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Review Article

Diagnostic Utility of Genes Associated with SARS-CoV-2: A Review

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Abstract

COVID-19 associated infection is caused by the virus SARS-CoV-2, the virus is single stranded positive sense binds with greater affinity to Angiotensin Converting Enzyme-2 present in human cells it is 30 kb pair long RNA genome which take over the host cell machinery, immune system of host cell with the help of accessory proteins leads widespread infection. COVID-19 contains structural protein spike, membrane, Nucleocapsid, and envelope. The epidemiology of COVID-19 is based on dynamics of RdRp, N, and E genes for diagnosis of COVID-19 and knowing the exact understanding of this infection. The protein RNA dependent polymerase (RdRp) is responsible which performs replication of virus in host cell. As there is not any homolog of RdRp is seen till now, so it can be a promising gene for COVID-19 associated diagnosis. There are mainly two techniques responsible for detection of COVID-19 associated infection one is immunological assay performed through antigen and antibody and other is molecular technique based of genome analysis real-time reverse transcriptase polymerase chain reactions (RT-PCR). Among both of them more reliable is RT-PCR which is based on diagnosis of RdRp gene, N-gene, E gene. Envelope protein is involved in assembly, budding and pathogenesis of virus also perform viroporin and interacts with host cell and CoV proteins. Nucleocapsid protein is involved in forming complexes membrane of virus and its assembly this protein is also involved in increasing the transcription efficiency of virus, recent studies had revealed that N-proteins are the multifunctional protein. This review article aim is to reveal the functioning of all the three genes (N, E and RdRp) associated SARS-CoV-2 infection.

Keywords: RdRp, N gene, E gene, Immunology.

INTRODUCTION

Coronavirus are the virions with diameter of 125 nm and spherical structure as reveled by the studies conducted recently with the help of tomography and cryo-electron microscopy [1]. The coronavirus feature club shaped is predominantly known with the spikes emerging through virion surface, spike are the one of the known defining feature of virion and gave it appearance of solar corona prompting the name coronavirus, virion consist envelope and it contains nucleocapsid. This is not found commonly in RNA virus with positive- sense and more common in RNA viruses with negative-sense. Coronaviruses found to be emerged as zoonotic virus in previous years and had leads to illness from common cold to harmful disease like Middle East Respiratory Syndrome (MERS) and Severe Acute Respiratory Syndrome (SARS) [2, 3]. SARS-CoV are transmitted from cats to humans and MERS are transmitted through camels to humans. Various other coronaviruses are still found to be circulating in animals which are not present in humans till now [4]. Among various coronaviruses newly emerged COVID-19 was firstly found in month of December (2019) and was not reported previously in humans. This virus was several times renamed after its discovery, firstly as β-coronavirus in Wuhan, after that on 12thJanuary 2020 World Health Organization (WHO) renamed it as 2019-novel coronavirus

(2019-nCoV). On 11th Feb 2020 the study group of Coronavirus named it SARS-CoV-2and the disease caused by virus called COVID-19 [5].

COVID-19 on 11th March 2020 was declared as global pandemic by World Health Organization (WHO), its main targets is respiratory organ with symptoms of fever, cough, dyspnea and dizziness. All of these symptoms vary from one to other human beings from mild to moderate with acute respiratory distress syndrome (ADS) syndrome to deaths few times. Its origin is not confirmed, its phylogenetic studies shown that it transmit through bats to humans which is not clear. SARS-CoV-2 consist largest RNA virus of genome size 31 kb all coronavirus strains consist of cap and poly (A) tail. Once coronavirus enters in the host cell its genomic RNA encodes structural protein it makes the particles of virus, non-structural protein which directs virus transcription, assembly, replication and controlling host cell and accessory proteins function has not been known. The coronavirus genome consist open reading frames ORF1 ab which is largest gene with overlapping ORF which encodes polyproteins 1ab and 1a, these polyproteins are cleaved to produce 16 non-structural proteins. Among all of the non-structural proteins RNA dependent RNA polymerase (RdRp) is most important for studying the functioning of COVID-19. RdRp is associated with replication and transcription of coronavirus, 3'end of the genomic RNA encodes all structural proteins which are spike (S), membrane (M) envelope (E) nucleocapsid protein (N) and various accessory proteins. SARS-CoV-2 enter in host cell through binding angiotensin I converting enzyme 2 (ACE-2-receptor for glycoprotein of spikes of human coronavirus). The examination of this binding process both functionally and structurally reveals that due to conformational alterations the entry of virus in host cell occurs, this process is improved by serine protease 2 host transmembrane after the entrance of virus in host cell it uncoats and release ssRNA in cytoplasm where it uses host cell translational machinery and translate viral mRNA into large polypeptide. These all polypeptides are cleaved through the specific protease in all the protein component of SARS-CoV-2. At that time ssRNA uses RdRp of its own for synthesizing more copies of ssRNA molecules. All 4 proteins spike, membrane, envelope, nucleocapsid and ssRNA are assembled matured and packed into new copies of virus which can exit the host cell by exocytosis.

