known to us [3, 4]. Corona viruses (CoVs) is a member from Coronaviridae family having positive stranded enveloped RNA with size ranging from 27-32 kb. Virions are spherical (120-160 nm) in nature (crown like peplomer spikes) with a large RNA which contains papain like protease (PLpro), main protease (Mpro), RNA-dependent RNA polymerase (RdRp),RNA helicase encoded by the replicase and other structural proteins (spike – S protein) [5]. This S protein is the main inducer of virus neutralizing antibodies. The The SARS-CoV is mainly constituted by four different proteins (a) S protein (Spike glycoprotein) which helps in attaching the virus to host cell thus promoting the entry to host body; (ii) Membrane protein which conserves the integrity of the membrane of the viral particle; (iii) Envelope protein, the smallest one which takes part in assembly and budding and (iv) Nucleocapsid protein which binds with the SARS-CoV RNA and backs the formation of nucleocapsid [6-1019-23]. The key receptor for the entry of SARS-CoV-2 in host cell is angiotensin-converting enzyme 2 (ACE2). Figure 1 shows a schematic illustraiton of the SARS-Cov-2 indicating RNA core, different structural proteins and its association with cell membrane receptor.

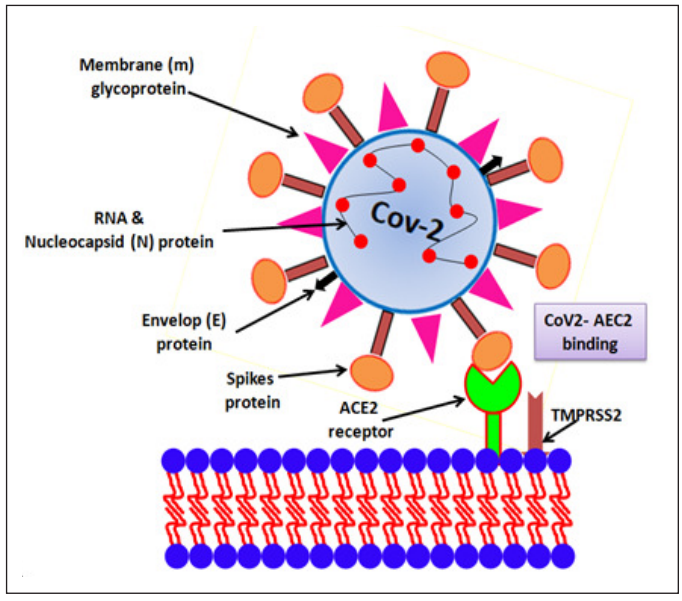

Fig 1: Schematic illustration of the SARS-Cov-2 binding with cell surface

Corona virus can adapt to new changes in environment through mutation and recombination thereby enabling to alter host range and tissue tropism effectively [11, 12]. There are four genres in ortho corona virinae subfamily namely; α, β, δ, γ coronavirus. Out of which α, β version cause the disease in humans and other mammals. In domestic as well as in livestock mammals, α, β- coronavirus are potential pathogenic in nature [13-16]. Among all viruses belonging to βcoronavirus lineage i.e. SARS-CoV, SARS-CoV-2 and MERS-CoV cause the most affective respiratory syndromes in human (The disease caused by SARS-CoV-2 known as COVID-19) [17]. Certain other strains (HCovKU1, HCoV-NL63, HCoV-OC43, HCoV-

229E) are there which cause mild symptoms in adult person. But these may be fatal for children and aged personnel [18]. The β coronaviruslineage A (HCoV-OC43 and HCovKU1) strain seem to originate from rodents whereas the α is probably coming from bats [19, 20]. Some reports have shown bats as the significant reservoir of the COVID-19 virus although the full epidemiology is not yet understood [21, 22]. There is almost 80% similarity between the genome of SARS-CoV-2 and SARS-CoV and a striking 96% with the Bat CoV RaTG13 [23].

Transmission of SARS Cov-2

The mode of transmission from human to human of this respiratory virus is either via air or via contact. The contact may happen from directly with infected individual or contaminated surface [24]. When an infected individual exhales, coughs, speaks or sneezes the virus bearing mucus is expelled in the form of small aerosols or large droplets. These expelled droplets can be directly inhaled by another person or it may deposit on surface or environmental objects (fomites). Later anyone touching the fomites followed by the mucus membrane of eye, nose, mouth will be infected. The droplets ejected from sneezing, coughing contains mature virus particles dispersed in lipid, NaCl or other biopolymers [25, 26].

This SARS-CoV-2 primarily upsets the respiratory system followed by systematic spreading into heart, liver, and kidneys [27]. It is still to be understood whether the virus infection cause direct organ damage or tissue injury. The AEC2 is heavily expressed in respiratory tract and other tissues present in cardiovascular system (CVS), central nervous system (CNS), GIT, female reproduction system. Lung and bronchial branches are also having the AEC2 expression thus acting as the primary sites for the SARS-CoV-2 binding [28, 29]. The higher binding of the SARS-CoV-2 with the AEC2 down regulate the signalling pathways. The virus can infect the CNS in mainly four different pathways. Firstly,via direct infection it crosses the blood brain barrier [30]. Secondly, it causes hypoxia injury due to infection in lung tissue, pulmonary edema along with other disorders [31]. Thirdly, it can attack the immune system by infecting the macrophages, microglia and astrocytes followed by brain damage [32]. Lastly, the interaction of SARS-CoV-2 with ACE2 present in the capillary endothelium affects the BBB [33].

Role of soft matter science in Covid management

The Covid-19 management requires urgent intervention by scientists in all fields of research. In particular, the expertise gained in the interdisciplinary soft matter science goes a long way in minimising the impact of virus transmission and therapy. The first and foremost in containing the spread of this virus is proper sanitisation of the surfaces. Different chemical agents have been recommended for sanitisation purposes, the most common one being alcohols and surfactants.

The interaction of surfactants with lipid bilayer systems such as vesicles have been studied in detail with the perspective of virus inactivation [34]. The ability of surfactant molecule in destabilising the structure of virus lipid membrane has been demonstrated [35]. Isothermal titration calorimetric studies on interaction of different types of surfactants such as potassium oleate, sodium laureth sulfate and and sodium lauryl sulfate with human (H3N2) and avian (H5N3) influenza viruses has been reported [36].

As of now there is no effective therapy available in the market against COVID-19, though vaccines have been marketed by a few companies worldwide. In general, during the course of vaccination a non-disease causing pathogen is injected into the human body followed by generation of specific antibody for encountering the disease causing antigen. Nevertheless, there are several types of vaccine platform based on the way how the weak antigen is presented to initiate our immune response. In this regard, mRNA vaccines are relatively new in the market in which mRNA vaccines are carrying the mRNA with the genetic code for preparing the antigen inside our body. As these materials are very much vulnerable to degradation at elevated temperatures, it has to be stored at very low temperature facility. Sometimes lipid based nanoparticles are used to increase the stability of the same. It has already shown great potential against the Zika virus. The front runners in COVID vaccine development programme, that is Pfizer/BioNTech vaccine (BNT162b2) and Moderna vaccine (mRNA-1273) candidate are using this mRNA platform. Usage of this platform is relatively safer as it does not introduce pathogen directly to the body and the manufacturing is also very rapid making it cost effective and scalable in this pandemic situation. The second type of platform is non-replicating viral vector which carry the genetic instructions of the disease causing pathogen with slight genetic modification of the same so that it cannot replicate inside the body. This vaccine does not require stringent temperature requirements for storage making them cost effective easily transportable candidate. AstraZeneca/Oxford (AZD1222) and Janssen (Ad26COVS1) are the ones following this platform. Protein subunit vaccines are the one which introduce the antigen protein directly to body for in-vivo generation of

the antibody. The influenza vaccine namely FluBlok is an example of this category. Currently, almost 70 COVID-19 vaccine candidates are in trial phase following this form. The fourth category is inactivated virus vaccines which are being grown through cell culture followed by introduction to human body. More than 25 vaccine candidates are in trial run for COVID vaccine development. As it requires high level of bio safety for handling the live virus, the production capacity here is limited.

Several chemical agents have been tried and tested for the probable treatment of COVID-19. Some of the drugs became popular also, likes of chloroquine or hydroxychloroquine [37]. Few other antibiotics as well as antiviral items were tested but without any significant outcome in the endeavour of vaccine discovery. Here in Table 1 we are listing some of the chemical agents tested against SARS-CoV, MERS-CoV and SARS-CoV-2 [38]. Unfortunately, very low bioavailability and uncalled for toxicity let them discarded candidates for the therapy. There are also very less reports regarding in-vivo as well as clinical reports of those candidates.

Repurposing the existing antiviral drugs together with novel nanocarriers for delivering biologicals like RNA, proteins etc have been the subject of great interest among reserachers. Targeted carriers with appropriate surface functionality can ensure the delivery of therapeutic agents to the site of interest. There have been efforts to develop products based on lipid-DNA nanoparticles, mRNA aerosol nanoparticles, exosome based delivery systems etc for theraputic applications [50-52].

Due to inefficacy of the bare chemical agents with respect to lesser target to non-target ratio nanostructured drug delivery systems (NDDS) have come into the picture for COVID vaccine generation. These are basically pharmaceutical formulations with specific properties due to their high surface to volume ration. They are skilled in incorporating, encapsulating and delivering the drug in particular area of interest. The major goals of the NDDS are prolong release profile along with lower dose frequency with minimum side effect. The current pandemic has demanded a critical analysis of all the available nanotechnology tools. Nanocarrier-based therapeutics actually come up with lot of opportunities to address the limitations of current antiviral therapy. Some rudimentary challenges such as poor aqueous solubility and low bioavailability can be resolved by nanocarrier-based antiviral drugs delivery. Certain active targeted nanocarriers also provides the prospect to cross biological barriers and attain therapeutic concentrations in sheltered viral reservoirs.Soft-based NDDS composed of lipids, as

Drug	Target	Assays	Usual treatment	Ref.
Arbidol	Binds to hemagglutinin	In vitro	Influenza, arboviruses	39
Atazanavir	Inhibition of the $3CL^{PRO}$	In silico, in vitro	HIV	40
Chloroquine	Immunomodulatory effects, increase endosomal pH required for virus	In vitro, clinical trial	Malaria	41,42
Darunavir	Protease inhibitor	In silico	HIV	43
Emodin	Blocked the interaction between the spike and ACE2	In silico, in vitro	Cancer	44
Hydroxychloroquine	Immunomodulatory effects, increase endosomal pH required for virus	In vitro, clinical trial	Malaria	41,45
Remdesivir	Inhibition the RdRp/Seems to inhibit of the 3CLPRO	In silico, in vitro, in vivo	HIV/Ebola	46
Toremifene	Destabilizing the virus membrane glycoprotein	In silico, in vitro	Cancer	47
Dolutegravir	Protease inhibitor	In silico	HIV	48
Favipiravir	RNA-dependent RNA polymerase (RdRp) inhibitor	In vitro, clinical trial	Influenza	49

liposomes, nanostructured lipid carriers (NLC), solid lipid nanoparticles (SLN), nanoemulsions (NE) and polymer nanoparticles of cyclodextrins (CD), dendrimers are NDDS with the highest antiviral activity reported so far. Figure 2 shows pictorial representation of a few commonly used delivery systems for potential application in covid therapeutics.

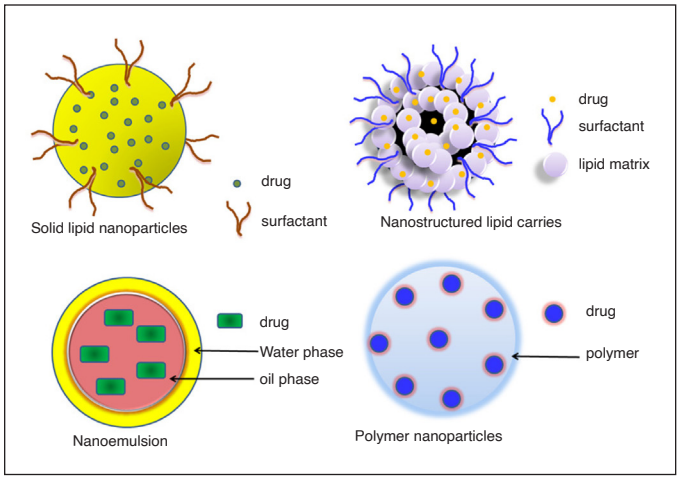

Fig. 2: Few delivery systems for potential application in covid therapeutics.

The compatibility of drug molecules can be further improved using particular type of nanocarriers with same physicochemical properties. For ionisable amphiphilic drugs lipid based carriers are the choice of the researchers [53, 54]. A modification with cholesterol have made the hydroxychloroquine loaded liposomes more efficient in reducing pulmonary fibrosis in a dose dependent manner [55]. Nanoparticles which are prepared using self-assembly of amphiphilic drug–drug conjugate have shown prolonged systemic retention and increased cellular uptake.

Combinatorial drug therapy is another prospect for treatment of COVID-19, offering numerous benefits such as lower dosages of the individual drugs causing fewer side effects, attaining manifold and complimenting therapeutic targets. It also reduces the chances of resistance development. Various such combinations for novel coronavirus treatment are documented in the WHO landscape information [56]. There are several strategies to overcome the traditional problems by co-encapsulation of both hydrophobic and hydrophilic drugs, co-delivery of RNAi/plasmidDNA + chemotherapeutics, codelivery of siRNA + micoRNA [57].One very important strategy which can be used to target the SARS-COV-2 that has migrated to the CNS is Multidrug-loaded pegylated-magneto-liposomal nanoformulations(antiretrovirals, latency reactivating agents, and drug abuse antagonist) that can easily cross the BBB with significant anti-HIV activity in primary CNS cells [58]. Loading of antigens on the surface of the nanocarriers depends on several factor like surface charge and the noncovalent interaction prevailing between them. Antigen immobilization on the surface of chitosan, dextran, Au nanoparticles are of main use depending on the nature of antigen [59, 60]. The mRNA based COVID-19 vaccine employing lipid nano particles (LNP) as carrier is already under clinical trial. LNPs are having size in the range of (80–200 nm) particles prepared by the self-assembly of an ionisable cationic lipid and possess the ability to deliver the mRNA to the site of action. Several other formulations made up of lipid (such as 1,2-dioleoyloxy-3-trimethylammoniumpropane (DOTAP) or dioleoylphosphatidylethanolamine (DOPE)) are suitably modified to increase the half-life of the vaccine. Figure 3 shows a schematic illustration of the encapsulation of mRNA inside the lipid bilayer capsule compose of PEGYlated lipids, DOTAP and DOPE. Other than LNPs, dendrimers, nanoliposomes with cationic peptides, cationic nanoemulsions, and several other nanoparticles made up of various polymers are the choice for mRNA delivery.

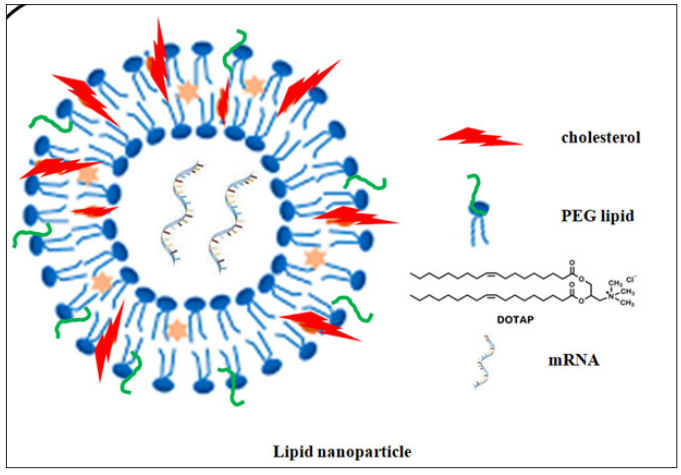

Figure 3: Schematic of lipid based mRNA vaccine delivery system.

Tai et al. have shown the preclinical studies to examine the pharmacokinetics (PKs) of liposomal hydroquinone in Sprague-Dawley rats. The nanocarrier loaded HQ has shown significantly higher lung exposure (~ 30-fold), half life (~ 2.5-fold) in lung in compared to the free HQ. It also has a lower blood and heart exposure in relative to the free HQ which reinstate the usage of nanocarrier for the delivery of COVID vaccines for better results [61]. A polymer based spermine-liposome for the delivery of siRNA to lung was developed and patented. This formulation with size ranging from (100-400 nm) is intranasal and has the ability to release the siRNA to the VERO cells. It has also shown

to inhibit the in-vivo MRS CoV gene expression in mouse model [62]. Several inorganic matrices like grapheme can also be potential treatment module for the COVID-19 therapy which was shown by Palmie et.al. [63]. The sheet like structure is the ultimate advantage for the graphene where the antibody can be attached by functionalization along with the inbuilt antiviral property of graphene. TiO_2 is also used by researchers to immobilize DNA fragment onto it which shows antiviral activities without a transfection agents [64].

Kong et al. demonstrated that virus receptor-functionalized nanodiscs composed of phospholipid bilayers and membrane scaffold proteins containing sialic acid can inactivate the H1N1 virus by selectively targeting the virion's surface proteins [65]. Iron oxide (Fe_3O_4) NPs that can catalyse lipid peroxidation in the viral envelope and destroy the surface proteins have been developed as an effective appropach for inactivating viruses [66].

MERS-CoV spike (S) protein NPs that can protect mice from MERS-CoV infection and stop replication in the lungs have also been demonstrated [67]. The apparent similarity of SARS Cov-2 with other types of viruses and previous knowledge about their immune response is of great help in developing a vaccine within reasonable time.

