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Coronavirus: Etiology of the COVID-19 Pandemic

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ABSTRACT

This article provides information on the etiological agent of COVID-19, the Coronavirus also named as SARS-CoV-2. It provides information on the viral taxonomy, structure, viral proteins, transcription and replication of the virus.

1. Introduction

Previous to this pandemic Coronaviruses (CoVs) were never taken seriously especially in humans. They were associated with common cold mostly which usually go unnoticed. However, these group of viruses had a higher profile in the Veterinary Sciences due to some very important diseases caused by them such as, Infectious Bronchitis (IB) that leads to heavy mortality and production losses in poultry, Porcine Haemagglutinating Encephalomyelitis (HEV), Porcine Epidemic Diarrhoea (PED) and Transmissible Gastroenteritis (TGE) leading up to 90% mortality in piglets (Dave, 2005)

It is not the first time that a coronavirus is causing a significant global health problem in the form of SARS CoV 2 and neither would it be the last one. Corona Viruses associated diseases came into prominence in the early 2003 when Severe Acute Respiratory Syndrome (SARS) was reported from the Chinese province of Guangdong and was attributed due to a corona virus. In 2012 out of nowhere Middle East Respiratory Syndrome (MERS) appeared in the Saudi Arabia which too was found to be due to coronaviruses (Lu et al., 2020). In the case of SARS there was more morbidity but less mortality but in the case of MERS there was a greater mortality. Thankfully MERS disappeared mysteriously as it appeared and was never a global threat. But with the SARS CoV 2 we are finding that we are in different situation altogether.

Taxonomy

According to the classification of The International Committee on Taxonomy of Viruses (ICTV), Coronaviruses belong to the subfamily Coronavirinae in the family Coronaviridae. In the family there are four genera; α-coronavirus (alpha CoV) and β-coronavirus (beta CoV) that are probably present in bats and rodents, while δ-coronavirus (delta CoV), and γ-coronavirus (gamma CoV) probably present in the avian species (Perlman and Netland, 2009; Lu *et al*., 2020; Yin and Wunderink, 2018) and a total of thirty eight unique species had been identified (Subissi et al., 2014). Corona Virus subfamily is rapidly expanding with the new generation sequencing applications improving detection and definition of novel CoV species and thus its classification is continually changing.

When we look into the evolution of SARS CoV 2 we find that the virus has a natural as well as zoonotic origin. Two scenarios can plausibly explain the origin of SARS CoV 2; either due to natural selection in an animal host before zoonotic transfer or natural selection in humans following

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zoonotic transfers (Lu *et al*., 2020; Yin and Wunderink, 2018). As of current update we recognize seven types of coronavirus that could infect humans viz., 229E (alpha coronavirus), NL63 (alpha coronavirus), OC43 (beta coronavirus), HKU1 (beta coronavirus), MERS-CoV leading to Middle East respiratory syndrome and SARS CoV, the virus responsible for severe acute respiratory syndrome (SARS) and SARS CoV 2 that started circulating in 2019 causing COVID-19 (Unhale *et al*., 2020).

Structure

Coronaviruses when observed under electron microscope have a typical crown like appearance which is due to the presence of glycoprotein spikes on its envelope (Perlman and Netland, 2009). Coronaviruses genome structure is best known among all RNA viruses. It is an enveloped positive strand RNA virus with the largest known RNA genomes of 30-32 kb with a 5'-cap and 3'-poly-A tail. Two-thirds of RNA encodes viral polymerase i.e., RNA dependent RNA polymerase (RdRp), RNA synthesis materials, and two large nonstructural proteins (ORF1a ORF1b). The other one-third of the genome encodes four structural proteins and those are spike (S), envelope (E), membrane (M) and nucleocapsid (N) proteins and the helper proteins. Although the length of the CoV genome shows great variability for ORF1a/ORF1b and four structural proteins, it is mostly associated with the number and size of accessory proteins (Luk et al., 2019; https://viralzone.expasy.org/785).