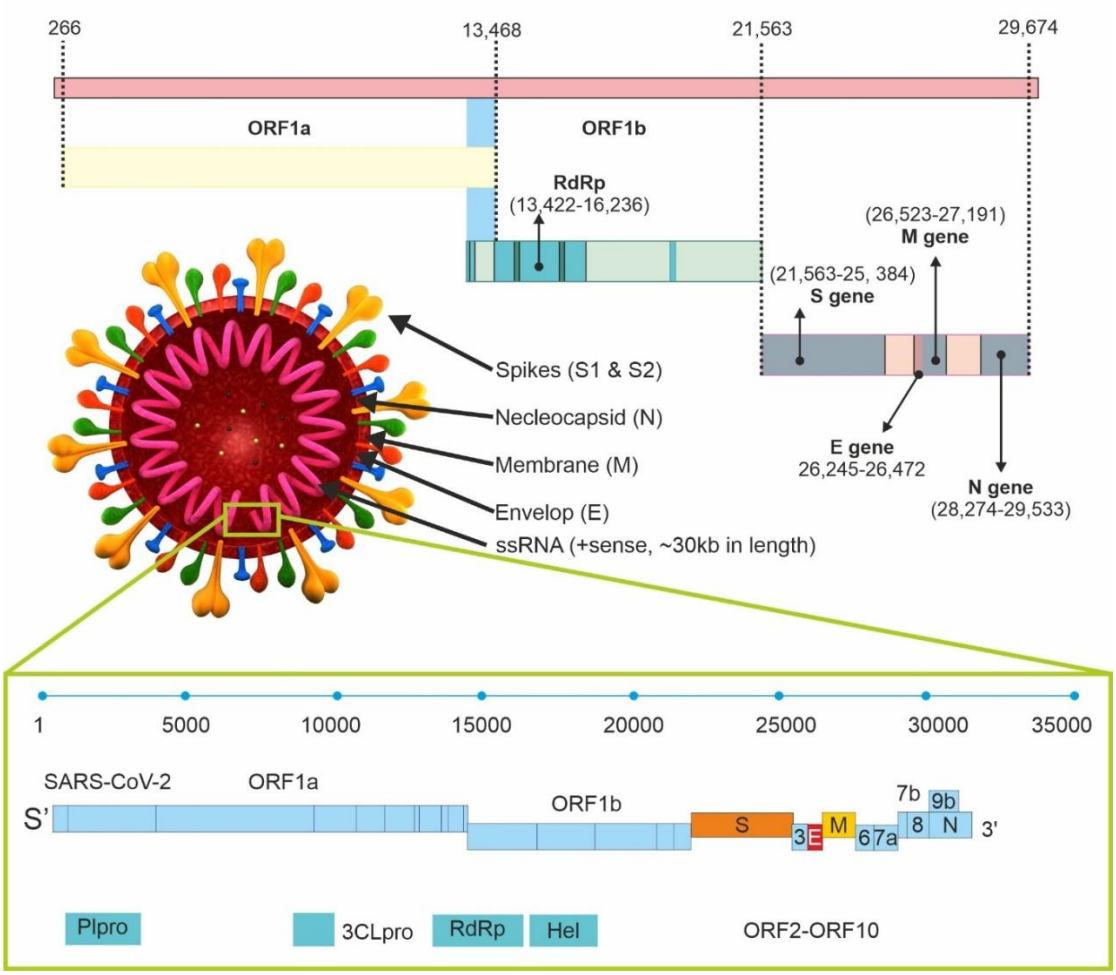

Fig 1:-Structure of COVID-19 virion and genome

The genome of covid-19 is single stranded positive RNA virus its 5' encodes replicase Protein from ORF1a and 1b through ribosomal frame shifting process which generates two poly protein which are further processed by papain like protease (Plpro) and 3C like Protease (3CLpro) it is sometimes referred as main protease (Mpro). ORF1ab polyprotein produces RNA dependent RNA polymerase which performs replication gene and at 3' end structural proteins are encoded S gene encodes spike protein, E gene encodes envelope protein, N gene encodes Nucleocapsid protein. Spike protein helps the virus for taking entry into the host cell by binding to angiotensinogen converting enzyme -2 through activation of TMPRSS2. Envelope protein performs assembly, budding and pathogenesis membrane Protein performs works as viroporin, nucleocapsid binds to the virus and leads to helical nucleocapsid formation. Among all of these few accessory proteins are also synthesized from ORF-2 -10 which exact function is not known.

Immune response against covid-19 infection

This disease effect body of human being very intensively if gets severe as this virus consist spike protein on its surface which gets internalized into the host cell by attaching to receptor Angiotensin-converting enzyme (ACE2). The enzyme furins present in host cell which play important role in virus for entering into the host cell [6]. Further the virus propagate through limited response of immune system which can be diagnosed through nasal swab and after propagation the virus reaches respiratory tract where it face more strong immune response. COVID-19 can be exhibited clinically at this stage and the innate immune response associated with cytokine can anticipating for consequent clinical course [7]. This disease is mostly mild and confined only to conducting airways upper portion [8] The infected patients approximately 20% form pulmonary infections and few of them forms dreadful disease [9]. Various studies revealed that persons suffering from comorbidities like hypertension, heart disease were more prone to forming moderate stage for this disease and finally leads to death [10-12]. The mechanism through which body fight against any infection is immune system also in case of SAR-CoV-2 infection the mechanism of immune system is a main factor which enhances the understanding for its diagnosis and prognosis. As there is yet not any medicine or vaccine registered for this disease so the best possible way to escape from this virus is to enhance your immune system because it is the weapon for fighting against any kind of infection (eg: virus, bacteria, fungi, protozoan and worms). As it had been known that as long as immune system is strong covid-19 infection remain unnoticed. There are 2 types of immunity innate, adaptive Immunity and the scenario of immune system if anyone get infected with COVID-19 will be. After getting infected by any viral disease the immune response is mediated by B-cell and T-cell which produces antibodies for specific antigen. The antibodies are very efficient in entirely blocking the entry of virus in the host cells and play intensive role in providing protection and at later stage prevent the relapse of viral infection. As we known the immunity are achieved through two methods one cell mediated and other humoral immunity, cell mediated immunity are seen in the infected cells which is mediated by T-cell and humoral immunity is seen in body fluids. Adaptive immunity is mediated by both types of T-cell T-helper and T-cytotoxic cells which play role in clearance of viral infected cells [13]. As before emergence of SARA-CoV-2 other strains of coronavirus MERS and SARS were existing which helped in acquiring knowledge of this virus and its escaping process from hosts immune response. Notable there was similarity in RNA sequence of coronavirus of MERS strain 50% and SARS-CoV 80% [14]. The virus SARS-CoV-2 consist additional genomic regions in comparison of SARA-CoV and MERS. Its evasion strategies found to be similar with other strains but many mechanism remains unknown [15, 16]. The result of viral infection heavily depends on mounting effect capability of antiviral response at time which can control the spreading of virus and can limit the injuries associated with organ and speedy recovery. COVID-19 induced immune response is both innate and adaptive and there are mainly 3 components which are crucial for this disease. Cytotoxic T-cell with CD-8 cell surface marker plays important role in defense from the virus resulting in apoptosis of infected cell and interaction of dendritic and macrophage cells with virus result in subsequent adaptive immune response. Also interferon system play role in response associated with infection of COVID-19 which is innate immune response inhibits the replication of virus in early phase. When coronavirus enter the cell body central part make antiviral immunity on the basis of interaction in between antigen and antigen presenting cell. The cells infected by COVID-19 disease are mainly recognized through cytotoxic T-cell and major histocompatibility complex (MHC) in case of cytotoxic T-cell antigen presentation depends on MHCI but in few cases where helper T-cell are present MHCII contribution is also seen. Various reports are known on the basis of relationship between several MHC polymorphism and their vulnerability for various coronavirus strains but less is known about its involvement in SARS-CoV-2 [17-19]. The invasiveness of this disease virus can be identified through innate immune system response through pathogen associated molecular patterns (PAMPs) in coronavirus PAMPs are basically the genomic RNA which is recognized through endosomes RNA receptor like TLR7,8,3 [20]. It can result in vigorous response in innate immune system cells to virus. Further leads in production of huge amount of interferons type I contains antiviral function. The appropriate innate immune response requires downstream cascade and interferons response. Interferon functions by directly interfering the replication ability of viruses and prevents generation of

multiple copies of virus in infected cells. The mechanism of evasion of SARA-CoV-2 is known to be somewhat similar to other strains of coronavirus SARS-CoV and MERS. The evasion strategy of SARA-CoV-2 involve inhibition of type-1 interferons recognition and downregulation of MHCs both class I and II molecules and signaling associated with interferons. As due to deregulation of Antigen Presenting Cell T-cell activation is diminished. On the other side humoral response against SARS-CoV-2 works by releasing antibodies especially IgM in the COVID-19 infected persons in few days of infection, further with by IgG production in a week [21-23]. As SARA-CoV-2 encodes various proteins both structural and non-structural among them nucleocapsid and spike protein are prominently immunogenic antigen. Primarily after COVID-19 infection antibody against nucleocapsid appears and present as a reliable and early serum marker for viral infection and later antibody in opposition of spike proteins forms and bind with envelope of virus. Current studies in this respect revealed that convalescent serum consist antibodies which counterbalance SARS-CoV-2 virus in cell cultures. So, antibody IgG against spike protein is found as both exposure marker and recovery marker of COVID-19 infections.