Conclusion and future prospects

The physicochemical properties of SARS Cov-2 viruses draw close analogy to various self assembled systems comprising lipid, proteins, RNA etc. Fundamental studies in the area of self assembly of lipids, proteins etc play a crucial role in understanding virus transmission and mitigating its effect on human population. Nano-engineered materials such as liposomes, virus like particles, lipid particles, dendrimers etc offer novel therapeutic platforms for the delivery of drugs or design of vaccines. So far no effective therapy aganist covid-19 has been approved. Preclinical animal models are decisive to accelerate the clinical translation, but the discrepancy between the model of disease in animals and human disease is the chief cause for the failure of the study. The researchers should amalgamate the various drug development tools along with the screening technologies, bioinformatics and proper animal models, etc. to inspect and authenticate nanoparticle combination therapeutics. Nanotechnology has to play the crucial pivotal role in defeating this COVID-19 virus to save the mankind from yet another disaster. The more information we can obtain regarding the structural morphology of the SARS-CoV-2 virus, it would be easier for the researchers to come up with appropriate remedies sooner than ever.

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Selenium and COVID-19: How important is this link?

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Abstract

Selenium is an important food component required in trace amount to maintain human health. Extensive biological studies over the years have established that selenium is needed for the synthesis of a special class of proteins called selenoproteins and these perform important physiological functions including immune functions of the body. Epidemiological as well as clinical studies have clearly established that selenium deficiency compromises immune functions and thereby increases the risk of viral infections. This has motivated the researchers to identify whether selenium deficiency is linked with corona virus disease -2019 (COVID-19) outcomes. The studies so far have revealed an inverse correlation between selenium status and the cure/mortality rate of COVID-19 patients. Thus selenium supplementation could be a potential treatment option for COVID-19 patients with low selenium status.

Key words: Micronutrient, selenium deficiency, viral infections, COVID-19

Introduction

Adequate nutrition is one of the most important requirements for the well being and proper health of an individual [1]. Nutritional requirement comprises both macro and micronutrients. Macronutrients are required in large quantities and include mainly proteins, lipids, and carbohydrates. On the other hand, micronutrients are required in trace quantities but are known to perform very important functions of body. Micronutrients comprise mainly vitamins and minerals and mediate their functions by acting as the cofactor and/or prosthetic group of enzymes involved in biosynthetic pathways, metabolism, energy production, signal transduction, and redox modulation [2]. As per world health organization (WHO) guidelines, as many as 21 micronutrients are needed for the growth and maintenance of the normal physiology of various cell types in the body [1]. The deficiency of micronutrients is marked by severe impairment in the physiological functions of the cells leading to growth defects, immune dysfunction, and onset of various diseases [2]. Although micronutrients have always been considered important to maintain the immune functions, the recent outbreak of a pandemic corona virus disease - 2019 (COVID-19) has prompted researchers to realize their role in fighting viral infections. In the last one year, since the outbreak of COVID-19, a lot of clinical studies have been done to correlate the availability of micronutrients in the body with the incidence, severity and mortality of COVID-19. All these studies have revealed that micronutrients like vitamin D, zinc (Zn), and selenium (Se) among others may play a critical role in fight against COVID-19 [3]. With this background, the present manuscript intends to summarize the link between body's requirement of selenium and COVID-19.

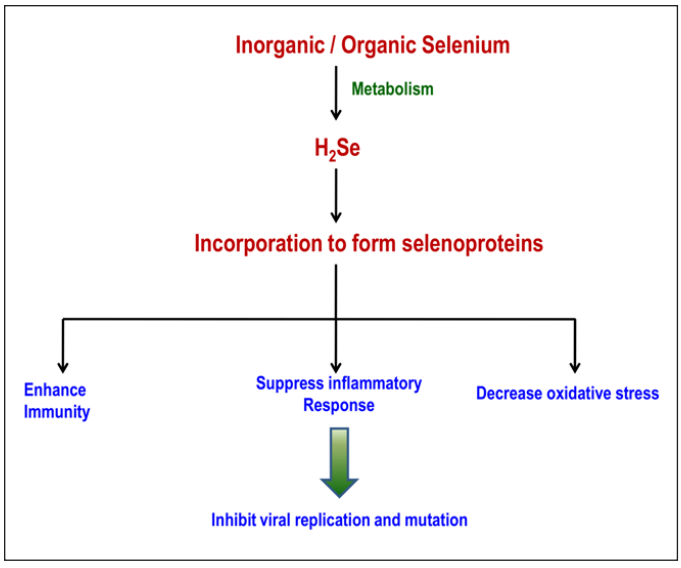
Selenium and immune responses

The nutritional requirement of selenium for human being ranges from 50 to 70 μg a day which is met mainly through consumption of plant and animal products [4,5]. Some of the food sources rich in selenium content are nuts, garlic, mushroom, broccoli, egg, spinach, asparagus, fish and meat among others. Brazil nut is the richest source of selenium [6]. The entry of selenium into the food chain follows the order: environment → soil → plant → animal → human. Soil contains selenium mainly in inorganic forms like selenite and selenate and once it reaches into biological systems (like plants and animals), it is converted into organic forms mainly as selenoamino acids [7]. Selenocysteine (SeCys) and selenomethionine (SeM), the selenium analogue of cysteine and methionine respectively are the two primary selenoamino acids present in biological systems which may further transform to give rise other modified selenoaminoacids like methyl selenocysteine and γ-glutamyl-Se-methyl selenocysteine [7]. Of these, SeM is synthesized in biological system by the substitution of sulfur with selenium into methionine. On the other hand, SeCys is considered as 21st amino acid and is predominantly synthesized in animals from inorganic selenium (H_2Se) through a complex biosynthetic pathway [8]. Subsequently, SeM and SeCys are incorporated into proteins during the translation of mRNA within cells. Importantly, cells do not have any machinery to incorporate SeM into protein and is therefore inserted mistakenly into proteins in place of

methionine. On contrary, cells possess specific tRNA to incorporate SeCys into proteins against UAG nucleotide sequence present in the mRNA [8]. The proteins containing SeCys in their sequence are called selenoproteins and are the mediators of the biological functions of selenium in living organism [7,8]. To date, about 25 different selenoproteins have been identified in humans. These proteins are mostly oxidoreductase in nature and perform wide range of functions like maintenance and storage of selenium, synthesis of selenoproteins, regulation of redox homeostasis, synthesis of thyroid hormones and controlling the growth and development of immune cells among others [9-11]. A few important selenoproteins are selenoprotein P and glutathione peroxidase (GPx) whose expressions or levels are highly responsive to the selenium availability in the body [8]. Accordingly the levels of these proteins are used as an indicator of the nutritional selenium status of a person. The selenium levels in the serum of healthy individuals ranges from 50 to 150 µg/dl [10,11]. Selenium deficiency in human has been attributed to the consumption of food products grown on soil with low selenium content and under such conditions, selenium supplementation up to 200 µg a day is recommended to maintain optimal health [5]. One of the serious concerns of selenium deficiency is the underdevelopment of immunity [5, 10]. The immunity is defined by the host ability to recognize and protect itself from foreign invading pathogens. Depending on the nature of immune responses, it can be classified into adoptive or acquired immunity and innate immunity. The innate immunity is the nonspecific reactions of immune cells against pathogens whereas adoptive or acquired immunity is the specific immune response against pathogens. Both innate and adoptive immunity are governed by cells of hematopoietic origin mainly the white blood cells. Macrophages, neutrophiles, mast cells and natural killer are some of the cell types involved in innate immune response whereas B and T cells provide adoptive immunity. The proliferation or maintenance and maturation of immune cells are highly redox dependant and selenium through its incorporation into antioxidant selenoproteins like GPx and thioredoxin reductase regulate the redox state of these cells and in turn plays a very important role in mounting effective immune responses of the host [12]. Extensive studies using cellular and animal models have established that selenium deficiency affects both innate and adoptive immune responses by reducing the number of lymphocytes, decreasing the phagocytic activity of macrophages and neutrophiles and inhibiting the proliferation of B and T cells and production of antibodies [12].

Selenium and viral infections

Pathogenic viruses have always posed serious health challenges to humans. These are tiny infectious structures of nanometer sizes and are biologically classified between living and nonliving [13, 14]. Structurally, they carry their own genetic material in form of DNA or RNA packaged within a coat comprised of proteins and/or lipids [13, 14]. The human pathogenic viruses have mainly RNA as the genetic material [13, 14]. Importantly viruses do not have machinery to replicate by themselves and is therefore considered as nonliving outside the host. However once they infect the host, they hijack host machinery to replicate their genetic material, synthesize their protein and to assemble new viral particles [13, 14]. Another important characteristic of viruses is that their genetic material is very prone to undergo mutation and this leads to the origin of newer more virulent strains of viruses [13, 14]. Since selenium deficiency compromises the immune response, it is also expected to increase the host susceptibility to viral infections. In last two decades or so several lines of evidences have emerged to justify these assumptions. The animal studies have confirmed that selenium deficiency indeed increases the infection and progression of several pathogenic viruses like Coxsackie, Influenza A, Human immunodeficiency virus-1 (HIV), Hepatitis B3 and Ebola (Scheme 1). On the other hand, selenium supplementation has been shown to reduce the viral load and associated pathologies in these studies [12]. Some of the mechanisms attributed for the antiviral effect of selenium are its purported role in enhancing immunity, inhibiting viral replication and mutation, suppressing inflammatory responses, reducing oxidative stress and decreasing hemorrhage through anti-coagulation effect [12]. In addition to these studies, there are also reports suggesting that selenium deficiency can increase the virulence of Coxsackie, Polio and Influenza viruses by inducing mutations in their genome [12-15]. Under selenium deficiency, host cells experience oxidative stress and this makes the intracellular environment unfavorable for viral existence. Sensing this as an alarm signal, virus increases its replication within cells and this also increases the probability of viral genome undergoing mutations to form virulent and/or drug resistant strains [12-15]. On contrary, adequate selenium status within the cells does not allow oxidative stress to set in and thus slows the viral replication and/or mutation. The most noticeable effect of selenium supplementation has been documented in suppressing the outcome of Ebola and HIV infections in human. The clinical studies have established that selenium supplementation (100 to 300 µg a day depending on the study) in patients with Ebola and HIV infections not



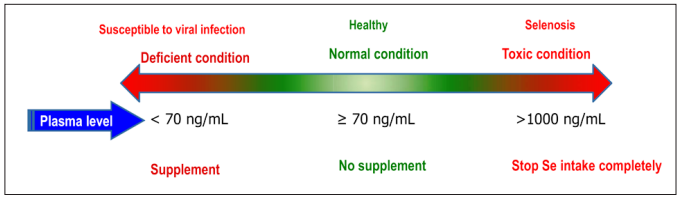
Scheme 1: *Role of selenium in controlling viral infections in host cell*

only improve immune (B and T cells) functions but also substantially reduce associated mortality [16,17].

Selenium and COVID-19

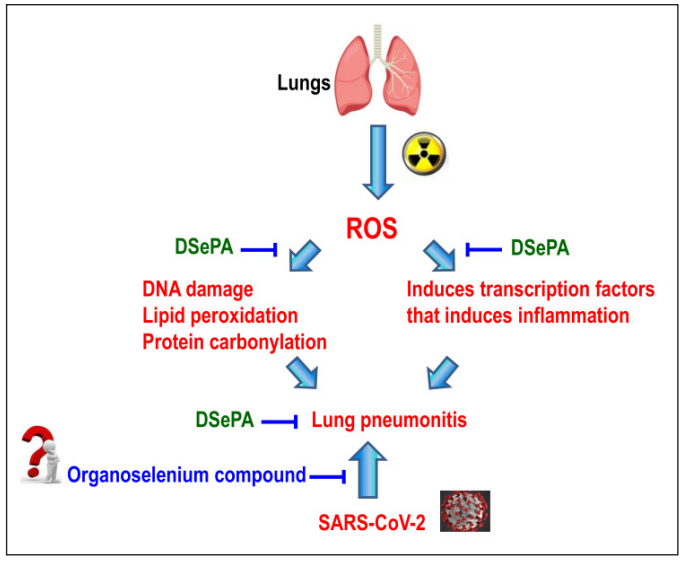
Over the years, mankind has seen several outbreaks of viral infections and the most deadly among these are corona viruses targeting the respiratory system. Especially the variants of corona virus have already caused three major outbreaks in the 21st century [13]. The first one named as Severe Acquired Respiratory Syndrome (SARS) happened in 2003, then Middle East Respiratory Syndrome (MERS) happened in 2012 and the ongoing COVID-19 which was initially reported from Wuhan China, but now has spread to entire world [18]. Within a year of this pandemic, it has already claimed more than 1.21 million deaths and about 47.4 million infections worldwide. The viral strain causing COVID-19 has been named as SARS-CoV-2 and is a new variant of the earlier discovered SARS-CoV-1 with about 80% of genetic similarity [18]. The absence of any specific drug or vaccine against this virus has led to unprecedented health crisis of present time and has forced many developed countries of the world to adopt social distancing as the only solution to stop the spread of this virus affecting their socio-economic conditions [18]. In the last one year, the scientific communities have made tremendous effort to understand the molecular biology of SARS-CoV-2, its role in patho-physiology of COVID-19 and to find the possible treatment strategy to manage this disease [19]. A lot of anti-viral and anti-inflammatory drugs have been evaluated for repurposing against COVID-19 and some of them like Remsdesivir, interferon-γ (IFN-γ) have also got food and drug administration (FDA) approval for the clinical management of COVID-19 [19]. Similarly a lot of new drugs as well vaccine candidates are either being developed or are already in Phase II or III of clinical trials and are expected to be available for general public by mid 2021 [20, 21]. Nevertheless this pandemic has also provided opportunity to clinician and scientist to study the correlation (if any) between the nutritional statuses of micronutrients specially selenium and the incidence, severity and cure rate/mortality of COVID-19. The necessity to perform such studies was realized from the consideration that selenium is vital for immune functions and its deficiency has also been implicated in the poor outcome of viral diseases caused by Ebola and HIV [12,15,16]. The first report associating selenium with COVID-19 appeared in the June 2020 issue of "American Journal of Clinical Nutrition" by a group of Chinese researchers in collaboration with Prof Raymen from UK [22]. This study meticulously performed the correlation analysis between hair selenium content of the population and the mortality/cure rate of patients suffering from COVID 19 in the different provinces of China [22]. China is one of countries known to have high variability in the selenium content of its soil. While some of the provinces including Heilongjiang (northeast region) of China are highly deficient in soil selenium content, the other province like Hubei has very high soil selenium content in one of its cities named Enshi. The results of this study indicated that population from cities known for low selenium intake or selenium content in hair exhibited a significantly higher percentage of mortality due to COVID-19. For example people from cities of Heilongjiang (selenium intake ~16 µg/day, selenium hair content ~ 0.26mg/kg) had significantly higher mortality rate of ~2.4% as compared to that of other provinces (~0.5%). On the other hand, people from the city like Enshi with high selenium intake (~16 µg/day) or hair content (~2.5 mg/kg) exhibited significantly higher cure rate of 36% as compared to 13% of other cities including Wuhan of Hubei province. Another similar study has been reported by a group of researchers from Germany [23]. This was a clinical study wherein researchers measured the level of selenium in the blood samples of COVID-19 patients (n=33) through X-ray fluorescence [23]. The results of this analysis have revealed a very interesting association between the selenium level in serum and the prognosis of COVID-19 patients. In general, COVID-19 patients exhibited a 2.5 times deficit in the selenium content in their blood as compared to the reference value of 84.4 ± 23.4 µg/L for European population. The patients having selenium level in the range of ≥53.3 ± 16.2 µg/L (close to reference value) exhibited better chance of survival as well as lesser days of hospitalization. On contrary, those

with low selenium status ≤40.8 ± 8.1 µg/L showed poor prognosis and had higher probability to succumb from the disease. Importantly, COVID-19 patient who did not recover from the disease also exhibited a consistent decrease in the selenium level in their serum. To further support these observations, a recent clinical study from Korea measured the nutritional status of COVID-19 patients (n=50) [24]. This study indicated that ~76% of the patients were vitamin D deficient and ~42% were selenium deficient. Taken together, above studies suggested an inverse correlation between selenium status in the body and COVID-19 outcome. It is also interesting to note that co-morbid conditions like aging, diabetes, cancer, kidney disease and cardiovascular diseases which have been clinically associated with the higher severity as well as mortality rate of COVID-19 have also been known to reduce the selenium status in the body [19,22,23]. Therefore, it is imperative to think that higher susceptibility of co-morbid patients could be due to selenium deficiency. However such assumptions need to be clinically validated. Similarly, the effect of selenium supplementation on the prognosis of COVID-19 has not been investigated yet and must be taken up in the coming future. The most widely used chemical form of selenium for human supplementation/nutrition is the inorganic form specially sodium selenite [19, 21]. Several clinical trials have been done in past to establish the safety and pharmacokinetics of selenite in humans. Although these studies have indicated that sodium selenite is quite tolerable (up to 600 µg in one dose) to human, it has a very narrow margin between its safety and toxicity (Scheme 2) [4]. The excess intake of selenium may cause severe disorders including respiratory and gastrointestinal abnormalities leading to even mortality [6]. Additionally selenium exposure has also been associated with incidences of cancer and diabetes [6].