Viral Proteins and Transcription

The surface spike (S) protein is involved in the attachment and entry into the host cell and is therefore the main target for neutralizing antibody and antiviral peptides (Ingallinella *et al*., 2004; Li *et al*., 2005; Liu *et al*., 2004; Simmons *et al*., 2004; Tripet *et al*., 2004). Once the RNA is inside the host cell the transcription works through the replication-transcription complex organized in double-membrane vesicles and via the synthesis of subgenomic RNAs (sgRNAs). Transcription termination occurs at transcription regulatory sequences, located between the open reading frames (ORFs) that work as templates for the production of subgenomic mRNAs (Letko et al., 2020). In the atypical CoV genome, at least six ORFs can be present. Among these, a frameshift between ORF1a and ORF1b guides the production of both pp1a and pp1ab polypeptides that are processed by virally encoded chymotrypsin-like protease (3CLpro) or main protease (Mpro), as well as one or two papain-like proteases for producing 16 non-structural proteins (NSPs) (Letko *et al*., 2020). Apart from ORF1a and ORF1b, other ORFs encode for structural proteins, including spike, membrane, envelope, and nucleocapsid proteins and accessory protein chains (Lei *et al*., 2018; Letko *et al*., 2020). Different CoVs present special structural and accessory proteins translated by dedicated sgRNAs.

Pathophysiology and virulence mechanisms of CoVs, and also of SARS CoV 2 have links to the function of the NSPs and structural proteins. Researchers have underlined that NSPs are able to block the host innate immune response (Cascella *et al*., 2020). Structural proteins like N protein together with M, E, and ORF7a are involved in the assembly of the virion (Fielding et al., 2006; Hsieh et al., 2005; Huang *et al*., 2006; Nelson et al., 2005; Wilson et al., 2004). ORF3a is an ion channel protein that is involved in viral budding and release (Lu *et al*., 2006).

Among the functions of the structural proteins, the envelope has a crucial role in virus pathogenicity as it promotes viral assembly and release.

Once sufficient viral genomic RNA and structural proteins have accumulated in the host cell, viral assembly and release by budding takes place. Endoplasmic reticulum as well as the Golgi apparatus of the host cell helps in the budding of helical nucleocapsid. For this to occur the triple membrane spanning M protein interacts with the N protein and viral RNA to generate the basic structure and interacts with the E and S proteins to induce viral budding and release. Unlike other coronaviruses, M protein of SARS CoV 2 also incorporates another triple-membrane-spanning protein of ORF3a into the virion (Ito *et al*., 2005).

Various diagnostic tests, antiviral agents, and vaccines are designed on the basis of the understanding of the structure and function of the various viral proteins involved in the life cycle of the virus. It has also been reported that the N protein is the most abundantly expressed viral protein in infected cells in which the mRNA levels were amplified 3 to 10 times higher at 12 h post infection than other structural genes (Hiscox *et al*., 1995) and is therefore an important target for immunohistochemistry and antigen detection in clinical specimens.

Genome changes resulting from recombination, gene exchange, gene insertion, or deletion are frequent among CoVs, and this will happen in the future outbreaks as well as has been observed in the present pandemic too. Clinical features and risk factors are highly variable among the various CoVs making the clinical severity ranging from asymptomatic to fatal (Phan, 2020). Most of the CoVs replicate in either or both respiratory and/or enteric tracts. The replication at a particular site or both sites is determined by the variants; i.e. one variant may have a tropism for the respiratory tract whiles the other for the enteric region. Variants having tropisms for both the sites usually causes pathology in only one of these regions, however, SARS CoV is an exception as it affects both systems (Unhale *et al*., 2020) whereas SARS CoV 2 (COVID-19) affects only respiratory tract.

Pathology

The first step in virus infection is the interaction of sensitive human cells with Spike (S) Protein. Genome encoding occurs after entering to the cell and facilitates the expression of the genes, which encode useful accessory proteins, which advance the adaptation of CoVs to their human host (https://viralzone.expasy.org/785). SARS CoV and MERS CoV that attach to the host cell respectively bind to cellular receptor angiotensin converting enzyme 2 (ACE 2) (SARS-CoV associated) and cellular receptor of dipeptidyl peptidase 4 (MERS CoV associated) (Lambeir et al., 2003). After entering the cell, the viral RNA manifests itself in the cytoplasm. Genomic RNA is encapsulated and polyadenylated, and encodes various structural and non-structural polypeptide genes. These polyproteins are split by proteases that exhibit