Beside this, laboratory findings revealed that 80% of COVID-19 cases include leukopenia and lymphopenia and depletion in CD-4 and CD-8 lymphocytes in inclusion of mild thrombocytopenia. Many investigators found changes in neutrophil/lymphocyte ratio in severe patients of COVID-19 [24-26]. Inflammatory markers were also found to be increased in COVID-19 cases like erythrocyte sedimentation rate (ESR), lactate dehydrogenase (LDH), C-reactive protein (CRP), aspartate aminotransferase (ASAT), troponin, ferritin, creatinine kinase (CK) and D-dimer. In severely ill patients high level of cytokine and tumor necrosis factor (TNF) had been observed (IL2, IL4, IL6, IL7, IL10). The severe acute respiratory syndrome consisting patients were observed with cytokine storms with previously seen cytokine and some other like CCL2, CCL3, CCL5, CXCL10 which causes various organ failure and finally death [27, 28]. As RT-PCR has been revealed as standard method for diagnosis of COVID-19. This testing is done for the 3 target in the virus (RdRp), Nucleocapsid (N), Envelope (E).

COVID-19 associated Genes and their processing

The genome of COVID-19 contains 2 large ORF-1a and 1b gene these ORF encodes non-structural protein including RNA dependent RNA Polymerase (RdRp) and at the other 3'end with small structural genomic region encodes spike (S), Nucleocapsid (N) Envelope (E) Membrane (M) gene . E gene encodes envelope protein, M gene encodes membrane protein, ORF 1a and 1b encodes RdRp, S gene encodes spike protein. Among all these Spike protein is involved in causing infection by entering in host cell with 5 accessory proteins which are ORF3a,6,7a, 8 and10 [29]. The Spike protein of virus combine with ACE2 receptor which results in conformational change in the virus and fusion with host cell membrane. The entry of cell needs the action of TMPRSS-2 protease which regulates the S-protein cleavage [30, 31]. The genes consist the information required for processing of functional molecules called proteins. So, all of these genes of SARS-CoV-2 infections are processed for forming proteins.

Among all the structural proteins Spike proteins uses N-terminal signal sequence for accessing its entry in Endoplasmic reticulum which is N-linked glycosylated [32]. S-protein is fusion protein and it mediates the attachment of virus to host receptor [33]. In various coronavirus Spike proteins is cleaved through furin-like protease into large receptor binding domain S1 and S2 form stalk of spike molecule. Membrane protein is abundantly found protein in the virion of (25-30 kDa) approx small in size with 3 transmembrane domains and give virion its shape. Despite being translated co-translationally M protein do not have signal sequence. Studies regarding M –protein shown that it exist as a dimer in the virion and adopt 2 distinct conformations allowing it to enhances the membrane as well as Nucleocapsid curvature [34]. Envelope protein is present in minute quantity in virion this protein is found in highly divergent form with common architecture [35]. E protein associated topology is not known very well but it has N-terminal ectodomain and C-terminal endodomain with ion channel activity. Recombinant viruses in opposition of other structural protein if lacking envelope protein is always found to be lethal [36]. E protein play role in causing infection by facilitating the assembly and release of the virus and also other functions.

Nucleoprotein is composed of two domain N-terminal and C-terminal and both of the domain have the capability of binding RNA in vitro but both of them binds RNA with distinct mechanism, optimal binding of RNA needs the contribution from both domain [37]. Nucleoprotein isphosphorylated and this is involved in changes in the structure and inducing the affinity for viral versus non-viral RNA [38]. Nucleoprotein have 2 RNA substrates TRSs and genome packaging signal [39]. N protein also binds to non-structural protein-3 which is a major component M protein and replication complex [40]. All these interaction of protein results in viral genome binding with replicase transcriptase complex and eventually packaging of genome encapsidated into virus particles. The hemagglutinin-esterase (HE) structural protein is present subset of β-coronaviruses binds to sialic acid on surface glycoprotein, consist acetyl-esterase activity [41]. All of these processes enhances the Spike protein associated entry in host cell and spreading the infection of virus through mucosa [42]. Synthesis of viral RNA is

performed by translation followed by assembly of viral replicase complex. Viral RNA forms both kinds of RNA genomic and sub-genomic RNA this provides messenger RNA for both accessory and structural gene which is present at downstream replicase polyprotein. These both RNA are produced through negative strand intermediate. In comparison of negative strand with positive strand abundance present only 1% and consist both poly-uridylate and anti-leader sequences [43]. At 3'end hypervariable region, stem-loop and pseudoknot is present but stem loop and pseudoknot not able to form simultaneously because they both overlap at 3'end [44]. Hence, these variable structure helps in regulation of alternate stages of RNA synthesis, exact role and mechanism is still not known. The most new feature of replication associated with coronavirus is leader and transcription regulatory sequence associated fusion at the time of production of subgenomic RNAs. It was originally found in +ve strand production, but now it is known to be present at the time of discontinuous extension of –ve strand RNA [45]. RdRp inhibits at any one trinucleotide repeat sequence is proposed in current model, further this inhibition of RdRp gene either elongates continuously to upcoming trinucleotide repeat sequence or escapes to amplify the at 5'end leader sequence of the genome. There is much evidence supporting this model in inclusion of anti-leader sequence presence at 3' end of the subgenomic RNAs negative strand. Coronavirus has also prominently known for their capability of recombination both homologous and non-homologous [46].

Recombination is a prominent factors in evolution of virus also the bases for recombination of target RNA. The reverse genetic technique is utilized for engineering of 3'end of genome of viral recombinants. Following synthesis and replication of sub-genomic RNA structure protein of virus are translated and inserted in endoplasmic reticulum (ER). All of these protein move through the secretory route of host cell. The genome of COVID-19 is encapsidated through nucleocapsid protein and bud into membrane of secretory pathway consisting structural protein of virus and mature virions are formed.

Membrane protein is associated with protein-protein interaction which is essential for coronavirus virion formation it cannot performed alone by membrane protein as virus-like particles (VLPs) are not formed by membrane protein solely. When both membrane protein and envelope protein are expressed together VLPs are formed showing that these two protein are required for forming coronavirus envelopes [47]. Nucleocapsid protein stimulates the formation of VLP which shows that fusion of secretory pathway with encapsidated genomes which enhances the viral envelopment. Spike protein is inserted into virions at this stage but it is not essential for assembly of virus. Spike protein incorporation in virus required interaction with membrane protein and trafficking to secretory pathway. Membrane protein is found in abundant quantity and envelope protein is found in fewer amounts. It is not known how envelope protein assists membrane protein in inducing membrane curvature. Although few studies revealed that envelope protein prevent aggregation of membrane protein. By the alteration in secretory pathway of host cell envelope protein also performed separate function in stimulating the release of virus. Membrane protein is also involved in binding with Nucleocapsid and it completes the assembly of virion. This interaction of membrane and Nucleocapsid is mapped at to C-terminal of membrane endodomain with carboxy terminal domain of nucleoprotein. Although the exact mechanism of Nucleocapsid complexed with RNA virus and its trafficking to ER-Golgi intermediate compartment (ERGIC) for interacting with membrane protein and incorporated into envelope of virus. The virions assembled and transported at the cell surface and further fuse with vesicles and finally exocytosis occurs. This process is unknown whether virions of SARS CoV-2 utilizes conventional pathway for transporting the large protein from Golgi or they have diverted a different way for its own exit.