Scheme 2: *Selenium concentration in plasma dictates its bioactivity.*

Therefore, selenium supplementation should be carefully monitored both in terms of chemical form used for consumption as well as the selenium status in the body. Selenium supplementation is recommended only in case of deficiency. Similarly, there is also a need to find the safer chemical forms of selenium for human supplementation. In this context, organic forms of selenium are known to be relatively lesser toxic than inorganic forms and hence should be considered for selenium supplementation [25]. Although there are several products available in market like selenium enriched mushroom, food grains, bread and other plant product that provide selenium supplementation in organic forms (mainly as selenoaminoacids), it is high time that synthetic organoseleniun compounds should also be evaluated for their potential as nutritional supplement. Our group at BARC has shown that 3-3′diselenodipropionic acid (DSePA), a synthetic derivative of selenocystine has



Scheme 3: *Possibility of organoselenium compounds in inhibiting lung pneumonitis caused due to COVID-19.*

not only a much lower LD_{50} (median lethal dose) value than most of the selenoamino acids present in plant product but also induces the synthesis of selenoproteins in experimental rodent models [25]. Additionally, this molecule is orally bio-available and has also been demonstrated to possess anti-inflammatory activity and to suppress radiation induced pneumonitis in mice model [25]. Therefore, it is postulated that DSePA has the potential to be developed as selenium supplement for the management of COVID-19 (Scheme 3). Apart from supplement, therapeutic roles of selenium is also being investigated in different chemical forms and one such compound Ebselen has shown excellent promise in the preclinical studies to fight against COVID-19 [26]. It is an organoselenium compound known for various pharmacological activities ranging from antioxidant to anti-inflammatory and anticancer activities [27]. Recently researchers from China have reported that this is also an excellent inhibitor of M^{pro} protein of SARS-CoV2 virus which is involved in maintenance and replication of the virus within the host cells [26]. Since Ebselen is already an FDA approved drug for bipolar disorders, it is being repurposed for COVID-19. Similarly, a lot of research work

has been reported in last one year on the virtual as well as *in vitro* screening of different classes of synthetic selenium compounds for the therapeutic inhibitions of various proteins of SARS-CoV 2 virus [28]. The knowledge gained from these studies would be helpful in exploring some of these for antiviral therapy in the coming future (Scheme 3).

Conclusions

The correlation studies of selenium and COVID-19 have mostly been reported from China and European countries. Importantly these countries are known to have systematic data on the mapping of selenium in their soil as well as in population and therefore it were helpful for the researchers from these countries to investigate the correlation of selenium with the outcome of COVID-19. Having understood that selenium deficiency may adversely affect the prognosis of COVID-19 patients, it is expected that regulatory agencies like WHO should include micronutrient specially selenium analysis mandatory as part of the treatment protocol of COVID-19 patients. Selenium supplementation in co-morbid patients as well as in those with low selenium status may be beneficial in improving the outcome of COVID-19 and therefore clinical trials should be undertaken to scientifically validate this hypothesis. Organic lesser toxic selenium compounds may be looked as the newer chemical forms for selenium supplementation. Finally country like India where selenium mapping is not available, the scientific communities are expected to take up research projects to address whether selenium deficiency is prevent in Indian population or not. Similarly it is the opportunity for synthetic chemist to design new selenium compounds for antiviral therapy as well as to develop nutritional supplement.

Acknowledgement

Authors thank Dr. Awadhesh Kumar, HOD, RPCD and Dr. A. K. Tyagi, Associate Director, Chemistry Group for the support and encouragement during the course of study.

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An Update on COVID-19: Role of Nanotechnology in Vaccine Development

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Abstract

Advancement in vaccine development has always been a crucial point in the health-care system, improvement in life expectancy. Despite that, there is still no robust and effective vaccine available for many diseases (tuberculosis, hepatitis, pneumonia, and the SARS-CoV-2 a threat to human existence). There are several limitations in developing vaccines like the ability to elicit an effective immune response and storage conditions. In the past two decades, nanomedicine played a significant role to overcome these limitations. Nanovaccines are the next generation vaccines, act as an intrinsic adjuvant and trigger an immune response to adaptive and innate immune systems. In the present review, the importance of nanovaccines for the management of COVID-19 is highlighted. Merits of nanovaccines over conventional vaccines are discussed because they could circulate in the bloodstream for a longer duration, better stability, ability to produce an improved immune response and effective for variants of SARS-CoV-2.

Keywords: Nanovaccines; SARS-CoV-2; Coronovirus; mRNA

Introduction

The first human coronavirus was discovered in the 1960s and found to infect the upper part of the respiratory tract. After that, the Severe Acute Respiratory Syndrome (SARS) and the Middle East Respiratory Syndrome (MERS) have infected humans in 2002 and 2012, respectively. The present universal pandemic is caused by the new coronavirus or novel SARS coronavirus; therefore, named as SARS-CoV-2, which has threatened the humanity all over the globe[1,2]. It is a single-stranded RNA (ssRNA) virus with a diameter of 80-120 nm[3, 4] and this nano-sized virus moves inside the body effecting the immune system and other host functions.[5, 6]As on date, no effective and promising medicine with no or permissible toxic effects is developed against infection for the human coronaviruses. However, few vaccines have been developed in 2020 and 2021 to combat the virus infections, although these will be active for one or two years or a booster dose will be required in future. It is also observed that the people who got infection from the virus even after vaccine shot did not seem life threat.

Novel coronavirus has infected 168.5 million people with casualties of 3.5 million globally, whereas 27.5 million people were infected and 0.319 million people died in India till May 28, 2021 as per World Health Organization (WHO) report. Currently, research is being conducted to develop novel and effective vaccines or drugs across the globe. In this context, nanotechnology impart a vital role starting from identification of gene (nanoparticles in respiratory viruses; 15-200 nm), prevention (nano-mask, nanofibers and pores, nano-based antimicrobial technology), diagnosis (nanosensors like quantum dots, carbon nanotubes & metal nanoparticles), treatment (nanomedicine, antiviral functions for nanoparticles, drug delivery), vaccination and research related to COVID-19.[7]

Nanovaccines are a new generation vaccines developed to deliver antigens (by acting as an intrinsic adjuvant).[8] There is evidence of nanocarriers' use to target the adaptive and innate immune systems, i.e., T-cells, B-cells, macrophages, monocytes, and neutrophils.[7] Currently, the vaccines approved by WHO for management of COVID-19 are mainly of four types. They can be based on the whole virus (attenuated), messenger RNA (mRNA), non-replicating viral vector, and protein subunit vaccine (Figure 1 and Table 1).[9,10] Although, the developed vaccines were given to humans, they could produce an immune response and may prevent future infections. COVID-19 vaccines based on messenger RNA (mRNA) was the first RNA-based vaccine being approved for clinical use as in Figure 2.[11]

Table 1: Vaccine Technology Developed so far for COVID-19 management.

Technology	Company (Vaccine Name)	Other Licensed Vaccines	Points	Remarks	Challenges	Ref
Whole Virus/ Whole-Virion Inactivated Vero Cell Vaccine	Bharat Biotech (COVAXIN)*, Sinopharm (BBIBP CorV), Sinovac (CoronaVac)	Hepatitis A, Polio, Seasonal Influenza, Rabies, Japanese Encephalitis	Inactive or weak form of virus used to develop the vaccine.	Well-established and time-tested technology Fast manufacturing and no storage issue	A booster shot may be required	[12, 13]
mRNA Vaccine (3 LNP-mRNAs/ nucleoside modified mRNA, LNP-encapsulated RNA)	Pfizer/BioNTech (BNT162b2)/ COMIRNATY Tozinameran, Moderna (mRNA1273)	No license but studies on Rabies, Zika virus, Influenza are available	mRNA initiating cells to develop proteins to produce - immunity	Faster manufacturing of the vaccines	Inadvertent properties Effective and efficient delivery into the body as RNA is highly unstable The first-time license is given for humans Needed low-temperature storage equipment (-80^{o}C)	[14, 15]
Viral Vector (non-replicating) Vaccine	Gamaleya Research (Sputnik V), Oxford-AstraZeneca (Covishield), Janssen/Johnson & Johnson (Ad26. COV2.S)	Ebola	Benign and engineered form of the virus 'the vector' used to pass on antigen's genetic code which producesantigens to trigger an enhanced immunity	Well-defined technology Produces strong immunity, it includes both B cells and T cells, as vector vaccines mimic the infection elicited by the pathogen, that is, virus	Efficacy may be reduced on prior exposure to vector Manufacturing is relatively slower	[16, 17]
Protein Subunit Vaccine	Novavax (NVX-CoV2373)	Hepatitis B, Pneumococcal disease, Shingles	Prefuse spike protein developed by recombinant nanoparticles to trigger an immune response.	Well-established technology, fast large-scale production	A booster shot may be required	[18, 19]
Other vaccine candidates based on DNA, Viral vector (replicating), Live attenuated virus, virus-like particle in the WHO list are under trials, *will get WHO approval soon						

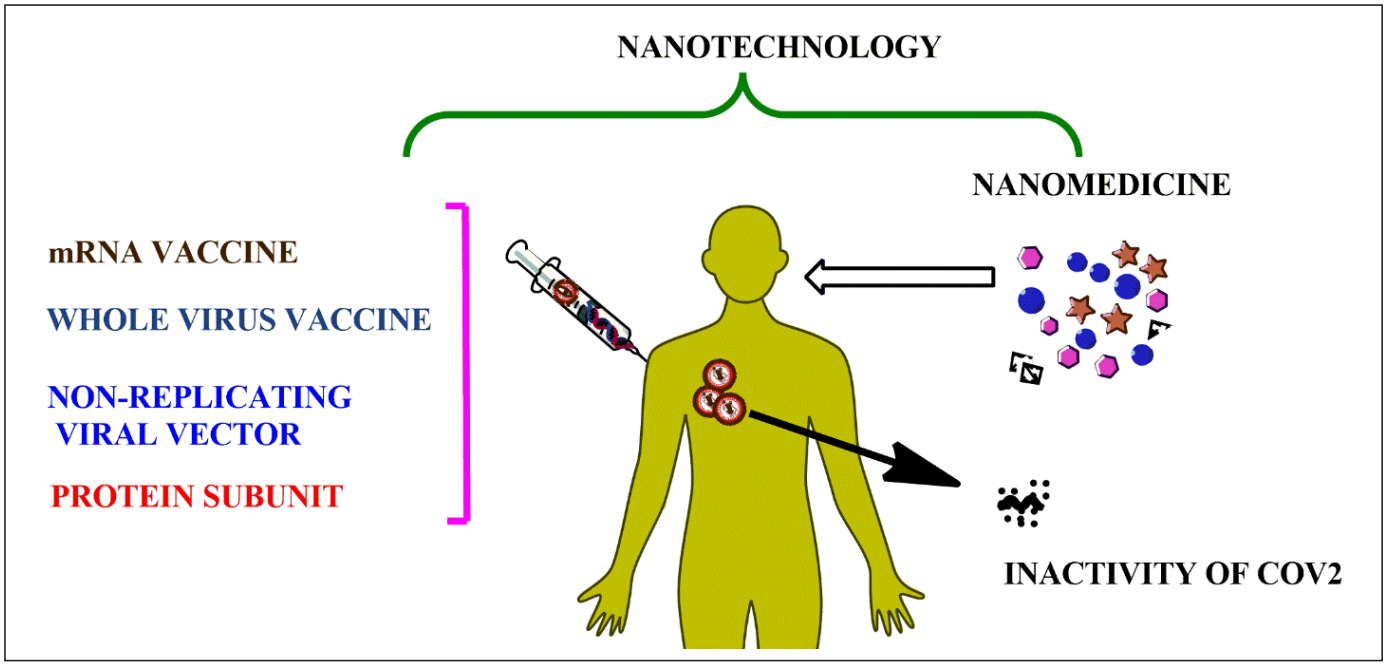

Figure 1: Vaccines and nanomedicine to combat SARS-CoV-2.

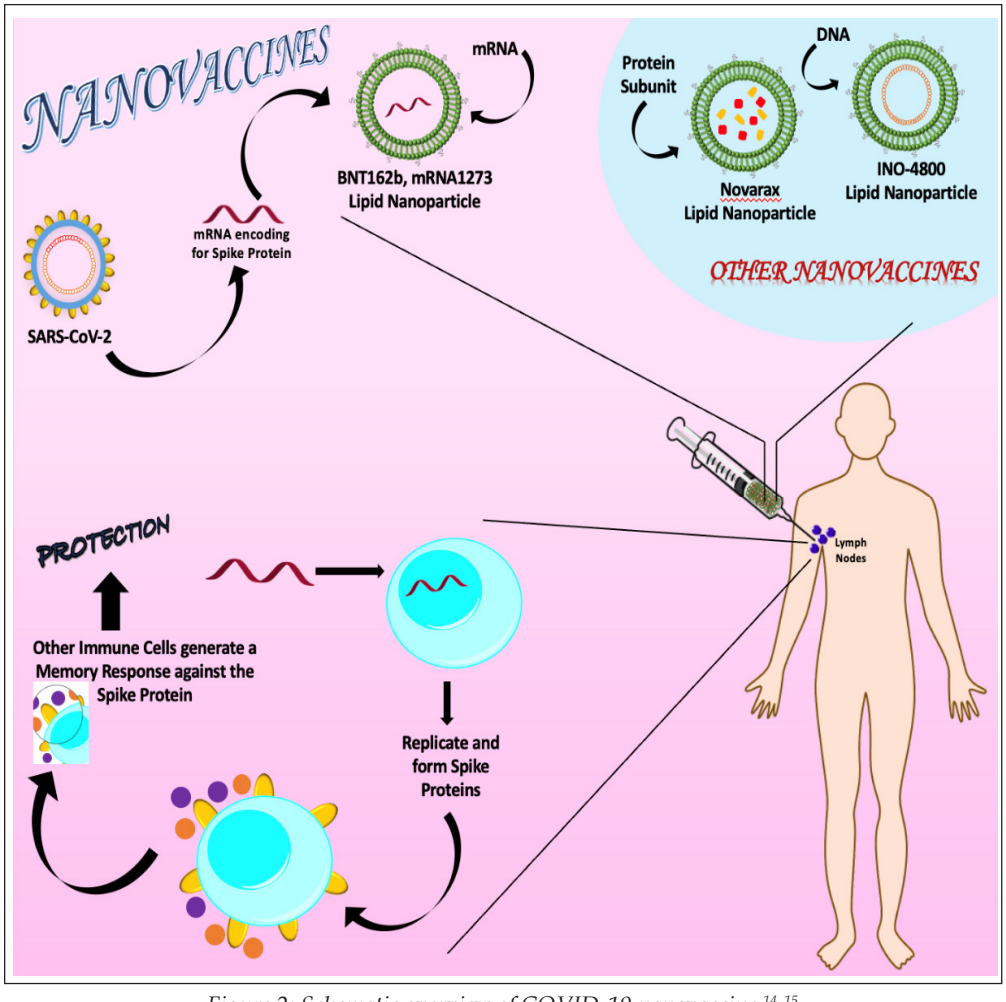

Figure 2: Schematic overview of COVID-19 nanovaccine.[14, 15]

Development of vaccines based on mRNA has gathered a lot of attention of researchers amidst the ongoing pandemic crisis because of the ability to deliver the specific genetic material of the target viral protein using the advent of nanomedicine. It makes them a safer alternative for the conventional attenuated vaccine candidates.[11, 20] mRNA is a potential choice for the nucleic acid-based vaccine because their inability to integrate with the host genome, thus, are non-infectious in nature; have a short half-life, are immunogenic and involves cytosolic processing. Further, the immunogenicity of mRNA can be modulated using molecular engineering strategies.[11, 21] Yet, mRNA-based nanovaccines need further innovation and improvement for effective transport *in vivo* using a nanocarrier such as LNPs

(lipid nanoparticles), Au or Ag, graphene, etc. For getting the approval from US Food and Drug Administration (USFDA), the delivery mechanism of nanovaccines is challenging.[22] Currently, two of the approved vaccines (BNT162b2[23] and mRNA-1273)[24]for COVID-19 management have efficacy of about 95%, are based on LNPs, nanocarriers.[21, 25, 26] LNPs are comprised of positively charged lipids such as cholesterol, polyethylene glycol, phosphatidyl choline, etc. and their rapid mixing techniques for synthesis are studied using cryo-transmission electron microscopy (cryo-EM), can generate liposomes.[25, 27, 28] Therefore, they can be used in therapeutics, drug delivery system, in several clinical trials for anticancer, antibacterial, antifungal, vaccines, etc. Further, nanocarriers could transport both hydrophilic and hydrophobic drugs within the closed interior and the lipid bilayer region, respectively. LNPs based delivery system was the first concept of nanomedicine considered for clinical applications. Doxil (for cancer treatment), Epaxal (a hepatitis vaccine) and several others are approved in therapeutics.[29] Intercalation of mRNA with positively charged lipids makes the mRNA resistant to degradation mediated by RNase. Hence, they attain more stability and can be administered through several routes for self-assembled particles. Vaccines developed by both Pfizer-BioNtech and Moderna can deliver mRNA encoding spike protein variants. However, a significant challenge faced in this case is posing hurdles to potential distribution due to long-term storage at low temperatures, that is, -70 - -80 oC.[11] Figure 2 demonstrates the mechanism of the mRNA nanovaccine to protect SARS-CoV-2. The specific mRNA encoding the sequence for spike protein was elucidated based on sequencing studies on the novel coronovirus. The mRNA was conjugated with the lipid nanoparticle (LNPs) (BNT162b and mRNA1273 vaccine) and were administered intramuscularly. The mRNA encapsulated in the LNPs enters the immune cells of the lymph node. It replicates and form spike protein (shown in yellow color), which further comes in contact with other immune cells (depicted in purple and orange color). This interaction is able to generate the antibodies against the spike protein of SARS-CoV-2, they are producing a memory response and providing protection against the COVID-19 infection.[13, 14]