chymotrypsin-like activity (Lambeir et al., 2003; https://viralzone.expasy.org/785). The resulting complex drives (-) RNA production through both replication and transcription. During replication, full-length (-) RNA copies of the genome are produced and used as a template for fulllength (+) RNA genomes (Luk *et al*., 2019; https://viralzone.expasy.org/785). During transcription, a subset of 7-9 sub-genomic RNAs, including those encoding all structural proteins, are produced by discontinuous transcription. Viral nucleocapsids are combined from genomic RNA and R protein in the cytoplasm and then are budded into the lumen of the endoplasmic reticulum. Virions are then released from the infected cell through exocytosis. The released viruses can infect kidney cells, liver cells, intestines, and T lymphocytes, as well as the lower respiratory tract, where they form the main symptoms and signs (Lambeir *et al*., 2003). MERS-CoV is able to affect human dendritic cells and macrophages in-vitro. T lymphocytes are also a target for the pathogen due to the characteristic CD26 rosettes. This virus can make the antiviral T-cell response irregular due to the stimulation of T-cell apoptosis, thus causing a collapse of the immune system (Chu *et al*., 2014; Zhou *et al* 2014).

Diagnosis

The challenge for diagnosis of SARS-CoV-2 was requirement of quick diagnosis as well as providing diagnostic facility to large number of patients avoiding false negatives and false positives. Thus keeping this in mind molecular assays were the obvious choice and real-time reverse transcriptase polymerase chain reaction (RT-PCR) with high sensitivity and specificity. At the start only S-gene was targeted to differentiate SARS-CoV-2 from SARS CoV-1 but sensitivity was limited (Zhou et al., 2020). Therefore other virus-specific genes were included like RdRp, N and E genes and WHO recommended the use of RdRp, E, N and S genes (WHO, 2020). Numbers of RT-PCR kits from various companies are available to carry out the detection of these four genes in a multiplex RT-PCR reaction. Thus RNA is extracted from the samples (nasopharyngeal swab, oropharyngeal swab, sputum, bronchoalveolar lavage) collected from the suspected patients and then complementary DNA is synthesized from the RNA extracted. This cDNA is then subjected to multiplex RT-PCR for detection of RdRp, N, E and S genes. Further the performance of these molecular assays will depend upon the ability of the virus to mutate further causing changes in the regions in which primers are hybridizing.

Immunodiagnostic point-of-care tests generating rapid results are less complex than molecular tests. Seroconversion of SARS-CoV-2 is found to occur between 7 and 11 days after onset of symptoms (Long *et al*., 2020). Thus antibody detection assays will be impractical for diagnosis of acute infection at an early stage. So these tests are helpful in epidemiological surveillance, contact tracing and research studies. Antibody detection tests are usually rapid -test lateral flow assays based on the principle of immunochromatography. These rapid test kits have the ability to detect IgM or IgG or also the viral antigen. Theoretically these assays decrease the time, cost and labour of testing in comparison to nucleic acid amplification assays.

Vaccines

The rapid spread and high mortality rate led to the need of development of effective vaccine to control COVID-19 pandemic. The S-protein of the coronavirus being important for virus-cell receptor binding and virus-cell membrane fusion is an effective target for CoV vaccine design. Also, it has been shown that antibodies generated against S-protein are long lasting and immunodominant in recovered SARS patients (Cao *et al*., 2020). Thus, S-protein serves an ideal vaccine target to induce neutralizing antibodies and protective immunity. Various other approaches for vaccine development too have been tried; protein subunit vaccine, virus-like particle vaccine, DNA vaccine, viral-vector vaccine, whole inactivated vaccine and RNA vaccines. Few vaccines to be named for SARS-CoV-2 are protein subunit vaccine by Novavax, Covaxx; RNA vaccine by Moderna, Pfizer, Curevac; viral-vector vaccine by AstraZeneca, Sputnik V by Gamaleya Research Institute, Johnson and Johnson; Inactivated virus vaccine by Sinovac, Wuhan Institute of Biological products, Bharat Biotech; Virus-like particle vaccine by Serum Institute of India; DNA vaccine by Cadila Healthcare limited. Thus this article provides concise information on coronaviruses being the cause of COVID-19 pandemic worldwide.

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