Diagnosis of COVID-19

Basically COVID-19 detection can be performed by two methods which are currently known and available, through genomic RNA of virus or production of antibody when anyone gets infected. Viral RNA can be detected through nucleic acid hybridization techniques. The antibody or antigen can be diagnosed through serological or immunological assay such as ELISA. Both of these categories of diagnosis complement each other. RNA detection of virus results to know the virus active stage and serological assay helps us to identify the people who have developed antibodies to fight the infections. The diagnosis based on RNA virus is Reverse transcription PCR (RT-PCR) is a kind of Polymerase Chain reaction (PCR) which works mainly on the principal of genes amplification. RT-PCR is a method uses enzyme reverse transcriptase for converting RNA to cDNA then further cDNA work as template sequence for PCR reaction. Quantitative PCR amplify the DNA molecule which can be visualized through fluorescent dye or DNA probe with fluorophore-attached. This technique RT-PCR is determined mainly for amplifying the genes, in SARS-CoV-2 infection this technique is used for detecting N, E, and RdRp gene.

Envelope (E) gene

The E gene encodes envelope protein which is short integral membrane protein of 76-109 amino acid with 12 kDa in size [48]. Its primary and secondary structure shows that it consist hydrophilic amino terminus short followed by larger domain which is hydrophobic transmembrane domain ends with carboxy terminal of hydrophilic type which had various proteins [49]. The region transmembrane domain of

hydrophobic consist at least 1 predicted α-helix with amphipathic region which oligomerizes for forming pore in membrane which is ion-conductive [50]. This protein is mainly present in Endoplasmic reticulum and Golgi complex where it performs budding, assembly and intracellular trafficking of coronaviruses [51]. E gene encoded protein of coronavirus is specific in terms of forming homotypic interactions, this permits to oligomerise and formation of viroporin ion channel protein. Biochemical studies revealed the capability of coronavirus envelope protein of forming homo-oligomer is associated with transmembrane domain (TMD). Many investigators have revealed that transmembrane domain residues are essential for coronavirus envelope protein homotypic interaction. Transmembrane domain associated mutation at asparagine to alanine and valine to phenylalanine residues had been found in disrupting the ion channel ability of coronavirus envelope viroporin [52]. Mutation mainly at asparagine and valine appears to be involved in disrupting oligomerisation of coronavirus envelope protein at some level. The monomers appearance in response to valine clearly indicates that this residue perform crucial role in oligomerisation. The capability of CoVE to form homopentameric structure is important in forming functional envelope viroporin of coronavirus. As coronavirus and also other viruses do not consist the machinery for replicating therefore they depend on host cell machinery for multiplying. The role of E gene in causing infection of coronavirus is interaction with 5 proteins of host which is BcL-xL (anti-apoptotic protein), PALS1(tight junction associated protein), sodium/potassium pump (Na/K+) [53]. The interaction between envelope protein and Bcl-xL is found to be associated with lymphopenia induction in coronavirus infected patients this was observed by Teoh *et al*., [54]. Beside enigmatic nature of envelope protein here various research conducted which had propose few function of envelope protein in infection of coronaviruses which are M and E protein interaction between cytoplasmic tails driving virus like proteins (VLP) production which shows that envelope protein participates in assembly of virus. Transmembrane domain hydrophobic portion of envelope protein is crucial for release of virions Eprotein function in pathogenesis of virus from various studies associated with virus revealed that few viral proteins are not associated with any definite function.

In spite of few viral genes deletion in life cycle of virus continues and shows that other viral proteins can repay for the deleted protein [55]. It is also an evidence for varied requirement of E protein for various strains of coronavirus and its reason is not known still. In few strains of coronavirus envelope deletion attenuates the virus where as in few strains virus propagate with envelope protein [56]. For in vitro or in vivo 3a could compensate for envelope protein loss but with different signaling pathway. The dynamics of this protein is not quite clear till now and warrants for further investigations.

Nucleocapsid (N) gene

The Nucleocapsid gene encodes Nucleocapsid protein which is found in abundant amount in coronavirus at the time of virion assembly. It binds to RNA of genome and result in helical Nucleocapsid formation. It has high hydrophilicity of Nucleocapsid protein is found to be contributed with high potential in providing immunity after infection. This protein is found to be highly immunogenic and expressed in vast quantity during covid-19 infection [57]. It has various functions as it is associated in binding with RNA of Coronavirus genome establishing ribonucleocapsid, replication, translation and transcription. In the infected cell nucleoprotein causes deregulation of cell cycle interferon production inhibition (Cyclooxgenase-2-transcription) COX-2 and Activator protein -1 (AP1) upregulation induces apoptosis in serum deficient cells all of these have pathological consequence.

N gene protein is of 46kDa with 422 amino acids, its N-terminal consist amino acids with positive charge which is associated with RNA binding. As predicted that nuclear localization signal is also present in between C-terminus which is lysine rich residue. The biophysical studies was performed by Chang et al. (2006) which showed that N protein is made up of two structural domain which are independent and a linker region, between these two domain a highly disordered region is found which serve as linker region. This region is associated in interaction between both membranes and (hnRNPA1) protein as established by Fang et al 2006, also this region has been known as a hot spot for phosphorylation. Hence, it concludes that this nucleoprotein has been classed into three different regions which are responsible for entirely distinct function in every stage of the virus life cycle. As N-protein is a capsid protein so its primary functions is to pack the genomic RNA in protective form. In order for achieving this N-protein must be provided with 2 distinct features such as , being capable for recognizing the RNA of genome and associate autonomously into oligomer for capsid formation. Among various one of the important properties associated with nucleoprotein is its encapsidation. Many labs have focused on this characteristic for developing the interference strategy which could be helpful for diminishing the propagation of virus. Studies initial performed in the labs by the use of yeast two hybrid assay which shown that N-protein are autonomous they self-associate through in labs by using a yeast two hybrid assay reveled that N-protein self-associate through its Carboxy-terminal end of amino acid (Surjit *et al*., 2004). A similar study was performed by He *et al.*, 2004 through mammalian two hybrid system and sucrose gradient fractionation they also established the same capability of N-protein to self-associate for forming oligomer. Further, mapping reveled the region of interaction with 184-196 amino

acid residue which encompasses the serine-arginine rich motif. Therefore, there were few differences regarding the interactions domain mapped in these studies. Afterwards, many studies biophysical and biochemical were performed extensively which enhanced our understanding of N-protein oligomerisation process. As in summary the study concludes that serine-arginine motif consist affinity of binding but particularly only for centrally present amino acid of another Nucleocapsid-protein molecule in spite of the serine-arginine motif itself. This region interestingly also consist RNA binding motif for N-protein which postulates that there may be a complementary interplay in between RNA-binding and oligomerisation activity of N-protein. Therefore, it revealed that once the protein tetramer is formed by interaction of protein-protein in between Nucleocapsid molecules binding of genomic RNA promotes Nucleocapsid structure assembly. Beside all of this N-protein is also involved in modulating host cell machinery thereby indicating regulatory role during the viral cycle. This protein directly inhibits the complex of cyclin-CDK leads to retinoblastoma protein hypo-phosphorylation and it happens due to up regulation of E2F1. Inhibition of CDK can be done by two ways, indirectly by downregulating the level of CDK-2 Cyclin E, A and another directly by CDK-2 cyclin complex associated binding of nucleoprotein, inhibition of interferon production which is produced at the viral response. Upregulation of cyclo-oxgenase 2 protein pro-inflammatory factor stimulated at the time of viral infection. The Nucleoprotein binds to NFK-B response element directly at COX-2 promoter and activates its transcription. Upregulation of Activator protein-1 was also seen in association of N- protein but its functional aspects is yet to be elucidated.