Another technology for vaccine development is based on the whole virus inactivated vero cell vaccine. The most widely used vaccine based on this technology is COVAXIN for the COVID-19 management. In this vaccine, the genome of the viral particle was modified or attenuated, so that it cannot cause infection.[30-33]The formulation of the BBV152 (COVAXIN) with Toll Like Receptors (TLR) 7/8 agonistin creases the levels of coronavirus specific INF-γ (interferon-γ) CD4 cells by inducing T helper cell 1 (Th1) biased antibody response.[32]

In viral vector (non-replicating) vaccines development, the viruses are genetically modified leading to a defect in its replication. So, the genetically modified virus attains an attenuated state, yet, still be able to produce an enhanced immunity. They have shown to trigger strong cellular immune response, that is, $CD8^+$ cells and also antibody-mediated response.[34] Viral vectors based COVID-19 vaccines are Covishield, Sputnik V and Janssen/Johnson & Johnson vaccine. The viral vector (modified virus) enters the cell and replicate to make its copy using the host cells machinery. The spike protein exist on the modified virus mimics the spike protein available on the surface of the SARS-CoV-2. Consequently, the hosts immune system recognizes, thereby eliciting an immune response and start producing antibodies and hence provide protection against the coronavirus.[35] This vaccine is based on the human adenoviral vector and a major limitation seen from the clinical trials conducted in US, eliciting thrombosis with thrombocytopenia syndrome (TTS), a rare syndrome and is similar to thrombocytopenia induced by heparin.[36]

Protein subunit vaccine is also another widely used strategy employed for vaccine development like Novavax. These vaccines are based on an isolated protein specific to the pathogen as an immunogen, in this case, the spike protein or main protease of the virus, which are bound to a carrier, eliciting an immune response.[37]

Currently developed vaccines for COVID-19 management were tested for prospective candidate against the mutant variants of SARS-CoV-2 such as the B.1.7 or hCoV-19/India/20203522 or 20B/501Y. V1 lineage (the UK variant possessing high transmissibility), the heterologous strain (hCoV27 19/India/2020Q11, and the Brazil variant (B 1.1.28.2) P2lineage. Recent research suggests that few COVID-19 vaccines could be a potential candidate to show good efficacy against the former mentioned variants as the results obtained suggests promising result as in Table 2.[38-41] The data obtained from the clinical trials showed the efficacy of Novavax towards B.1.1.7 and B.1.351 variants. Further clinical trials of Novavax for the South Africa (501Y.V2) variant are found to be less effective.[42, 43] As per reports, Pfizer-BioNTech and Oxford could be updating the vaccines against original corona virus, to target new coronavirus mutants.[41]

Table 2: Vaccine against SARS-CoV-2 Variants.[38-41]

Vaccine	Efficacy towards Variants
Pfizer/BioNTech	B.1.617.2 (Delta) -87.9% B.1.1.7 (Alpha) -89% B.1.351 (Beta) – 75%
Moderna	P.1 (Gamma)
Sputnik V	-
Novavax	B.1.1.7(Alpha) – 85.6% B.1.351 (Beta) – 60% 501Y.V2 – <50%
Johnson & Johnson	B.1.351 (Beta) – 57%
Sinopharm	-
COVAXIN	Promising results B.1.617.2(Delta) trials still cont..
The efficacy towards vaccines cannot be compared due to different clinical trial designs	

Nanoparticles can mimicstructural and functional characteristics of viruses that could be helpful to develop novel medicine.[44-46] At present, nano-based vaccines are approved for clinical utilization.[47-50] During pandemic conditions, the conventional vaccine production approach could not produce such mass production. For this purpose, immune informative, next-generation sequencing, human challenge studies, reverse vaccinology or genetic, nanomedicine and nanovaccine type of novel methods is more useful.[51] Both the viruses and nanoparticles (NPs) occurs at the nanoscale, making nanotechnology a dominant approach for vaccine development.[44] To develop efficient delivery systems, nano experts focus on the mechanism of vectors at the molecular level.[52, 53]

Remarkably, vaccines based on nanomaterials could induce an enhanced protective immune response over conventional vaccines. Au, Ag, Ag_2S, TiO_2, Zr, and graphene have been proposed.[54] To develop vaccine, the spike-proteins of nCoV should be the targeted. Nucleic acid (mRNA) based vaccines have lower production costs and easy purificationvaccines by BioNTech and Moderna's. The delivery of vaccine to target cells is challenging because of stability and specificity. A delivery system enabled with nanomedicine could be effective. Nanocarrier mingled with RNA is a system design that can be effective in autoimmune disease, neurological diseases, malignancies and infections by distributing small interfering RNA (siRNA), combining mRNA-1273 vaccine with R drug substance. Nanocarriers enabled the transport of antigens, prevent premature degradation, and therefore, can reduce the side effects.[55] Conjugated antigens with NPs developed higher antibody and cytokine reactions in contrast to non-conjugated antigens due to vaccine antigen stabilization. For example, Influenza H1N1 antigen coalesced to chitosan NPs and Au NPs coated *Yersinia pestis* F1-antigen and immunogenicity was improved.[56]

Different types of engineered nanomaterials (ENMs) are used selectively for drug delivery due to tunable physicochemical properties having negligible side effects and lower cost. Virus-cell interactions can be tunes by altering the shape, size, and surface of ENMs.[57, 58] Viral antigen-loaded ENMs can be used and include viral genome or immobilized protein, i.e., Au NPs combine with the membrane protein of the West Nile virus and anti-hepatitis C virus (HCV) DNA vaccine. LNPs that include solid-lipid nanoparticles (SLNs), nanoemulsions, and nanosuspension,[59, 60] polymer-based nanoformulations,[61, 62] dendrimers,[63] nanocapsules,[64] nanospheres,[65] peptides based NPs,[66, 67] Carbon nano-formulations include carbon nanotubes, graphene and graphene oxides NPs,[68, 69] Quantum dots (QDs),[70, 71] Inorganic NPs and magnetic NPs[72] are amongst the most typical nanomedicine components and can be used in SARS-CoV-2 management.[1]

Challenges and Future Outlook

Earlier approaches based on nanomedicine have led to underwhelming outcomes because of the complexity and lack of understanding of the continuous interaction between nanomedicine and biological molecules (nano-bio interplay). However, evidence implies the odds for a nanomedicine-based approach in the development of vaccines are more favorable.[73, 74] mRNA could be modulated via standard laboratory techniques and since nanomedicine-based strategies are scalable and versatile, they can rapidly adapt to produce new vaccines.[11] The success of BNT162b2 and mRNA-1273 would help overcome the current health crisis of unprecedented dimensions globally. Further, it insinuate the impact of nanomedicine globally.[26, 75]

Despite the innovation of RNA based vaccines for the COVID-19 management is a big milestone to be achieved. According to the research findings, an estimated 1.1 cases per million of allergic immune reaction after the first dose of Pfizer-BioNtech vaccine are reported.[29] Researchers have shown that these rare allergic reactions because of the PEG-lipid component of the vaccines.[29] PEG is being used in several formulations and was found to trigger anaphylaxis, a life-threatening situation causing rash, breath shortening, increased heartbeat and drop in blood pressure. But it is believed that people who are earlier exposed to PEG, possess large numbers of antibodies against PEG and are therefore at risk for an anaphylactic

reaction to the BNT162b2 vaccine.[76]

Another challenge is associated with RNA-based nanovaccines, viruses with high mutation rates need to be tackle. But successful integration of nano-agent delivery system could be a worthwhile possibility to mitigate the heterogenous COVID-19.[7, 8]

There is a need sound of surveillance system to pick up past disease threats. No nation has enough personal protective equipment and diagnostic tests. Therefore, there is a need to have more security around healthcare staff and more vital disease surveillance and monitoring.

Despite several advantages, LNPs based liposomes have specific limitations such as lack of stability and selectivity, also short bloodstream circulation. To overcome these limitations, ligand modifications at the surface of the liposomes could enhance target selectivity. Further, stimuli-responsive liposomes have been devised to restrain the release of anen capsulated drug. Owing to the complex architecture of LNPs, the physical stability of the nanocarriers needs advancement. In recent time, a stable cubosomes has been developed.[25, 29]

Conclusion

Nanomedicine has been shown to emerge as a powerful interdisciplinary approach for providing nanoagents and nanovaccines with their potential role to mitigate the disease burden. The development of RNA-based vaccines such as BNT162b2 and mRNA-1273 for the COVID-19 is a massive achievement and therefore, represents a big milestone in the field of nanomedicine. The odds for a potential nanomedicine-based approach in vaccine development were put forward to be favorable. Due to scalability and versatility of mRNA, the approach based on nucleic acid nanovaccines can be adopted to develop promising vaccines for infections in future by different microbes. Herein, authors have highlighted the role of nanocarriers or nanovaccines and it could be a prospective approach to curb the disease burden and manage future pandemics. The most prominent literacy from the pandemic will be an improved public understanding of the value of science as the new generation grows up with great respect for science.

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SARS-CoV-2 mutations and immune response: Why mutated variants are more dangerous

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Abstract

COVID-19 is a viral respiratory illness caused by a new coronavirus called SARS-CoV-2. The World Health Organization declared the SARS-CoV-2 outbreak a global public health emergency. The genomic analysis of SARS-CoV-2 have revealed novel mutations in the virus. These continuous mutations have risked the possibility of the deadlier viruses into the nature. Currently, variants B.1.1.7, P681H, B.1.427, B.1.429, B.1.351, P.1 has been emerged as SARS-CoV-2 variants. Mutations such as D614G, L37F, P10S, P13L, A97V, T1198K has been reported in several sample studies in relation to the spike protein. The emergence of this variant raises concerns of a potential increase in transmissibility or propensity for SARS-CoV-2 re-infection of individuals. Mutations in the viral genome enables them to have easy escape from the host immune system, which makes the disease more worsen with having increased transmission rate. Understanding the mechanism of variant generation in SARS-CoV-2 is a major factor to develop vaccines or effective drugs against it.

Keywords: SARS-CoV-2, COVID-19, mutation, immune escape

Introduction

SARS-CoV-2 has been quite prevalent across the globe and currently making India as the most affected country in the world. Researchers isolated the virus that caused pneumonia in December 2019 and found it to be similar to a strain of β-coronavirus (CoV). SARS-CoV-2 has reportedly shown a high nucleotide sequence homology with two severe acute respiratory syndrome (SARS)-like bat coronaviruses which are bat-SL-CoVZC45 and bat-SL-CoVZXC21 (88% homology) and a 79.5% homology with SARS-CoV, while only 50% homology with the Middle East respiratory syndrome coronavirus (MERS) CoV [1,2]. The virus, now named SARS-CoV-2, contains a single positive stranded RNA (ribonucleic acid) of 30 kilobases, which encodes for 10 genes [3]. Studies on SARS-CoV-2 has demonstrated that virus enters the host cells by binding the angiotensin-converting enzyme 2 (ACE2), through its receptor binding domain in the spike protein. The RNA viruses acquire the capability to mutate causing genetic variations thereby increasing the infectivity and mortality rate among the host population. Mutations mainly takes place during the error that takes place via the replication of RNA virus to form new replicons [4].

The virus mutation has been surprisingly taking place since its emergence. During the replication process the virus acquires certain mutations which creates novel variants having different characteristics. As a consequence, vaccination has been taken as an effective rapid solution against the viral spread. This has also raised the concern towards virus variants. Substitution of nucleotides has considered to be one of the prominent factors contributing towards viral evolution in nature. Similar to the betacoronaviruses, the genome of SARS-CoV-2 has a long ORF1ab polyprotein at 5'end, followed by four structural proteins which also includes the spike protein, small envelope protein, matrix protein and nucleocapsid protein [5]. Mutations in viral variants have been focussed upon the spike (S) of viral protein. S protein has a vital function in incorporation and viral entry into target cells. The S1 subunit of S protein binds to host surface receptor ACE-2 via its receptor binding domain (RBD). TMPRSS2 activates S protein subsequently, S2 protein facilitates fusion of the viral and cellular membrane allowing the delivery genome into the host cell [6]. Therefore, S protein determines the infectivity of the virus and its transimibility into the host. As, S protein is the major antigen-inducing protective responses. This protein is most focussed by scientists as a target for drugs and neutralizing antibodies.

RNA viruses are reported to have more mutation rates than the DNA viruses. Amino acid changes in the surface protein can significantly alter viral function and/or interactions with neutralizing antibodies. For example, A226V of Chikungunya virus E1 protein facilitated its

adaptability in the vector Aedesalbopictus, resulting in an increased transmissibility [7]. As of May 6, 2020, 329 naturally occurring variants in S protein have been reported in public domain. Notably, there were only 13 amino acid sites with a rate of more than 0.1%. Preliminary study suggested that the increased fatality rate may be associated with the most dominant variant D614G. Presumably, this change may have induced a conformational change in the S protein, thereby resulting in the increased infectivity. However, it remains largely unclear as to whether these reported variants could influence viral infectivity, transmissibility, or reactivity with neutralizing antibodies. It is also well documented that mutations affecting glycosylation of viral proteins could also profoundly affect viral life cycle and its interaction with the host. For example, N-glycosylation at specific sites of the HIV-1 Env protein is critical for Env expression and assembly [8]. Deletion of certain glycosylation sites could decrease the binding of Env protein to the CD4 receptor, abolishing the infectivity of the resulting viral particles. It is of note that although the S protein of SARS-CoV-2 is much more heavily glycosylated, with 22 potential N-glycosylation sites, how these glycosylation sites could affect viral infectivity and antibody-mediated neutralization remains unknown.

One of such variant strain currently with high infectivity rate prevailing across India and other countries is B.1.6.17 which is a triple mutant SARS-CoV-2 variant. This variant was initially detected in Maharashtra in October and was thought to be a double mutant with two mutations of E484Q and L452R [9]. Later scientific studies revealed the presence of three mutations adding P681R into the list and terming it as triple mutant variant. Scientists also believe the presence of additional mutations such as E154K and Q1071H. The variants from UK, South Africa and Brazil has reported to have the same mutations of concern. The L452R variant first reported in California upon infection escapes the immune system showing greater virulence [10]. The Bengal strain which is termed as B.1.618 variant of SARS-CoV-2 was first found in West Bengal similarly, escapes the immune system and has increased viral spread. The B.1.618 is characterised by E484K mutation which is majorly responsible for immune-escape. This carriers two deletions in the spike protein, called H146del and Y145del [11]. Both of these deletions has been associated with immune escape.

D614G variant of SARS-CoV-2 has been identified as the most common clade located in a B-cell epitope with a highly immunodominant region and may therefore affect vaccine effectiveness [12]. Perhaps, scientists have identified 14 other variants besides D614G even though when amino acids are quite conserved in this epitope. Most of the variant strains having D614G mutation also have a mutation in the protein responsible for replication (ORF1ab P4715L; RdRp P323L), which shifts the focus that it might affect replication speed of the virus. This protein is the target of the anti-viral drugs such as remdesivir and favipiravir. The susceptibility for mutations suggests that treatment resistive strains may emerge quickly. Mutations in the receptor binding domain of the spike protein often relates to the theory that variants would have large binding affinity with ACE2, since that would decrease the fitness of the virus. Samples from the United States had mostly mutations V483A and G476S, whereas V367F is found in samples from China, Hong Kong Special Administrative Region, France and the Netherlands [13]. The mutant variants having mutations V367F and D364Y have been reported to increase the structural stability of S protein and helps in more efficient ACE-2 receptor binding. In summary, studying the functional and structural changes in spike protein is very crucial to design and develop effective treatment options.

SARS-CoV-2 Variants

Since, viruses are continuously mutating, it can cause a new variant, or strain, of a virus to form. A variant usually doesn't affect how the virus works. But sometimes they make it act in different ways.

Scientists around the world are tracking changes in the virus that causes SARS-CoV-2. The major focus is to understand the SARS-CoV-2 variant mechanism of viral spread and its infectivity rate among host. Also, to develop effective vaccines understanding mutant variants is important. The current SARS-CoV-2 variants exhibit high transmissibility and immune escape ability as a result of several co-occurring mutations in the S glycoprotein. Increased transmissibility is conferred by H69/V70 and N501Y in B.1.1.7, E484K and N501Y in B.1.351, E484K in B.1.1.28, and L452R in B.1.429. Immune escape is contributed to by Y144 in B.1.1.7, E484K and K417N in B.1.351, E484K in B.1.1.28, and L452R in B.1.429 [14, 15].

B.1.1.7: This variant was first detected in the United States in December 2020. It was initially detected in the United Kingdom.

This variant has reported a mutation in the spike protein's receptor binding domain (RBD) at position 501, where the amino acid asparagine (N) has been replaced with tyrosine (Y). This mutation is denoted in shorthand as N501Y. This variant also has reported to have many other mutations, which includes the deletion in 69/70 which has

occurred spontaneously many times and likely leads to a conformational change in the spike protein

P681H: this mutation is reported to be in the S1/S2 furin cleavage site, a site with high variability in coronaviruses [16]. This mutation has also emerged spontaneously multiple times. This variant was earlier reported in UK during September 2020. But since December 2020 it has been reported in several other countries with cases of having B.1.1.7 lineage. This variant reportedly affects the transmissibility rate causing rapid and efficient transmission of the virus. In January 2021, scientists from UK reported evidence that suggests the B.1.1.7 variant may be associated with an increased risk of death compared with other variants. Early reports found no evidence to suggest that the variant has any impact on the severity of disease or vaccine efficacy.