RNA dependent RNA polymerase (RdRP) gene

SARS-CoV-2 genome contains ORF1a/1b which encodes nonstructural protein including RdRp gene, which is translated to RdRp protein. It is associated with replication of genetic material of virus inside the cell to produce mass of proteins. The newly form genetic material and proteins then coalesce into new viral particles that come out from host cell ready to infect neighboring cells. RdRps are encoded by all RNA virus and few DNA virus which participates in transcription and replication of virus [59]. RdRps viral genome in all the viral classes can be positive, negative and dsRNA it share tertiary structure and multiple sequence motif. RdRp structure resembles a right hand with finger, thumb, and palm domains completely. Among 5 in 7 RdRp consist conserved domain in palm while other two in finger domain [60]. The RdRp core conserved domain and conserved RdRp core and its related motif are catalytic important for function and helps in targeting of drugs associated with COVID-19 treatment. At the time of host cell of COVID-19 infection viral gene areinvolved in gnome replication machinery by combining with other elements [61]. In COVID-19 RdRp gene is one of the target associated with diagnosis by the RT-PCR technique, it is a variant of PCR method which utilizes enzyme reverse transcriptase for converting RNA into cDNA. On the other side Quantitative real time PCR recognizes the DNA molecule with fluorescent dye or DNA probe attached with fluorophore.[62] Real-time PCR (RT-PCR) is performed by the process of RNA isolation, synthesis of cDNA with enzyme reverse transcriptase, mixing buffer, primer for the target portion of gene, deoxynucleoside triphosphate, fluorescent dye, main replicating enzyme DNA polymerase, then incubating the mixture with distinct temperature for performing thermal cycling and measurements through fluorescence for calculating cycle threshold Ct. [63] Result of PCR is considered positive if Ct value will be found to be lower than 40 [64]. This ct value diminishes at the third week of COVID-19 infection and at later stages not be detected. The positivity of PCR decreased steadily in sputum and could be positive, it is found to be negative in nasopharyngeal swab.COVID-19 associated test of RT-PCR gives positive results before the one day initiation of symptoms but in many cases due to less load of virus patients infected with COVID-19 were not detected before the start of symptoms.[65] SARA-CoV-2 associated detection in samples of saliva was performed with viral culturing methods and RT-PCR both which shown positive results in 91.7% patients and after hospitalization decreased in RNA level was observed. On the other hand viral culturing reveled live viruses presence in saliva of few patients. [66] RT-PCR test was done from swab of nasopharynx and saliva for detecting the RdRp, E,N genes in COVID-19 infection. This reveled sensitivity for 89 to 77% for saliva and nasopharyngeal swabs samples. There was not any significant difference found in between the specimen of saliva and swab. The sample when collected afterwards at the time of illness there was greatest difference observed between both the samples may be due to less virus load at this stage. The replacement of saliva with nasopharyngeal swab when any patient cannot tolerate the nasopharyngeal swab mainly when concentration of virus is greater in upper portion of respiratory tract. The swab of nasopharyngeal should be checked for second specimen in patient with high index of clinical suspicion and their swab of saliva is negative [67].In comparison with RdRp reported in 30 European laboratories and many more with which reveled lowest detection limit in RdRp/Hel. COVID-19 association with RdRp/Hel was shown to positive for the patients whom RdRp-P2 assay was negative [68] Corman *et al*., had designed the SARS-CoV-2 associated workflow with the nucleic acid technology as we know due to the nature of this virus there is major drawback of non-availability of samples or virus isolates of patients [69]. They utilized the envelope gene as primary line of screening for the assay and for confirming the test from RdRp gene assay. The best results from E gene and RdRp gene and N gene show slower sensitive. The limitation of detection for E gene

and RdRp gene was found to be 3.9 and 3.6. They also formed a specific probe that is RdRp-P2 for specifically diagnosis of genomic RNA of COVID-19 infections [70]. All of these concludes that COVID-19-RdRp/Hel assay was found to be significant highly sensitive than RdRp-P2 assay for diagnosis of the RdRp gene of COVID-19. The sensitivity of both assays was not found to significantly different in the sample of sputum and rectal swabs.

As RdRp initiates governs and elongates the RNA strand includes the 100-100 nucleotides addition. The analogs of nucleotide can inhibit the elongation of RNA catalyzed through RdRp once it is inserted in newly formed RNA chain. RNA of virus synthesis through RdRp both de novo primer and primer dependent at 3'end of RNA template. Primer independent synthesis of RdRp capable of synthesis of RNA by nucleotide usage in absence of primer. On the other hand primer dependent RNA synthesis is dependent oligonucleotide of few nucleotide and serve as aprimer for RNA synthesis. RdRp is essential in virus life cycle and do not have any host homolog. This gives an opportunity developing antiviral drug and decrease chances of getting affected in humans. Usually RdRp are known to be low fidelity enzyme because of lack in proofreading activity. Thus there are various chain terminators or mutagens which were found to the target the RdRp enzyme. There were few nucleoside analogs in form of derivatives of adenine or guanine which will inhibit the RNA synthesis from RNA viruses including human coronaviruses. As RdRp catalyzes the incorporation of Nucleotide triphosphates during elongation so the Polymerase elongation template element assay is utilized for detecting this elongation activity of RdRp. This test approaches the 5' end of oligonucleotide of an RNA template which is fluorescent labeled probe for measurement polarization of fluorescence. The signal of polymerization through probe enhances as its mobility deceases with elongation of newly form RNA chain which is produced from the enzyme RdRp. The inhibition of enzyme RdRp by any inhibitor reduces its fluorescence polarization signal as the complementary RNA chain elongation stops. Another assay which was utilized for RdRp is alkaline phosphate- coupled polymerase assay based. This assay approach is also based on nucleotide analog as polymerase proceeds, modified nucleotide analog is incorporated which leads to fluorophore emission allowing RdRp detection. As if a modified analog 2- [2-benzothiazoyl]-6-hydroxybenzothiazole) which is conjugated with adenosine triphosphate had been inserted in RNA chain which is growing and synthesizing through RdRp results in formation of pyrophosphate as a byproduct. Which reacts subsequently with alkaline phosphate for producing highly fluorescent BBT. A study was conducted in this context by screening 40,572 compounds utilizing this assay which revealed that RdRp as dengue virus inhibitor. One application of this assay is to screen hepatitis C virus inhibitors RdRp. The acquired knowledge from the other strains of coronaviruses shows that RdRp protein alone cannot exhibit more activity without its complex partners. An assay Fluorometric RdRp Activity is largely been used for RNA and DNA both. It utilizes fluorophore to detect both dsRNA and ssRNA template. It application is found in detecting the inhibitor of virus like hepatitis C and RdRp by the use of RNA template Poly C (one strand is polyinosinic acid and other is polycytidylic acid involve in inducing interferon formation which is experimentally involve as antiviral agent). RdRp catalyzes the synthesis of dsRNA in primer independent manner which was revealed by the fluorescent dye PicoGreen. It is also found to be adapted effortlessly for RdRp inhibitors compound screening and also various other viruses.

As the assays which are cell free have several advantage for high productivity and capability for screening but it has certain loop holes like enzyme assay do not need any compound to pass through the membrane so if any inhibitor identified through the assay will not enter the cells will be inactive in cell based assay and in animals models. The cellular metabolic effects will be not measured and enzyme assay will not utilized for determining the activities of prodrugs (is a compound which after administrating is metabolized into active drug) which is hydrolyzed by cellular enzyme for converting into active component. Few enzymes which are found in cell free enzyme are found to be active probable may not be found to be active in cell based enzyme because it can be inactivated through intracellular enzymes. In assay based on cells transfection is performed for viral RdRp expression or complex is formed with luciferase RNA in orientation of –ve sense which is then transcribed to +ve sense luciferase RNA through RdRp for protein synthesis of reporter [71]. It results are obtained through the measurement of luciferase signal by its intensity and the signal is proportional to intracellular RdRp activity level. RdRp has been utilized for one of the targets for developing drug for SARS-CoV-2 due to its virus replication capability and one of the critical proteins for increasing copies of COVID-19 virus. A main advantage of targeting RdRp as a drug has been in frequent progress for forming drug like inhibitor of RNA polymerase in few years back. RdRp assay will also be crucial for designing a new candidate drug for COVID-19. The structural information of RdRp will be helpful in explaining the halting process of antiviral drug remdesivir and favipiravir associated with inhibition of RdRp basically this drug is antiviral [72]. Additionally RdRp inhibitor also targets other protein of virus which can provide better COVID-19 therapy efficacy for its treatment.

CONCLUSION

As we all are aware of currently known respiratory disease which is caused by severe acute

respiratory syndrome coronavirus-2 (SARS-CoV-2) known as COVID-19. This disease had progress rapidly and became a global health problem. SARS-CoV-2 virus with RNA genome consist positive sense single stranded and consist two third of portion associated in encoding RdRp and two large non-structural protein involved in host response modification. The other part encodes four structural proteins spike, envelope, membrane, Nucleocapsid and other proteins. Among all the proteins three of them are majorly involve in diagnosis of COVID-19 are RNA dependent RNA polymerase (RdRp) Nucleocapsid protein, Envelope protein. The enzyme RdRp is associated with host cell replication of virus and is found to be the most important fascinated and promising for discovery of drug targets for SARS-CoV-2. As RdRp do not have any host cell homolog so its inhibitor can be developed for diagnosis of SARS-CpoV-1 which will enhance the potential and decrease the target effects against the host proteins and are effective and safer for treating COVID-19 patients. On the other side envelope and Nucleocapsid protein are involved in different aspects. Envelope protein is involved in envelope formation budding and pathogenesis, Nucleocapsid in virion assembly enhancing virus transcription.As recent studies had revealed that Nucleocapsidis multifunctional protein. So it concludes that these all among N gene, E gene and RdRp for diagnosis all of them are important and for establishment of vaccine for COVID-19 RdRp is the major gene.

ABBREVIATIONS

RdRp - RNA dependent RNA polymerase
ACE-2- Angiotensin I converting enzyme 2
MERS- Middle East Respiratory Syndrome
SARS- Severe Acute Respiratory Syndrome
TMPRSS 2- Transmembrane protease, serine 2
HE - Hemagglutinin-esterase
VLPs- Virus-like particles
BcL-xL- B-cell lymphoma-extra large
CDK- cyclin-dependent kinases
NFK-B- nuclear factor kappa-light-chain-enhancer of activated B cells
COX 2- Cyclooxygenase-2
ORF- Open reading frame

REFERENCES

1. Huan, P., Wang, H., & Cao, Z. (2018). A rapid and specific assay for the detection of MERS-CoV. *Front Microbiol*, 20, 33-37.
2. Yin, Y., & Wunderink, R. G. (2018). MERS, SARS and other coronaviruses as causes of pneumonia. *Respirology*, *23*(2), 130-137.
3. of the International, C. S. G. (2020). The species Severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. *Nature microbiology*, *5*(4), 536-544.
4. Lu, R., Zhao, X., Li, J., Niu, P., Yang, B., Wu, H., ... & Tan, W. (2020). Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *The lancet*, *395*(10224), 565-574.
5. Centers for Disease Control and Prevention. (2020). First travel-related case of 2019 novel coronavirus detected in United States. 1, 21.
6. Walls, A. C., Park, Y. J., Tortorici, M. A., Wall, A., McGuire, A. T., & Veesler, D. (2020). Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein. *Cell*, *181*(2), 281-292.
7. Tang, N. L. S., Chan, P. K. S., Wong, C. K., To, K. F., Wu, A. K. L., Sung, Y. M., ... & Lam, C. W. K. (2005). Early enhanced expression of interferon-inducible protein-10 (CXCL-10) and other chemokines predicts adverse outcome in severe acute respiratory syndrome. *Clinical chemistry*, *51*(12), 2333-2340.
8. Wu, Z., & McGoogan, J. M. (2020). Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in China: summary of a report of 72 314 cases from the Chinese Center for Disease Control and Prevention. *Jama*, *323*(13), 1239-1242.
9. Mason, R. J. (2020). Pathogenesis of COVID-19 from a cell biology perspective. *Eur Respir J*, 55, 2000607.
10. Chen, N., Zhou, M., Dong, X., Qu, J., Gong, F., Han, Y., ... & Zhang, L. (2020). Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *The lancet*, *395*(10223), 507-513.
11. Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y., ... & Cao, B. (2020). Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *The lancet*, *395*(10223), 497-506.
12. Qin, Y., Zhen, F., & Jiao, X. (2020). April Analysis of risk factors of severe COVID-19 patients. *Research Square*, 22, 1-14.
13. Kumar, S., Nyodu, R., Maurya, V. K., & Saxena, S. K. (2020). Host immune response and immunobiology of human SARS-CoV-2 infection. In *Coronavirus disease 2019 (COVID-19)* (pp. 43-53). Springer, Singapore.
14. Lu, R., Zhao, X., Li, J., Niu, P., Yang, B., Wu, H., ... & Tan, W. (2020). Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *The lancet*, *395*(10224), 565-574.
15. Prompetchara, E., Ketloy, C., & Palaga, T. (2020). Immune responses in COVID-19 and potential vaccines: Lessons learned from SARS and MERS epidemic. *Asian Pacific journal of allergy and immunology*, *38*(1), 1-9.
16. Felsenstein, S., Herbert, J. A., McNamara, P. S., & Hedrich, C. M. (2020). COVID-19: Immunology and treatment options. *Clinical immunology*, *215*, 108448.
17. Wang, S. F., Chen, K. H., Chen, M., Li, W. Y., Chen, Y. J., Tsao, C. H., ... & Chen, Y. M. A. (2011). Human-leukocyte antigen class I Cw 1502