B.1.351: This variant was first detected in the United States at the end of January 2021. It was initially detected in South Africa in December 2020.

This variant has multiple mutations in the spike protein, including K417N, E484K, N501Y [17]. Unlike the B.1.1.7 lineage detected in the UK, this variant does not contain the deletion at 69/70.

This variant was initially reported in samples from Nelson Mandela Bay, South Africa beginning of October 2020, and cases have since been detected outside of South Africa, including the United States. The variant also was identified in Zambia in late December 2020, at which time it appeared to be the predominant variant in the country.

Currently there is no evidence to suggest that this variant has any impact on disease severity.

P.1: This variant was first detected in the United States in January 2021. P.1 was initially identified in travelers from Brazil, who were tested during routine screening at an airport in Japan, in early January.

The P.1 variant is a branch of the B.1.1.28 lineage and was initially reported in Japan via the samples collected during the Haneda airport routine screening process from four travellers from Brazil by the National Institute of Infectious Diseases (NIID). The P.1 lineage contains three mutations in the spike protein receptor binding domain: K417T, E484K, and N501Y [17].

Reports suggest that some mutations in the P.1 variant has an effect on the transmissibility and antigenic profile, which might hinder the ability of antibodies function that has been generated through a previous natural infection or through vaccination to recognize and neutralize the virus. The emergence of this variant raises concerns of a potential increase in transmissibility or propensity for SARS-CoV-2 re-infection of individuals.

B.1.427 and B.1.429: These two variants were first identified in California in February 2021.

These variants seem to spread more easily and quickly than other variants, which may lead to more cases of SARS-CoV-2. An increase in the number of cases will put more strain on healthcare resources, lead to more hospitalizations, and potentially more deaths.

The *ACE2* allele frequencies included six interaction-booster variants (S19P, I21V, K26R, T27A, N64K, and H378R) and eight interaction-inhibitor variants (E37K, N51D, K68E, F72V, M82I, G326E, Q388L, and P389H) which have been shown to vary significantly between populations [18].

Some of the potential consequences of emerging variants are the following:

- **Spreads at faster rate.** Mutated variant of SARS-CoV-2 with the mutation D614G has been reported with evidences that it confers increased ability to spread more quickly than the wild-type SARS-CoV-2 [19]. *In vitro* reports suggest that 614G variants replicate at increased rate in human respiratory epithelial cells, outcompeting 614D viruses. There also is epidemiologic evidence that the 614G variant spreads more quickly than viruses without the mutation.
- **Causes moderate to severe disease conditions.** The new variants of SARS-CoV-2 reported in January 2021 namely, B.1.1.7 variant showed to be associated with an increased risk of death compared to other variants. More studies are needed to confirm this finding.
- **Ability to escape detection by specific viral diagnostic tests.** The most effective and specific test reverse-transcription polymerase chain reaction (RT-PCR) used for the detection of SARS-CoV-2 infections have multiple targets to detect the virus, such that even if a mutation impacts one of the targets, the other RT-PCR targets will still work [20].
- **Decreased sensitivity towards therapeutic agents such as monoclonal antibodies.**
- **Ability to evade natural or vaccine-induced immunity.** The SARS-CoV-2 infection into the host by both vaccinations against and natural infection produces a "polyclonal" response that targets several parts of the spike protein [21]. To evade such immune related responses, the virus would likely need to accumulate multiple mutations.

Among these possibilities, the most concerned factor is the ability of virus to evade vaccine-induced immunity because once a large proportion of the population is vaccinated, there will be immune pressure that could favour and accelerate emergence of such variants by selecting for "escape mutants."

The double mutant variant in India

The B.1.617 variant of SARS-CoV-2 carries two mutations, E484Q and L452R. Both are separately found in many other coronavirus variants, but they have been reported together for the first time in India.

The two mutations are found in the virus's spike protein. The spike protein helps the virus to bind itself to the human cell's receptors and gain entry into a host cell.

The E484Q mutation is similar to E484K, a mutation found in the United Kingdom (lineage B.1.1.7) and South Africa (B.1.351) variants of the coronavirus.

The L452R mutation has been found in fast spreading variants in California (B.1.427 and B.1.429). It can increase the binding power of spike proteins with ACE2 receptors on human cells, making it more transmissible. L452R can also potentially enhance viral replication [22].

Together, E484Q and L452R are more infectious, and can evade antibodies.

B.1.617 has been spreading quickly in India. It is now the dominant strain in the state of Maharashtra in southwestern India. Maharashtra is India›s second most populous state and home of India›s financial centre at Mumbai.

Back in December 2020, an estimated 271 million people (about one-fifth of India›s population) were already infected with COVID-19, based on seroprevalence data from surveys across 21 states in India. Modelling studies suggested that India may have already reached herd immunity through natural infection. India›s health minister announced that the country had successfully contained the spread of the virus.

Three months later, India is battling its biggest COVID-19 surge yet. Infections are at the highest daily average reported, with over 340,000 new infections reported daily, and experts believe the actual number of infections and deaths may be under-estimated.

The second wave has created a huge impact in the country putting down few questions to be answered. Is the new variant that has to be blamed for the current surge? Or is it due to the irresponsible measures taken by the people by avoiding masks, having large gatherings, travel, thinking that India was already immune.

SARS-CoV-2 immune escape

Continuous process of evolution has made the battle of survival between hosts and pathogens a major determinant of natural selection. As, the host develops defence mechanisms to tackle infections, the infectious agents develop and evolve to counteract these defence mechanisms and therefore survive. The current SARS-CoV-2 pandemic can also be seen in this context. Although the human body has a strong barrier of its immune system which acquires the ability to eliminate any microbe, the SARS-CoV-2 has created its own strategies to trick the human immune system.

There has been continuous study on SARS-CoV-2 since its emergence. Scientists claim similarity among the mechanisms of other coronaviruses such as MERS and SARS-CoV, they can be extrapolated to understand more about the SARS-CoV-2. As most of pathogens, SARS-CoV-2 can be recognized by the innate immune receptors known as pattern recognition receptors (PRRs) [23]. Eventually, this process of recognition allows the innate immunity to eliminate the infection or to activate the adaptive immune responses. However, SARS-CoV-2 can use its proteins such as M, N, NSP1 and PLpro to interfere with these defence mechanisms [24]. Interferons which are known to be potent antiviral cytokines has been shown to be inhibited in the COVID-19 patients with the help of viral proteins. This less or delayed induction of IFN is able to shift the response to the production of proinflammatory cytokines. These proinflammatory cytokines acts by sending more macrophages and inflammatory cells into the lungs thereby causing leakage of vascular vessels and also impairs the adaptive immune responses. By this mechanism, the virus doesn't just halt the immune response but rather induces exaggerated cycles of inflammation that damages the lung. This uncontrolled release of pro-inflammatory cytokines i.e. dysregulation and excessive immune responses that may cause immune damage to the body tissues is termed a cytokine storm.

Natural killer (NK) cells are also a part of the innate immunity and effectively has the ability to eliminate the virally infected cells. SARS-CoV-2 studies have revealed the reduction in the number of activated NK cells making them functionally exhausted and downregulate genes necessary for tackling the virus [25]. The mechanisms by which the SARS-CoV-2 induces these changes are still not known. Although the SARS-CoV-2 virus primarily infect cells of the respiratory tract, it can also infect some immune

cells such as monocytes, macrophages and dendritic cells; a process which can attenuate the immune cells functions. These cells are known to be Antigen-Presenting cells (APCs) and are capable of presenting the viral antigens to the lymphocytes in the lymph nodes to trigger an adaptive immune response. However, more studies demonstrated that SARS-CoV-2 can downregulate major histocompatibility complex (MHC) molecules which are necessary for this presentation [26]. The virus also limits the dendritic cells maturation and renders them incapable of activating the adaptive immune cells. Adaptive immunity a long- lasting and very specific mechanism of immunity and is the major player in fighting viral infections. This arm of the immune system needs an activation by the innate immune cells which lost their functions due to SARS-CoV-2 infection. Not only this, but also the cytokine storm is hypothesized to induce the clinically observed lymphopenia in COVID-19 patients [27]. Other hypotheses of how the SARS-CoV-2 affects the adaptive immunity include that the infection can result in exhaustion of T cells, where they can't perform their functions anymore and also that the infection can interfere with the expansion of T cells through downregulating some genes necessary for the lymphocytes proliferation.

The mechanisms by which SARS-CoV-2 manipulated the immune system are not so well understood yet. The great inter-individual variability of the immune response and why some individuals can control the infection while others can't is still not clear. Further studies are still needed to understand the interaction between the virus, the environment and the host. Understanding such mechanisms will help us better understand how our immune systems function and can be considerable points in developing novel immunotherapies and vaccines against COVID-19.

Conclusion

The pandemic of COVID-19 has caused more than 16.7 cr confirmed cases and more than 34.7 lakh deaths globally as of 25 May 2021 since the first case was reported from Wuhan, China. The confirmed cases and deaths are rising quickly, and the fast evolution and transmission of SARS-CoV-2 has generated several particular mutations across geographic regions. Since there were no vaccine and treatment-based selective pressures in the early pandemic, the host genetic variability could drive adaptive evolution by selecting for increased genetic diversity in SARS-CoV-2 across geographical regions. Mutations in the S glycoprotein have been shown to enhance viral transmissibility and immune escape ability, however, no current mutations increase viral pathogenicity or COVID-19 severity. The recent emergence of B1.1.7 (prevalent in the United Kingdom), B.1.351 (in South Africa), B.1.1.28 (in Brazil), and B.1.429 (prevalent in California, USA) show variants with several mutations in the S glycoprotein, especially within the RBD. Some mutations have been found to enhance viral infectivity (ΔH69/V70, N501Y, and P681H) or contribute to immune escape (ΔY144, ΔL242/244, E484K, L452R, and N501Y). Understanding such mechanisms will help us better understand how our immune systems function and can be considerable points in developing novel immunotherapies and vaccines against COVID-19.

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Impact of ACE-2 mutations on severity of COVID-19 pandemic

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Abstract

COVID-19 disease is a respiratory problem caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) which has put global health care system under immense pressure. This highly infectious virus has spread in many countries. It is evident that SARS-CoV-2 is mutating and some mutations (herewith referred to as point mutation resulting into substitution of single amino acid by another in synthesized protein) in the spike protein result into improved transmission, virulence of virus and aid in slipping past the host immune response. However, the ability of SARS-CoV-2 to infect humans is invariably associated with their binding strengths to human receptor angiotensin-converting enzyme 2 (ACE2). Therefore, the mutations in the key binding region of ACE2 is also a dictating factor controlling the affinity and infection severity of SARS-CoV-2in human hosts. This article encompasses the key mutations harbored by ACE2 receptors and their impact on the SARS-CoV-2 transmission ability.

Overview of COVID 19

Severe respiratory disease coronavirus disease 2019 (COVID-19) is latest addition in the series of major pathogenic zoonotic disease outbreaks of the last two decades. The major outbreaks were primarily caused by the family of viruses known as beta coronaviruses; Severe Acute Respiratory Syndrome coronavirus (SARS-CoV) which emerged in 2002 caused ~8000 infections while MERS-CoV which emerged in 2012 was responsible for infecting around 2300 people. SARS-CoV 2 which was first reported in China in late December 2019 has rapidly grown into catastrophic situation and has put modern health care system under immense pressure. Subsequently world health organization has categorized this spread as pandemic. Although, SARS-CoV 2 has a lower mortality rate as compared to SARS-CoV and MERS-CoV, it has claimed millions of lives owing to its higher virulence. The morbidity and mortality has increased exponentially around the globe. The primary reason for this increase is the significant mutations that have taken place in the virus making it more virulent and aiding it in evading immune response. [1-3]

Overview of Coronaviruses

Coronaviruses belong to a large group of animal virus from coronaviridae family. This coronaviridae family is further divided into four sub-groups namely alpha, beta, gamma and delta coronavirus. All the viruses that have caused viral transmission in humans belong to beta group. These beta viruses affecting humans primarily cause serious human respiratory tract infections. Similar to most of the emerging viruses infecting humans in recent times, coronaviruses also have RNA as their genomic material. These are enveloped viruses having relatively large single stranded, positive sense RNA genomes ranging from 27 to 32 kilobases (kb). The virus envelope under electron microscopy appears like a crown and hence it is given the name "coronavirus" which is derived from the Greek word for crown [1, 4].

Like other coronaviruses, SARS-CoV-2 also possesses a single stranded positive sense RNA which is ~29990 nucleotides in length. These nucleotides code for 29 proteins which include 4 structural proteins namely spike (S) glycoproteins, membrane (M) glycoproteins, envelope (E) and nucleocapsid (N) proteins (Fig. 1). Rests of the 25 proteins are divided into accessory and non structural proteins.

The genome organizations of SARS-CoV-1 and SARSCoV-2 are remarkably similar, with ~79% identity at

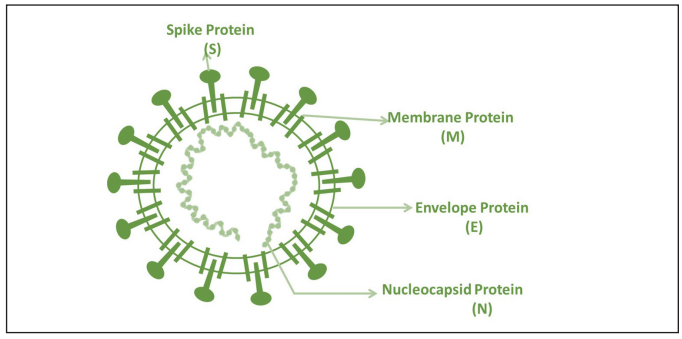

Figure 1: Structure of SARS-CoV-2

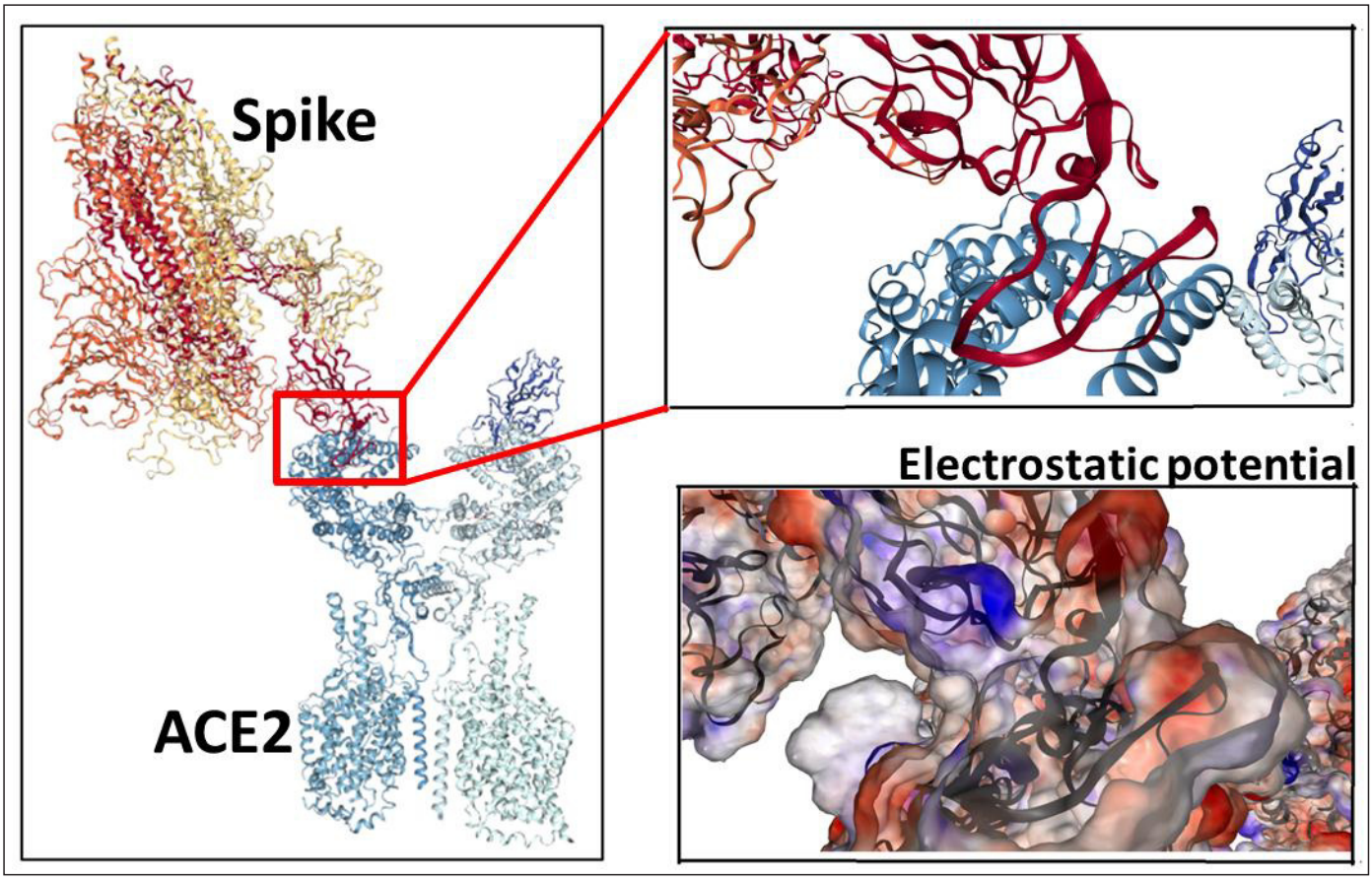

Figure 2: Cocrystal ACE2 with S protein of SARS-CoV-2. (image is formulated using the COVID-3D tool at osiq.unimelb.edu.au/covid3d/protein/QHD43416/ACE2 BOAT).

the nucleotide level. Both viruses exploit ACE2 as a receptor for gaining entry into human cell and are so far the only human corona viruses in lineage B of betacoronaviruses.