and class II DR 0301 genotypes are associated with resistance to severe acute respiratory syndrome (SARS) infection. *Viral immunology*, *24*(5), 421-426.
18. Keicho, N., Itoyama, S., Kashiwase, K., Phi, N. C., Long, H. T., Van Ban, V., ... & Quy, T. (2009). Association of human leukocyte antigen class II alleles with severe acute respiratory syndrome in the Vietnamese population. *Human immunology*, *70*(7), 527-531.
19. Hajeer, A. H., Balkhy, H., Johani, S., Yousef, M. Z., & Arabi, Y. (2016). Association of human leukocyte antigen class II alleles with severe Middle East respiratory syndrome-coronavirus infection. *Annals of thoracic medicine*, *11*(3), 211-213.
20. Akira, S., & Hemmi, H. (2003). Recognition of pathogen-associated molecular patterns by TLR family. *Immunology letters*, *85*(2), 85-95.
21. Zhou, P., Yang, X. L., Wang, X. G., Hu, B., Zhang, L., Zhang, W., ... & Shi, Z. L. (2020). A pneumonia outbreak associated with a new coronavirus of probable bat origin. *nature*, *579*(7798), 270-273.
22. Meyer, B., Drosten, C., & Müller, M. A. (2014). Serological assays for emerging coronaviruses: challenges and pitfalls. *Virus research*, *194*, 175-183.
23. Huang, L. R., Chiu, C. M., Yeh, S. H., Huang, W. H., Hsueh, P. R., Yang, W. Z., ... & Chen, P. J. (2004). Evaluation of antibody responses against SARS coronaviral nucleocapsid or spike proteins by immunoblotting or ELISA. *Journal of medical virology*, *73*(3), 338-346.
24. Singh, A., Shaikh, A., Singh, R., & Singh, A. K. (2020). COVID-19: From bench to bed side. *Diabetes & Metabolic Syndrome: Clinical Research & Reviews*, *14*(4), 277-281.
25. Wang, Y., Wang, Y., Chen, Y., & Qin, Q. (2020). Unique epidemiological and clinical features of the emerging 2019 novel coronavirus pneumonia (COVID- 19) implicate special control measures. *Journal of medical virology*, *92*(6), 568-576.
26. Tan, C., Huang, Y., Shi, F., Tan, K., Ma, Q., Chen, Y., ... & Li, X. (2020). C- reactive protein correlates with computed tomographic findings and predicts severe COVID- 19 early. *Journal of medical virology*, *92*(7), 856-862.
27. Li, X., Geng, M., Peng, Y., Meng, L., & Lu, S. (2020). Molecular immune pathogenesis and diagnosis of COVID-19. *Journal of pharmaceutical analysis*, *10*(2), 102-108.
28. Pan, Y., Li, X., Yang, G., Fan, J., Tang, Y., Zhao, J., ... & Li, Y. (2020). Serological immunochromatographic approach in diagnosis with SARS-CoV-2 infected COVID-19 patients. *Journal of Infection*, *81*(1), e28-e32.
29. Chen, Y., Liu, Q., & Guo, D. (2020). Emerging coronaviruses: genome structure, replication, and pathogenesis. *Journal of medical virology*, *92*(4), 418-423.
30. Hoffmann, M., Kleine, W. H., & Schroeder, S. (2020). SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell*, 22, 54-59.
31. Guo, Y. R., Cao, Q. D., Hong, Z. S., Tan, Y. Y., Chen, S. D., Jin, H. J., ... & Yan, Y. (2020). The origin, transmission and clinical therapies on coronavirus disease 2019 (COVID-19) outbreak–an update on the status. *Military Medical Research*, *7*(1), 1-10.
32. Beniac, D. R., Andonov, A., Grudeski, E., & Booth, T. F. (2006). Architecture of the SARS coronavirus prefusion spike. *Nature structural & molecular biology*, *13*(8), 751-752.
33. Bosch, B. J., Van der Zee, R., De Haan, C. A., & Rottier, P. J. (2003). The coronavirus spike protein is a class I virus fusion protein: structural and functional characterization of the fusion core complex. *Journal of virology*, *77*(16), 8801-8811.
34. Neuman, B. W., Kiss, G., Kunding, A. H., Bhella, D., Baksh, M. F., Connelly, S., ... & Buchmeier, M. J. (2011). A structural analysis of M protein in coronavirus assembly and morphology. *Journal of structural biology*, *174*(1), 11-22.
35. Godet, M., L'Haridon, R., Vautherot, J. F., & Laude, H. (1992). TGEV corona virus ORF4 encodes a membrane protein that is incorporated into virions. *Virology*, *188*(2), 666-675.
36. DeDiego, M. L., Álvarez, E., Almazán, F., Rejas, M. T., Lamirande, E., Roberts, A., ... & Enjuanes, L. (2007). A severe acute respiratory syndrome coronavirus that lacks the E gene is attenuated in vitro and in vivo. *Journal of virology*, *81*(4), 1701-1713.
37. Chang, C. K., Sue, S. C., Yu, T. H., Hsieh, C. M., Tsai, C. K., Chiang, Y. C., ... & Huang, T. H. (2006). Modular organization of SARS coronavirus nucleocapsid protein. *Journal of biomedical science*, *13*(1), 59-72.
38. Stohlman, S. A., & Lai, M. M. (1979). Phosphoproteins of murine hepatitis viruses. *Journal of virology*, *32*(2), 672-675.
39. Molenkamp, R., & Spaan, W. J. (1997). Identification of a specific interaction between the coronavirus mouse hepatitis virus A59 nucleocapsid protein and packaging signal. *Virology*, *239*(1), 78-86.
40. Hurst, K. R., Koetzner, C. A., & Masters, P. S. (2013). Characterization of a critical interaction between the coronavirus nucleocapsid protein and nonstructural protein 3 of the viral replicase-transcriptase complex. *Journal of virology*, *87*(16), 9159-9172.
41. Klausegger, A., Strobl, B., Regl, G., Kaser, A., Luytjes, W., & Vlasak, R. (1999). Identification of a coronavirus hemagglutinin-esterase with a substrate specificity different from those of

influenza C virus and bovine coronavirus. *Journal of virology*, *73*(5), 3737-3743.
42. Cornelissen, L. A., Wierda, C. M., Van Der Meer, F. J., Herrewegh, A. A., Horzinek, M. C., Egberink, H. F., & De Groot, R. J. (1997). Hemagglutinin-esterase, a novel structural protein of torovirus. *Journal of virology*, *71*(7), 5277-5286.
43. Sethna, P. B., Hofmann, M. A., & Brian, D. A. (1991). Minus-strand copies of replicating coronavirus mRNAs contain antileaders. *Journal of virology*, *65*(1), 320-325.
44. Liu, Q., Johnson, R. F., & Leibowitz, J. L. (2001). Secondary structural elements within the 3′ untranslated region of mouse hepatitis virus strain JHM genomic RNA. *Journal of virology*, *75*(24), 12105-12113.
45. Sawicki, S. G., Sawicki, D. L., & Siddell, S. G. (2007). A contemporary view of coronavirus transcription. *Journal of virology*, *81*(1), 20-29.
46. Keck, J. G., Makino, S., Soe, L. H., Fleming, J. O., Stohlman, S. A., & Lai, M. M. (1987). RNA recombination of coronavirus. In *Coronaviruses* (pp. 99-107). Springer, Boston, MA.
47. Bos, E. C., Luytjes, W., Van Der Meulen, H., Koerten, H. K., & Spaan, W. J. (1996). The production of recombinant infectious DI-particles of a murine coronavirus in the absence of helper virus. *Virology*, *218*(1), 52-60.
48. Corse, E., & Machamer, C. E. (2000). Infectious bronchitis virus E protein is targeted to the Golgi complex and directs release of virus-like particles. *Journal of virology*, *74*(9), 4319-4326.
49. Nieto-Torres, J. L., DeDiego, M. L., Verdiá-Báguena, C., Jimenez-Guardeño, J. M., Regla-Nava, J. A., Fernandez-Delgado, R., ... & Enjuanes, L. (2014). Severe acute respiratory syndrome coronavirus envelope protein ion channel activity promotes virus fitness and pathogenesis. *PLoS pathogens*, *10*(5), e1004077.
50. Verdiá-Báguena, C., Nieto-Torres, J. L., Alcaraz, A., DeDiego, M. L., Torres, J., Aguilella, V. M., & Enjuanes, L. (2012). Coronavirus E protein forms ion channels with functionally and structurally-involved membrane lipids. *Virology*, *432*(2), 485-494.
51. Yuan, Q., Liao, Y., Torres, J., Tam, J. P., & Liu, D. X. (2006). Biochemical evidence for the presence of mixed membrane topologies of the severe acute respiratory syndrome coronavirus envelope protein expressed in mammalian cells. *FEBS letters*, *580*(13), 3192-3200.
52. Torres, J., Parthasarathy, K., Lin, X., Saravanan, R., Kukol, A., & Liu, D. X. (2006). Model of a putative pore: the pentameric α-helical bundle of SARS coronavirus E protein in lipid bilayers. *Biophysical journal*, *91*(3), 938-947.
53. Steinmann, E., Penin, F., Kallis, S., Patel, A. H., Bartenschlager, R., & Pietschmann, T. (2007). Hepatitis C virus p7 protein is crucial for assembly and release of infectious virions. *PLoS pathogens*, *3*(7), e103.
54. Teoh, K. T., Siu, Y. L., Chan, W. L., Schlüter, M. A., Liu, C. J., Peiris, J. M., ... & Nal, B. (2010). The SARS coronavirus E protein interacts with PALS1 and alters tight junction formation and epithelial morphogenesis. *Molecular biology of the cell*, *21*(22), 3838-3852.
55. Liu, B., Panda, D., Mendez-Rios, J. D., Ganesan, S., Wyatt, L. S., & Moss, B. (2018). Identification of poxvirus genome uncoating and DNA replication factors with mutually redundant roles. *Journal of virology*, *92*(7), e02152-17.
56. Almazán, F., DeDiego, M. L., Sola, I., Zuñiga, S., Nieto-Torres, J. L., Marquez-Jurado, S., ... & Enjuanes, L. (2013). Engineering a replication-competent, propagation-defective Middle East respiratory syndrome coronavirus as a vaccine candidate. *MBio*, *4*(5), e00650-13.
57. Cong, Y., Ulasli, M., Schepers, H., Mauthe, M., V'kovski, P., Kriegenburg, F., ... & Reggiori, F. (2020). Nucleocapsid protein recruitment to replication-transcription complexes plays a crucial role in coronaviral life cycle. *Journal of virology*, *94*(4), e01925-19.
58. Surjit, M., Liu, B., Jameel, S., Chow, V. T., & Lal, S. K. (2004). The SARS coronavirus nucleocapsid protein induces actin reorganization and apoptosis in COS-1 cells in the absence of growth factors. *Biochemical Journal*, *383*(1), 13-18.
59. Gorbalenya, A. E., Pringle, F. M., Zeddam, J. L., Luke, B. T., Cameron, C. E., Kalmakoff, J., ... & Ward, V. K. (2002). The palm subdomain-based active site is internally permuted in viral RNA-dependent RNA polymerases of an ancient lineage. *Journal of molecular biology*, *324*(1), 47-62.
60. Mestas, S. P., Sholders, A. J., & Peersen, O. B. (2007). A fluorescence polarization-based screening assay for nucleic acid polymerase elongation activity. *Analytical biochemistry*, *365*(2), 194-200.
61. Graci, J. D., & Cameron, C. E. (2006). Mechanisms of action of ribavirin against distinct viruses. *Reviews in medical virology*, *16*(1), 37-48.
62. Kozlov, M., Bergendahl, V., Burgess, R., Goldfarb, A., & Mustaev, A. (2005). Homogeneous fluorescent assay for RNA polymerase. *Analytical biochemistry*, *342*(2), 206-213.
63. De Clercq, E., & Li, G. (2016). Approved antiviral drugs over the past 50 years. *Clinical microbiology reviews*, *29*(3), 695-747.
64. Eltahla, A. A., Lackovic, K., Marquis, C., Eden, J. S., & White, P. A. (2013). A fluorescence-based high-throughput screen to identify small compound inhibitors of the genotype 3a hepatitis C virus RNA polymerase. *Journal of biomolecular screening*, *18*(9), 1027-1034.

65. Kocabas, F., Turan, R. D., & Aslan, G. S. (2015). Fluorometric RdRp assay with self-priming RNA. *Virus genes*, *50*(3), 498-504.
66. Sáez-Álvarez, Y., Arias, A., Del Águila, C., & Agudo, R. (2019). Development of a fluorescence-based method for the rapid determination of Zika virus polymerase activity and the screening of antiviral drugs. *Scientific reports*, *9*(1), 1-11.
67. Gong, E. Y., Kenens, H., Ivens, T., Dockx, K., Vermeiren, K., Vandercruyssen, G., ... & Kraus, G. (2013). Expression and purification of dengue virus NS5 polymerase and development of a high-throughput enzymatic assay for screening inhibitors of dengue polymerase. In *Antiviral Methods and Protocols* (pp. 237-247). Humana Press, Totowa, NJ.
68. Nasiri, A. H., & Nasiri, H. R. (2018). Polymerase assays for lead discovery: An overall review of methodologies and approaches. *Analytical biochemistry*, *563*, 40-50.
69. Corman, V. M., Albarrak, A. M., Omrani, A. S., Albarrak, M. M., Farah, M. E., Almasri, M., ... & Memish, Z. A. (2016). Viral shedding and antibody response in 37 patients with Middle East respiratory syndrome coronavirus infection. *Clinical Infectious Diseases*, *62*(4), 477-483.
70. Gong, E. Y., Kenens, H., Ivens, T., Dockx, K., Vermeiren, K., Vandercruyssen, G., ... & Kraus, G. (2013). Expression and purification of dengue virus NS5 polymerase and development of a high-throughput enzymatic assay for screening inhibitors of dengue polymerase. In *Antiviral Methods and Protocols* (pp. 237-247). Humana Press, Totowa, NJ.
71. Su, C. Y., Cheng, T. J. R., Lin, M. I., Wang, S. Y., Huang, W. I., Lin-Chu, S. Y., ... & Wong, C. H. (2010). High-throughput identification of compounds targeting influenza RNA-dependent RNA polymerase activity. *Proceedings of the National Academy of Sciences*, *107*(45), 19151-19156.
72. Gao, Y., Yan, L., Huang, Y., Liu, F., Zhao, Y., Cao, L., ... & Rao, Z. (2020). Structure of the RNA-dependent RNA polymerase from COVID-19 virus. *Science*, *368*(6492), 779-782.