SARS-CoV-2 S protein

Spike protein, a transmemberane glycoprotein protruding from the viral surface is mainly responsible for the entry of SARS-CoV-2 into the host cell. The Spike protein is a trimeric protein. Each monomer unit of the S protein is functionally divided into two subunits, S1 and S2 that mediate attachment and membrane fusion respectively. In SARS-CoV-2, Receptor Binding Domain, located in the S1 subunit initiates the attachment with ACE2. RBD region contains a Receptor Binding Motif (RBM) which contains most of the residues that mediate the binding of SARS-CoV-2 to the host receptor. The two functional subunits (S1 and S2) of the S protein remain non-covalently bound in the pre-fusion state stabilized by the RBD of the S1 subunit. Binding of S1 to the host receptor results in substantial structural rearrangement that destabilizes the trimer. These conformational changes are crucial for membrane fusion since this exposes the S1/S2 cleavage site to host proteases. It is believed that the presence of multiple arginines (multi basic site) in the cleavage site is responsible for the activation of proprotein convertases, like furin, that is likely to facilitate the cleavage of S1 and S2 subunits. It is evident that the mutations in and around the RBD are of serious concern as they can significantly alter the binding affinity of S viral protein with ACE2 which may result into the increased transmission of virus and also assist in escaping from the host antibodies. Based on the mutations various lineages have emerged in different parts of the world. Some of them have been tagged as variant of concern (VOC) by world health organization (WHO) depending on their infecting and escaping ability [5, 6].

Angiotensin Convertase Enzyme 2 (ACE2)

SARS-CoV-2 exploits human receptor ACE2 for host cell entry as shown in Fig 2. This human receptor consists of 805 amino acids which are divided into two functional domains i.e. the N terminal peptidase domain and a C terminal domain. It is the peptidase domain of ACE2 that harbor the active site. ACE2 is primarily responsible for the maturation of the peptide hormone angiotensin that controls blood pressure and vasoconstriction. Additionally,

it also cleaves a number of biologically active peptides like kinetensin, neurotensin,casomorphins and apelins. ACE2 is has widespread presence in human body organs such as alveolar epithelial lung cells, intestine, kidney,heart, adipose tissues and blood vessels. Furthermore a high expression of ACE2 in the oral cavity has also been reported. This could potentially increase the risk of SARS-CoV-2 infection as it grants easy access to the host [7-10].

The interaction of ACE2 with SARS-CoV-2 has been found to be stronger than previous SARS-CoV. This is mainly due to the mutations acquired in the S protein of SARS-CoV-2. For instance, presence of glycine rich stretch between 482-485 amino acid sequence forms more compact RBD structure which creates better contacts with ACE2. Furthermore, substitution of Leu472 in SARS-CoV with Phe486 in SARS-CoV-2 could produce stronger van der Waals interaction with ACE2. Similarly change from Val404 to Lys417 increases the interaction of SARS-CoV-2 RBD and ACE2 due to the formation of a salt bridge between Lys417 of S protein and Asp30 of ACE2. Apart from these, crystallographic studies suggest that the SARS-CoV-2 S protein has better steric compatibility than SARS-CoV S protein due to which former binds to ACE2 with 10 to 20- fold higher affinity, which may be the reason for higher infectivity and transmissibility [3, 11, 12].

ACE2 mutations and their impact on transmission

SARS-CoV-2 primarily depends on ACE2 for fusion and entry. Thus mutations in S protein of virus or host receptor could potentially alter the binding affinity

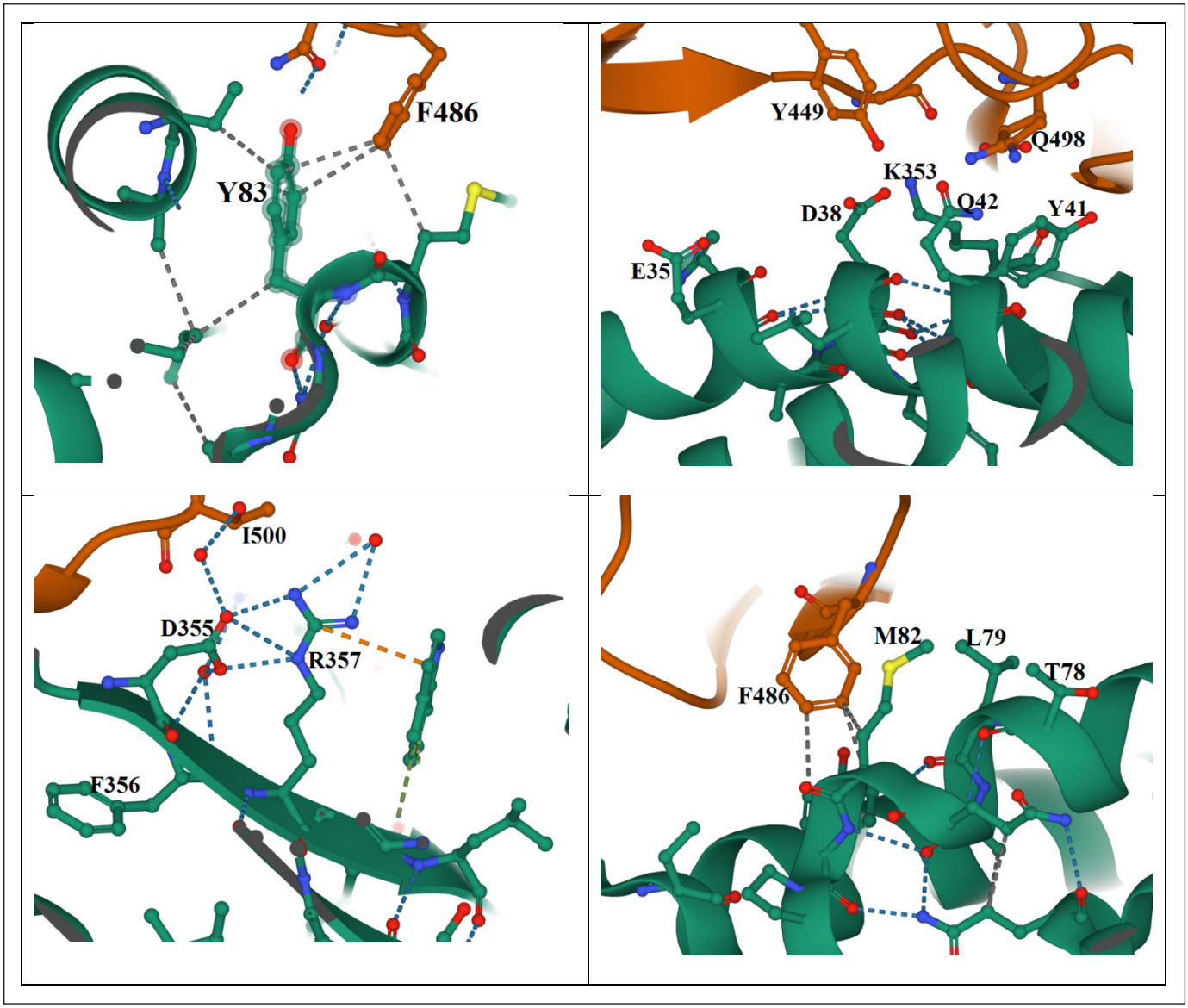

Figure 3: Key residues of ACE2 having key mutations. (Image has been formulated at RCSB website http://www.rcsb.org/structure/using data available in Protein Data Bank Figure, PDB ID: 6LZG)

and susceptibility to infection. From the reports it is evident that genetic polymorphisms in the ACE2 gene due to mutations may amend the intermolecular interactions with the spike protein of SARS-CoV-2 and/or contribute viral transmission.

In a study conducted by Cao et al., thirty-two ACE2 mutations that included 7 hotspot mutations (Lys26Arg, Ile468Val, Ala627Val, Asn638Ser, Ser692-Pro, Asn720Asp, and Leu731Ile/Leu731Phe) have been identified. Among these ACE2 variants, 13 mutations were found to improve binding affinity towards SARS-CoV-2 while 18 mutations were designated as interaction suppressor. Among the 13 mutations that improved binding, mutations Ser19Pro, Ile21Thr, Lys26Arg, Thr27Ala, Asn64Lys, and His378Arg were found to be abundant in all population groups. In the same line, a comprehensive analysis of a large data set consisting of 290,000 genomic samples identified multiple ACE2 mutations. The data suggested that ACE2 variants Ser19Pro, Ile21Val, Glu23-Lys, Lys26Arg, Thr27Ala, Asn64Lys, Thr92Ile, Gln102Pro and His378Arg found in the binding region increased infection vulnerability whereas the variants Lys31Arg, Asn33Ile,His34Arg, Glu35Lys, Glu37Lys, Asp38Val, Tyr50Phe, Asn51Ser,Met62Val, Lys68Glu, Phe72Val, Tyr83His, Gly326Glu,Gly352Val, Asp355Asn, Gln388Leu and Asp509Tyr exhibited lower binding affinity for SARS-CoV-2 S protein. Lys26 is located near the N terminal region and forms a hydrogen bond with Asn90. The mutation Lys26Arg is likely to induce a destabilization in secondary structure of ACE2 protein. Gly211, a short carbon chain amino acid which is present at the turning point of a loop assist Val212 to form strong hydrophobic interaction with Leu91 that stabilizes ACE2 structure. A substitution of achiral and flexible glycine with polar hydrophilic arginine weakens the Val212-Leu91 interaction and possibly destabilizing the ACE2 structure. The mutations close to theTMPRSS2 cleavage sequence could affect virion intake. ACE2 variants from Genome Aggregation Database identified 12 deleterious missense variants - Ser19Pro, Asp206Gly, Gly211Arg, Arg219His, Arg219Cys, Lys341Arg,His378Arg, Ser547Cys, Ile468Val, Arg697Gly, Ser692Pro,Leu731Phe. Based on structural analysis, nine of these variants could disrupt protein structure or its interaction with the RBD of SARS CoV-2 [3, 4, 13-17].

In computational modeling by Ge and coworkers, protein-protein binding affinity was estimated. Based on the calculations, it has been found that out of the 13 missense (point) mutation under consideration, six mutations (D38E, M82I, Y83F, K353H, R357A, and R357S) enhances the binding of ACE2 with S protein while seven mutations (S19P, K31D, Y41A, M82N, M82T, D355A, and D355N) were found to retard the interaction [18].

From protein molecular modeling carried out by Hussain's group, it has been found that amino acid substitution (K26E) and (M82I) may adversely affect the stability of the encoded protein compared with the wild type (unmated or pre-existing version). Moreover they also observed that All ACE2 variants were found to bind with the viral spike protein with topologynearly identical to the resolved ACE2-SARS-CoV-2 complex structure (PDB Id: 6LZG). By and large, the nature and number of intermolecular contacts between SARS-CoV-2 spikeprotein and humans ACE2 variants were found to be comparable [19].

Conclusions

This article highlights the impact of mutations in human ACE-2 on the SARS-CoV-2 transmission. Whereas some mutation improves the binding affinity of ACE2 with S protein, there are several mutations that suppress the interactions between the host receptor and viral S protein. The individual chemical nature of substituting amino acid in ACE-2 mutation is critical in controlling the chemical interaction, structural flexibility and biological functionality which in turn is governing the infectivity of SARS-CoV-2.

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Covid-19: Time to flatten the curve

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Abstract

The novel human coronavirus has caused a pandemic that the world's population is struggling to overcome. While the then oblivious coronaviruses were known to humans since the 1960s, it has become a familiar term nowadays. This review takes a peek into the history of coronaviruses until the SARS-nCoV-2, the various strains that have evolved from 1960s until 2020, the spread in the population of SARS-nCoV-2 rising from panic to the precaution phase, the vaccine trials and ultimately the Biology behind the virus so as to formulate a specific diagnosis.

Introduction

The biodiverse world comprises bacteria, fungi, protists, algae, plants and animals that largely fall under the two major categories of prokaryotes and eukaryotes. Viruses do not fall into either of these categories and are considered as non-living entities requiring a host to propagate while displaying living principles. Hence, a separate classification for viruses based on the hereditary material (genome) is used. Four such types include double-stranded DNA (dsDNA) or RNA (dsRNA) and single-stranded DNA (dsDNA) or RNA (ssRNA). Coronaviruses are ssRNA types that were quite unpopular among Biologists and humankind since the 1960s, but have today become a home-name and are branded as 'COVID-19' with about anything and everything ranging from sanitizers to sleeping pillows! In fact, an entire dictionary of words, expressions and phrases has been and are still being built using the terms coronavirus and covid! The 2019 novel coronavirus (2019-nCoV) or the severe acute respiratory syndrome coronavirus 2 (SARS-nCoV-2) as it is now called, originated on 17th November 2020 in the Wuhan City of Hubei Province of China and rapidly spread to the rest of the world with 220 countries being affected to varying degree (Lu *et al*, 2020). Till 17/01/2021 around 95,168,510 cases of coronavirus disease 2019 (COVID-19) and 2,034,614 deaths have been reported with the worst affected countries being USA, India and Brazil (Chen *et al*, 2020; Wang *et al*, 2020).

History

All known coronaviruses belong to the family Coronaviridae that are large positive sense single-strand RNA viruses with an envelope (diameter, 60-140 nm) bearing on its surface spike-like projections that makes it look like a crown when observed under the electron microscope; hence the name coronavirus (Zhou *et al*, 2020). Their genomes range from 25-32 kilobases with the virion size of 118-136 nm in diameter. Two subfamilies, namely *Coronavirinae* and *Torovirinae* exist; the former has four genera, *alpha coronavirus*, *beta coronavirus, delta coronavirus* and *gamma coronavirus*, while the latter has two genera, *torovirus* and *bafinivirus*. Mammalian hosts are infected by *alpha coronaviruses* and *beta coronaviruses*, whereas birds are exclusively infected by *gamma coronaviruses* and *delta coronaviruses*. In 2003, the severe acute respiratory syndrome (SARS) coronavirus was first identified (Peiris *et al*, 2003), an intensive search for novel coronaviruses was carried out. This unexpectedly resulted in the detection of a diverse number of novel coronaviruses from their natural reservoirs, viz. domesticated animals, humans, bats, avians and wildlife (Snijder *et al*, 2003; Poon *et al*, 2005; Wevers *et al*, 2009; Woo *et al*, 2009; Zaki *et al*, 2012; Chu *et al*, 2011; Vijaykrishna *et al*, 2007; Woo *et al*, 2012). Exhaustive phylogenetic analyses of coronaviruses from several species with explicit modeling variation, keeping in mind natural selection over time, has led to the understanding that the most recent ancestor common for all coronaviruses has likely existed millions of years ago on this planet (Wertheim *et al*, 2013).

Normally known to cause a range of conditions in humans and animals, coronaviruses are also known to be zoonotic. The conditions post-infection could be a mild cold, fever, cough, severe pneumonia, respiratory discomfort and death. Coronaviruses affecting the human population were first noted in the 1960s albeit with mild respiratory illnesses. For several centuries these human coronaviruses have likely circulated among the human population, and include 229E (alpha coronavirus), NL63 (alpha coronavirus), OC43 (beta coronavirus) and HKU1 (beta coronavirus) in order of how these appeared in their infectious forms on the earth. Of these that cause mild respiratory infection, HKU1 can also cause gastrointestinal

infection. The newer coronaviruses or the 'true emerging infectious diseases' which causes severe acute respiratory syndrome includes SARS-CoV (2002-2003) and was found to be transmitted from civet cats to humans (Li *et al*, 2020). MERS-CoV (2012) was found to be transmitted from dromedary camels to humans and causes Middle East respiratory syndrome and now the novel SARS-nCoV-2 causing COVID-19 has struck a pandemic; all these have undergone recent animal-to-human transition. Such transitions or crossovers from a natural host (animals) to humans is always associated with viral mutations. Thus far, no viruses have really evolved enough to become persistent human pathogens; it involves years of acclimatization. In addition, methods used in controlling the human-to-human transmission alleviates the virulence thereby reducing the risk of new viruses from emerging.

Origin and Spread of COVID-19

It was in December 2019 that a cluster of adults from Wuhan, the capital city of Hubei province presented themselves with severe pneumonia (of unknown origin) to local hospitals. It seemed that several of these patients had some kind of exposure to the Huanan wholesale seafood market, one that sold fish, meat and traded live animals. Etiological investigations of the respiratory samples of patients were initiated (Wang *et al*, 2020; Coronavirus Outbreak (2020) Updates available at: https://www.worldometers.info/ coronavirus/). Within a month or so, (31st December 2019), Chinese authorities notified an outbreak of a pneumonia-causing virus to the World Health Organization with a statement that the Huanan seafood market was the likely origin of the virus. Scientists that immediately went into genome analysis of the virus soon (7th January 2020) revealed that the virus was identified as a coronavirus which had >95% homology with bat coronavirus and >70% similarity with the SARS-CoV. Subsequent environmental samples in and around the Huanan seafood market also tested positive, confirming the place of its origin (Xinhua, 2020). It was only on 1st January 2020 that the market was closed.

Further to this, human-to-human transmission was confirmed when the cases beyond the first cluster of individuals reported no exposure to the Huanan market (Huang *et al*, 2020). Although the first fatal case was reported on 11th Jan 2020, the epidemic was recognized only after 2 months into the outbreak at Wuhan. By the time the spread could be curbed, Chinese (probably asymptomatic at that point) had travelled outside of Wuhan (different parts of China and around the world) to celebrate the Chinese New Year on 25th January 2020; their main destinations being south asian countries (Thailand, South Korea and Japan). Because no checks were in place, USA and the European continent followed inevitably in quick succession. Within this span, 0.1 million cases of infection suddenly erupted around the globe and by 23rd January, the first lockdown was announced in the Hubei province of China. While some countries started placing checks/screening mechanisms at airports, most others had yet to realize the intensity of this epidemic. At the same time, individuals with no history of travel to China reported infection and that too at a rapid rate, suggesting that local human-to-human transmission was occurring in these countries (Rothe *et al*, 2020). It was not until 11th March 2020 that WHO declared this viral epidemic as a pandemic because by this time >118,000 infected cases in 114 countries were reported, and 4,291 people had lost their lives. A loud and clear warning from WHO for countries to detect, trace, test, treat, and isolate people, especially travellers so as to avoid an infected individual from becoming clusters and clusters eventually leading to community transmission. By mid-April, however, several countries had announced lockdowns and all this depended on some countries struggling with a lack of capacity, others struggling with lack of resources, and yet others struggling with a lack of resolve. WHO literally announced the 'mantra' of Prevention, Preparedness, Public health, Political leadership and most of all, People.

SARS-CoV-2: the virus, the infection/reinfection, the symptoms, the treatment

Coronaviruses have an interesting zoonotic history behind them, starting from avians to camels to bats and then humans. In the late 1920s, North America reported an acute respiratory infection of chickens. Upon isolation and closer examination of the pathogen causing this illness, it was identified as coronavirus (Estola, 1970). About a decade later (1931), two scientists (Arthur Schalk and M. C. Hawn) reported yet another respiratory infection, once again in chickens, this time in North Dakota, USA. Both these illnesses were observed at mortality rates of 40–90% (Fabricant, 1998). In 1933, the virus was successfully isolated and named infectious bronchitis virus (IBV; Bushnell and Brandly, 1933). Cultivating this virus was not easy, and was brought about in 1937 by Charles D. Hudson and Fred Robert Beaudette, hence the name Beaudette strain (Decaro, 2011). With another decade for a gap (late 1940s), two more animal coronaviruses were reported; one that caused murine encephalitis and another that caused mouse hepatitis, both respectively known as, JHM and MHV (McIntosh, 1974). These independent investigations did not manage to converge the relatedness of these viruses, then (Lalchhandama, 2020).

The first human coronavirus was discovered after a gap of two decades (Kahn and McIntosh, 2005; Mahase, 2020). In 1961, a common cold virus designated B814 was identified (not cultivable, Monto, 1984) by E. C. Kendall and co-workers affiliated to the Common cold unit of the British Medical Research Council (Kendall *et al*, 1962; Richmond, 2005). Incidentally, this virus could not be cultivated using conventional methods that were successfully demonstrated for other known common cold viruses such as rhinoviruses, adenoviruses, etc. In 1965, yet another common cold virus designated as 229E was isolated and cultivated using kidney cells in the USA (University of Chicago) by Hamre and Procknow (1966). Then in 1967, an interesting and useful insight was provided by a virologist, June Almeida who compared the various parameters associated with the three viruses, viz. IBV, B814 and 229E. Using electron microscopy, she demonstrated that all three viruses were morphologically related; all had similar shapes and the distinctive club-like spikes on their surfaces (Table 1). Simultaneously, another research group in the same year at the National Institute of Health, USA isolated yet another common cold virus with a morphology similar to those viruses as elucidated by June Almeida, and this virus was termed OC43 (Almeida, 2008). Common features of all these viruses include the presence of RNA as the genetic material, illness is mainly an acute respiratory syndrome (like the common cold), covering made up of lipids (as the viruses could be killed by ether) and the typical club-shaped spikes that earned the name coronavirus (Almeida and Tyrrell, 1967; McIntosh *et al*, 1967a; McIntosh *et al*, 1967b; Schmeck Jr. 1967). The 21st century saw the constant emergence of SARS-CoV from animals to humans and in November 2002 the first individual with the infection was identified in Foshan, Guangdong, China. Gradually, 8000 people from 29 different countries all over the world were infected given an average fatality of 9.6%. Over a period of ~2 years, the virus and the disease affected 30 countries (WHO release, 2004).

Another enveloped, positive-sense, ssRNA human coronavirus HKU1 (HCoV-HKU1) in January 2004 was identified causing an upper respiratory disease with symptoms ranging from a common cold to pneumonia and bronchiolitis in Hong Kong (Xinyi *et al*, 2016; Woo, *et al*, 2004). Further research indicated that HCoV-HKU1 enters its host cell by binding to the N-acetyl-9-O-acetylneuraminic acid receptor (Xinyi *et al*, 2016). The same virus was identified in the respiratory sample from a 35-year-old woman with pneumonia (Lau *et al*, 2006). In the same year between May and August, HCoV-HKU1 virus was identified in respiratory samples of 10 patients (all children) in northern Australia (Sloots *et al*, 2006). In July 2005, six cases were reported in France (Sloots *et al*, 2006). Upon back-tracing, it was observed that between December 2001 and February 2002 in the Connecticut area of USA of the 851 infants and children, nine were infected with the same human coronavirus HKU1 (Hurdiss *et al*, 2020). In late 2004, another species of enveloped, positive-sense, ssRNA human coronavirus from among the Alphacoronavirus genus was identified in a 7-month-old child in the Netherlands who was affected with bronchiolitis (Abdul-Rasool and Fielding, 2010) and seemed to be spreading during the winter months of November and March (Fehr and Perlman, 2015). The virus seems to have infected children, the aged and those with co-morbidity with symptoms that included mild to moderate upper respiratory tract infection, severe lower respiratory tract infection, croup and bronchiolitis. In

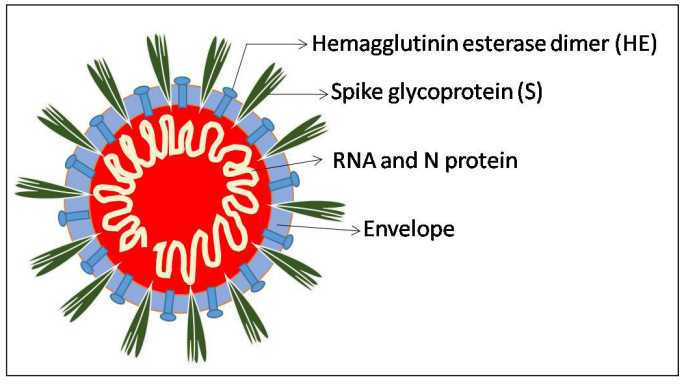

Figure 1: *Cartoon representation of SARS-nCoV-2.*

Table 1: The human SARS-CoV that have caused an epidemic/pandemic in the 21st century.

Virus name	SARS-CoV	HCoV NL63	HCoV HKU1	MERS-CoV	SARS-CoV-2
Place where it was first found	Foshan, Guangdong, China	Netherlands	Hong Kong	Middle East	Wuhan, China
Organism found	Humans, bats, palm civets	Humans, Palm civets, bats		Humans, bats, camels	Humans, bats, pangolin
Infection caused	SARS	SARS	SARS	SARS	SARS
	2002	2003	2004	2013	2020

2012, a species of the genus Betacoronavirus (enveloped, positive-sense, ssRNA) was isolated in a patient with flu-like respiratory illness in Saudi Arabia and was named as Middle East respiratory syndrome-related coronavirus (MERS-CoV). This virus enters its host cell by binding to the DPP4 receptor (de Groot *et al*, 2013; Fehr and Perlman, 2015). Incidentally, this slow-spreading virus, probably incubating and alternating its hosts was then reported by July 2015 in over 21 countries, suspecting to be a cause for a future epidemic (Alnazawi *et al*, 2017; Hemida *et al*, 2014). Then, the virulent strain of fast-spreading SARS-nCoV-2 was identified from the Wuhan district of China causing the severe acute respiratory syndrome (Gorbalenya *et al*, 2020) and is now responsible for the COVID-19 pandemic being popularly named as the **2019 novel coronavirus** (**2019-nCoV**). Further sequence analysis by the U.S. National Institutes of Health indicated it to be a successor to SARS-CoV-1 (van Doremalen *et al*, 2020). The route of entry for the virus was found to be the receptor angiotensin converting enzyme 2 (ACE2) present on human cells (Zhou *et al*, 2020; Letko *et al*, 2020; Hoffman *et al*, 2020; Wu, 2020).

A cartoon representation of SARS-nCoV-2 virus indicates the presence of an enveloped, positive-sense, single-stranded RNA (Figure 1). The virus was known to transmit through an infected individual's sputum, sneeze, cough. The non-infected individual could contract it through the mouth, nose and eyes. It is now known that surfaces contaminated with the virus could also be sources of the infection. Once inside, it is known to attach to the ACE receptor present on the surface of respiratory cells and invade it. The immense viral load released into the surrounding leads to a 'choked' pharynx and depending on this load, symptoms take 7-14 days to appear (He *et al*, 2020). In an interesting study conducted by Scientists from the University of North Carolina, USA, it was found that the nasal cavity is the primary site of infection with the virus further seeding into the lungs (Hou *et al*, 2020). The symptoms caused by all coronaviruses are similar and include cough, fever, and shortness of breath. However, to diagnose a potential COVID-19 case, healthcare professionals have been making use of diagnostic tests that are specific towards the spike protein of SARS-nCoV-2 so as to rule out influenza (flu) and other infections with similar symptoms. In the current scenario, it has been observed that individuals with COVID-19 have had a wide range of symptoms, ranging from mild to severe illness. Some infected individuals may not exhibit symptoms, some others require ventilator support, and few others have known to die. Symptoms may appear 4-14 days after exposure to the virus and may include: cough, fever, chills, congestion or runny nose, conjunctivitis, fatigue, headache, muscle pain or body aches, sore throat, shortness of breath or difficulty breathing, loss of taste or smell, nausea or vomiting, rash on skin, or discolouration of fingers or toes, and/or diarrhea. An individual may not develop all these symptoms, but a subset of these have been observed among all those infected with SARS-nCoV-2. Since it has now been well-established that the virus spreads from human-to-human transmission, an individual with any of these symptoms may likely harbour the virus and could spread it. However, infected individuals that do not develop any of the symptoms are considered as asymptomatic carriers. Such individuals place 'the vulnerable' human beings at risk as they are considered without symptoms and therefore are normal! They continue with their normal day-to-day activities *versus* those who are sick and show symptoms! In a case of re-infection, one 33-year old patient from Hong Kong was found positive in March and then re-appeared in August after being discharged from the hospital in April. Interestingly, using whole-genome sequencing, it was found that both the isolates from this patient showed the virus belonging to different clades (Kai-Wang *et al*, 2020). Another case was found in a 25-year old man from Nevada, USA and his re-infected isolate was a variant from the one he was infected with in the first place (Tillett *et al*, 2020). The need of the hour was to test each and every individual for the presence/absence of the virus.

Treatment: The world over, there is a struggle to find a treatment for SARS-nCoV-2 and some drugs have been recommended, although are not the guarantee for cure. Providing oxygen support and dexamethasone (a corticosteroid) are the most highly recommended forms of treatment, currently. Combinations of drugs such as remdesivir, hydroxychloroquine, lopinavir/ritonavir and interferon regimens appear to have been administered and have produced mixed benefits. Hydroxychloroquine has not been shown to offer any benefit for treatment of COVID-19. These and other ACE inhibitors, angiotensin-receptor blockers as well as statins have helped certain patients from recovering. The recent-most strategy has been to use convalescent plasma. The repurposing of the drug baricitinib (Olumiant) used in rheumatoid arthritic patients has been approved by the FDA to be used to treat COVID-19 in some cases. Meanwhile, two monoclonal antibodies, one called bamlanivimab, and the second, a combination of two antibodies called casirivimab and imdevimab have been used to treat mild to moderate COVID-19 in people who have a higher risk of developing serious illness due to COVID-19. Each reported COVID-19 positive individual is soon administered one intravenous infusion in an outpatient setting so as to avoid hospitalization.

The vaccine - Is it a silver bullet?

With the pandemic gaining strength worldover, an inevitable search for candidates for the purpose of vaccination was carried out. About 200 such candidates were in the pipeline, globally. Interestingly, 50 of these have been under clinical trials and 152 under preclinical investigations. The Chinese vaccine candidate was already in human trials in the month of April 2020. The vaccine used was the recombinant adenovirus type-5 (Ad5-nCoV) vector with the spike glycoprotein of SARS-nCoV-2 inserted and the results of the efficacy, immunoreactivity and safety were considered clinically insignificant. The second trials were to commence and the date of completion was announced as 31st January 2021. The Sponsor of this study was revealed as the Institute of Biotechnology, Academy of Military Medical Sciences, PLA of China with Collaborators as CanSino Biologics Inc., Jiangsu Province Centers for Disease Control and Prevention, Hubei Provincial Center for Disease Control and Prevention and Zhongnan Hospital (Zhu *et al*, 2020).

Overall, the candidates for vaccination include either the attenuated (inactivated) virus, or viral protein subunits, or are nucleic acid-based. At present, Pfizer/BioNtech vaccine (BNT162) has been in the lead and the major breakthrough in the 'vaccine gaming' scenario! They revealed that their RNA-based vaccine is 95% effective and is to be given in two doses, 21 days apart. The UK has received 40 million doses and on 2nd December, it became the first in the world to approve the vaccine and administered it first on 8th December 2020 on a 90-year old woman. The vaccine must be stored in a freezer (-70°C) and would have to be transported in a cold-chain system. On the other hand, the vaccine developed by Oxford University/AstraZeneca (named Covishield) have also indicated significant protection. It would probably be one of the easiest to handle as it can be transported at non-freezing, but cold temperatures. It is an attenuated form of a genetically engineered chimpanzee virus that causes common cold. The Moderna and Sputnik (Russia) vaccines have also been designed like that of Pfizer and would be the next to be rolled out. In India, the Serum Institute of India is conducting human trials for the vaccine developed by Oxford University and AstraZeneca. Hyderabad-based pharmaceutical Biological E. was conducting trials for Dynavax Technologies Corporation in collaboration with Baylor College of Medicine, Houston, USA. Hetero and Dr. Reddy's were following suit for Sputnik. The indigenous vaccines from Bharat Biotech/ICMR-India and Zydus Cadila will be rolled out for the public in early 2021.

It is still suspected by many that the vaccine, however effective it might be, could not serve to be a silver bullet! That said, one must know how vaccines work in the human body. All vaccines are made either from the attenuated, inactivated, dead pathogen or from their epitopes that are antigenic/immunogenic. It is meant to demonstrate an immunogenic response towards the pathogen such that an infection post the vaccine administration has the capacity to eliminate the pathogen and avoid the infection altogether. After the vaccine is produced in the wet laboratory of the Scientist, these are tested on cell lines first whose success is then translated to preclinical trials (with animals). When the vaccine is demonstrably safe on animals, it gets the necessary approvals (FDA) for human/clinical trials that occur in three phases. Each phase basically increases the number of individuals (always volunteers) undergoing vaccine administration; wherein each individual is carefully followed up for reactions of any kind (fever, itching, inflammation, intense swelling at the area of inoculation, allergy, etc). A vaccine is then determined as 'safe' when even at the third phase of human trial no adverse reactions are noted.

On 21st September 2020, WHO released a document asking countries the world over to prepare themselves for manufacturing and administering of COVID-19 vaccine under the VIRAT scheme (COVID-19 Vaccine Introduction Readiness Assessment Tool). There is no assurance of 100% efficacy for the vaccine and its lasting protection. Until some related investigations are carried out, it is a 'wait-and-watch' approach strategy. A parallel best strategy is to step out of the house only if necessary, wear a mask when outside the home, use sanitizers, keep social distance and avoid all crowded places.

Biology behind the virus and diagnosis

The six genome sequences of the human coronaviruses were compared with each other (Table 2). The average genome size is ~28 kb, with 6-11 genes encoding 6-12 proteins. The RNA sequence of SARS-nCoV-2 has ~50-80% identity with other SARS-CoVs. Importantly, there are four major structural proteins common to all these viruses, viz. the Envelope (E), Membrane (M), Nucleocapsid (N) and Spike (S), all respectively sharing >90% identity across these viruses. The conserved domains of the SARS-nCoV-2 spike (**S**) protein sequence were predicted using https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi (Figure 2A). The CoV **S** protein is an envelope glycoprotein and has a role to play in viral attachment and fusion with the host membrane, thereby gaining entry into host cells. It has therefore served as a major target in the development

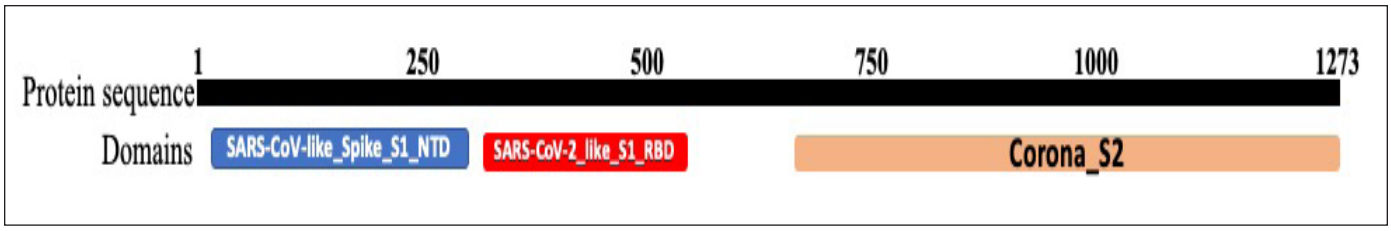

Figure 2*: Domain organization of the spike protein. Note the presence of S1_NTD and S2.*

of crucial antibodies that would inhibit entry and as a candidate for vaccine, too. Synthesized as a precursor protein in the host cells, it is cleaved into the N-terminal S1 subunit (~700 amino acids) and a C-terminal S2 subunit (~600 amino acids) that respectively mediate attachment to the host and fusion with the membrane. Trimers of S1/S2 heterodimers are eventually formed and protrude out on the viral envelope (Figure 1). The receptor-binding domain (RBD) lies in the S1 subunit and is used by the virus to bind to the angiotensin-converting enzyme 2 (ACE2) receptor present on host (human and bat) cells, thus causing viral attachment. Neutralizing this attachment has been one of the strategies of disrupting viral entry into hosts. On the other hand, the S2 subunit contains a hydrophobic fusion peptide and two heptad repeat regions (Figure 2). A multiple alignment of the **S** protein from representative strains of all the six human CoVs was performed (Figure 3). The S2 part of the protein seems to be more conserved than the S1. This raises the possibility that the RBD of S1 is probably evolving at a rapid rate giving rise to various strains since the first SARS-nCoV-2 was isolated by Chinese scientists (Figure 3). This also explains the fact that drugs that targeted the **S** protein RBD of earlier SARS-CoVs did not prove useful therapeutic molecules (peptides and antibodies specific to this region of **S** protein; Wrapp *et al*, 2020). The genome sequence reveals 15 ORFs from 5′ to 3′, viz. ORF1a, ORF1b, Spike (S), ORF3a, ORF3b, Envelope (E), Membrane (M), ORF6, ORF7a, ORF7b, ORF8, ORF9b, ORF14, Nucleocapsid (N), and ORF10. When the sequence is compared with those of other SARS-CoVs it shows an absence of the 8a protein, 8b is relatively longer, 3a is the shortest, and spike protein is the longest. Some tiny insertions and deletions have also been observed (Lu *et al*, 2019).

Given this, specific nucleotide fragments of the genome could in practice be used for identification of the virus in a patient. Being an RNA, it is first converted into cDNA (complementary DNA) using an RNA-dependent DNA Polymerase enzyme. The cDNA can then be used in a real-time PCR machine with specific primers for amplification and identification. The reaction relies on the specific annealing of a primer to a region on the target gene that is extended (synthesized) by a heat-stable DNA Polymerase and through several rounds of the same reaction, amplification occurs. The reaction can be tracked in real-time by using a fluorescent dye or a probe that is sequence-specific and fluorescently labeled along with a quencher. The RdRp, N and E genes have been used in RT-PCR for identification (Corman *et al*, 2020). Additionally, specific regions of ORF1ab and the gene encoding the unique residues of the S protein have also been used. Most of these have now been sold as commercial kits. With use of multiple gene targets, appropriate positive and negative controls in the assay kit, false positives and negatives can be easily eliminated and good sensitivity achieved. Most such kits are now available around the globe (Igloi *et al*, 2020). Since the virus infects the respiratory system, a nasopharyngeal/nasal swab, or lavage from the broncho-alveolar region or tracheal aspirate can be the source of the virus (sample) for analysis. Based on the number of days post infection, the upper or lower respiratory tract could serve as a source. In some cases, the virus has been found in the blood and stool samples, as well. While this is a real-time, rapid and high-throughput assay, viral loads <10 have gone missing. Suggestions to circumvent this problem have also been offered. These include combining with nested PCR or serological testing for specific antibodies towards SARS-nCoV-2. RT-PCR assays involve expensive instrumentation and skilled personnel to perform the test. Commercial antibodies raised against viral proteins that are specific to SARS-nCoV-2 are also being used to indirectly identify the viruses and might prove relatively easier with the personnel with less expensive gadgets. The need for a point-of-care diagnosis that not only reduces the time, but is sensitive and reliable was being sought simultaneously. The real-time Reverse Transcription-Loop-Mediated Isothermal Amplification (RT-LAMP) is a four primer-based PCR that makes use of a single temperature throughout the reaction. Of the four sets of primers, one set, viz. the inner primers (forward and reverse, FIP and BIP) form the base for the heat-stable reverse transcriptase enzyme (Bst from Geobacillus stearothermophilus) with an inherent helicase-like activity to form the first cDNA strand. The second set of primers, viz. the outer primers

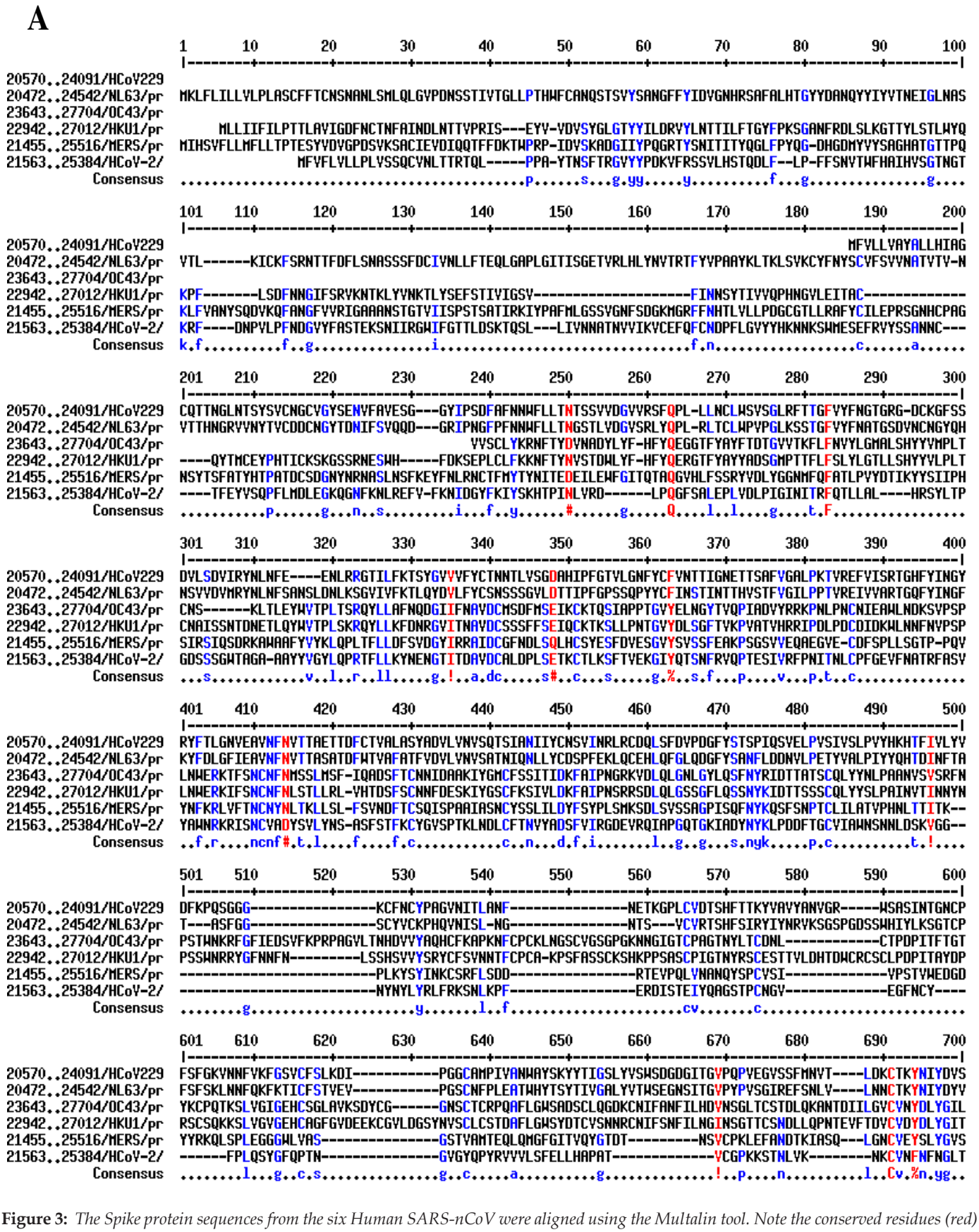

Figure 3: *The Spike protein sequences from the six Human SARS-nCoV were aligned using the Multalin tool. Note the conserved residues (red) in the S2 (B) part of the sequence than that of S1 (A). Multalin tool: http://multalin.toulouse.inra.fr/multalin/*

B

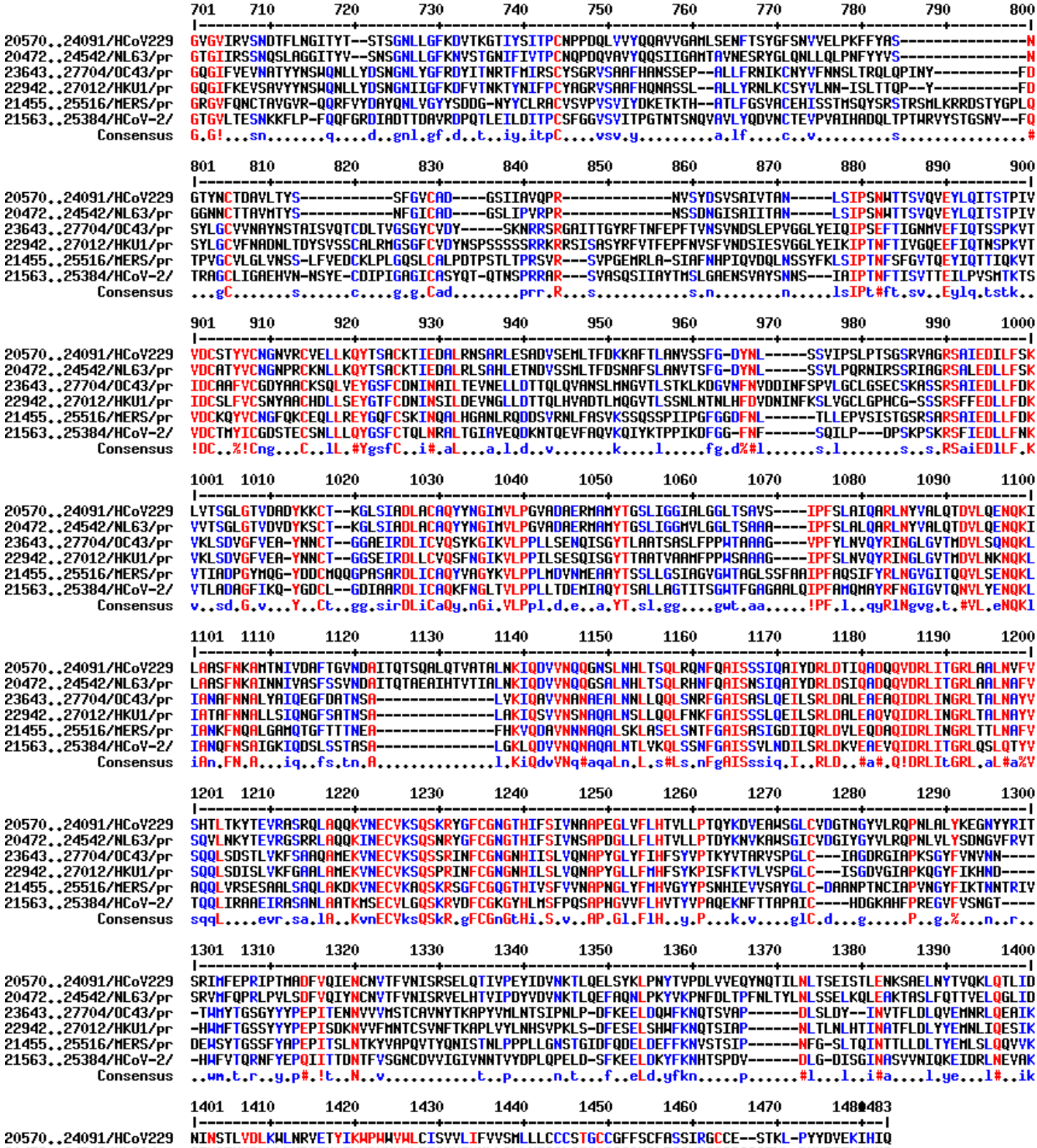
701 710 720 730 740 750 760 770 780 790 800
20570..24091/HCoV229
20472..24542/NL63/pr
23643..27704/OC43/pr
22942..27012/HKU1/pr
21455..25516/MERS/pr
21563..25384/HCoV-2/
Consensus
801 810 820 830 840 850 860 870 880 890 900
901 910 920 930 940 950 960 970 980 990 1000
1001 1010 1020 1030 1040 1050 1060 1070 1080 1090 1100
1101 1110 1120 1130 1140 1150 1160 1170 1180 1190 1200
1201 1210 1220 1230 1240 1250 1260 1270 1280 1290 1300
1301 1310 1320 1330 1340 1350 1360 1370 1380 1390 1400
1401 1410 1420 1430 1440 1450 1460 1470 1480 1483

Table 2: Comparison of some genomic features of human SARS-CoVs.

Name	RefSeq	INSDC	Size (kb)	GC (%)	Protein	Gene
HCoV 229E	NC_002645.1	AF304460.1	27.32	38.3	8	7
HCoV NL63	NC_005831.2	AY567487.2	27.55	34.5	6	6
HCoV OC43	NC_006213.1	AY585228.1	30.741	36.8	10	8
HCoV HKU1	NC_006577.2	AY597011.2	29.93	32.1	8	8
MERS-CoV	NC_038294.1	KC164505.2	30.11	41.2	10	9
SARS-nCoV-2	NC_045512.2	MN908947.3	29.751	38.0	12	11

(FP and BP) participate in the formation of the subsequent rounds of amplicons known as LAMP cycling. This gives rise to double stranded looped DNA structures with alternating inverted repeats recognized by a DNA indicator dye (Thai *et al*, 2004; Zhang *et al*, 2020). Yet another specific method involves Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR), a family of nucleotide sequences that can be recognized by bacterial enzymes known as CRISPR-associated enzymes (Cas). One method makes use of SARS-nCoV-2-specific guide RNA that triggers the excision of reporter RNA sequences (Zhang *et al*, 2020). Yet another makes use of the Cas12a enzyme to cleave the reporter RNA that is further amplified isothermally (Broughton *et al*, 2020). Although ~115 different types of assay have been coined for detecting SARS-nCoV-2, 90% are based on either PCR or RT-PCR, ~6% make use of isothermal amplification tools, 2% make use of CRISPR-based technology and ~2% make use of hybridization techniques such as microarray. While this review is not a comprehensive attempt in diagnostic methods for SARS-nCoV-2, the reader is encouraged to refer to those outlined elsewhere (Carter *et al*, 2020 and References therein). The diagnostic methods that make use of molecular tools in identifying the virus do not however report the progression of the disease *per se* and hence warrant the use of immunological/serological methods that rely on the response of the immune system during the entire course of infection.

The immune system, with organs positioned throughout the human body uses its defence strategies to combat pathogens, including viruses and foreign material to produce antibodies as arsenals against them. In case of SARS-nCoV-2, there is minimal innate response in the first few days of infection. In fact, IgM antibodies are detectable in the first few days to a couple of weeks post infection. It is only after the virus has propagated into the respiratory tract, is the innate response generated strongly. At this point, the disease is relatively mild in majority of the infected individuals, but it is clinically manifest and specific antibodies against the virus can be found at this juncture in the patient's blood. It has been observed that a 'full-blown' disease invokes both innate and adaptive immune responses. The IgM or IgG titres specific to any of the viral antigens (proteins) including, but not limited to S, E, N have been commercially developed by several manufacturers (Du *et al*, 2020). These make use of the conventional Enzyme-Linked Immunosorbent Assay (ELISA), Neutralization assay that makes use of patient antibodies that neutralizes the pathogen (virus) added to a cell-line (Postnikova *et al*, 2019; Whiteman *et al*, 2020), the point-of-care Lateral-flow Immunoassay that makes use of chromatographic technique on whose matrix an immobilized viral antigen captures specific antibodies from the patient sample, Rapid Antigen test developed specifically towards the N protein (Yang and Sun, 2005) and the most sensitive Luminescent Immunoassay (Cai *et al*, 2020). An interesting Biosensor-based technology has been developed by PathSensors, Inc (PathSensors News and Press, March 24, 2020. www.pathsensors.com/psi-sars-cov-2-biosensor/) which uses Surface Plasmon Resonance (SPR) chips as biosensors for capturing the virus specifically. Taken together, a complete prognosis involves enlisting not only the symptoms but also identifying the virus or the antibodies produced by the immune system using serological tests.

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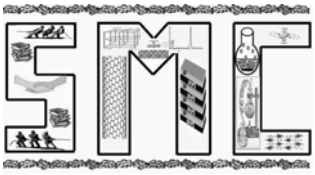

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