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REVIEW ARTICLE OPEN ACCESS

SARS-CoV-2 infection: Pathogenesis, Immune Responses, Diagnosis

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Abstract

COVID-19 has emerged as the most alarming infection of the present time instigated by the virus SARS-CoV-2. In spite of advanced research technologies, the exact pathophysiology and treatment of the condition still need to be explored. However, SARS-CoV-2 has several structural and functional similarities that resemble SARS-CoV and MERS-CoV which may be beneficial in exploring the possible treatment and diagnostic strategies for SARS-CoV-2. This review discusses the pathogen phenotype, genotype, replication, pathophysiology, elicited immune response and emerging variants of SARS-CoV-2 and their similarities with other similar viruses. SARS-CoV-2 infection is detected by a number of diagnostics techniques, their advantages and limitations are also discussed in detail. The review also focuses on nanotechnology-based easy and fast detection of SARS-CoV-2 infection. Various pathways which might play a vital role during SARS-CoV-2 infection have been elaborately discussed since immune response plays a major role during viral infections.

Keywords: COVID-19, SARS-CoV-2, Pathogenesis, Immune Response, Diagnosis

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INTRODUCTION

The end of the year 2019 marked the beginning of what we are now seeing as a pandemic, coronavirus infection. In December 2019, Wuhan, China reported the first case of this pandemic. The infection had been named a novel coronavirus infectious disease 2019 (COVID-19) on 12th January 2020 by the World Health Organization (WHO).[1,2] Coronaviruses (CoVs) cause pathogenicity in humans, birds, bats, cats, etc. It first affects the respiratory system and later may involve other vital systems such as the central nervous system, gastrointestinal tract and hepatobiliary system.[3] The international virus classification commission has given the term severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) to the novel coronavirus. Coronavirus has also been the causative agent for earlier epidemic diseases like severe acute respiratory syndrome (SARS) and the Middle East respiratory syndrome (MERS).[4] In the coronavirus family, CoVs causing infection in humans are 229E, OC43, HKU1, NL63, SARS CoV, and MERS CoV, SARS-CoV-2 is the newest addition to this list and belongs to the Betacoronavirus family.[5] The total number of COVID-19 cases worldwide reached up to 49.1Cr on April 4, 2022, of which 61.5 lac deaths have been reported. Transmission of the virus can occur through symptomatic and asymptomatic patients; hence the absence of a vaccine makes the control of disease outbreaks a difficult and challenging task.[6] The common features of COVID19 infection resembles common viral infections like fever, sore throat, cough, chest, muscle pain, dyspnea, and headache. These symptoms resemble the upper respiratory tract infection so diagnosis may be confused with seasonal upper respiratory tract viral infection. These symptoms might worsen resulting in acute respiratory distress syndrome. The coronavirus infection may also affect the cardiovascular, hepatobiliary and nervous systems.[7] This review helps in understanding the body's genomic structure, the immune mechanism during SARS-CoV-2 infection, and early diagnosis strategies. The review also focused on early and fast detection techniques such as nanotechnology for coronavirus infection. The aim of the present review is to improve the overall understanding and immune responses involved during SARS-CoV-2 infection in humans and its early diagnosis approaches.

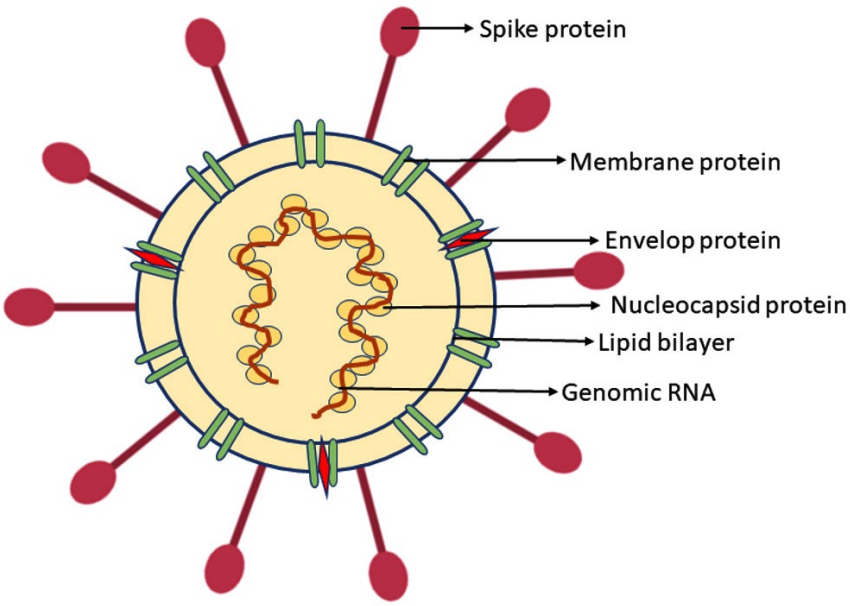

Figure 1. Schematic representation of SARS-CoV-2 virus depicting proteins and viral genome.

Morphology and Genomic Structure of SARS-CoV-2

SARS-CoV-2 is an encased, spherically shaped virus containing a positive single-stranded RNA. RNA is made up of matrix protein and related to a nucleoprotein within a capsid. The envelope of the virus consists of club-shaped glycoprotein projections or spikes, membrane proteins, and envelope proteins embedded in host-derived lipid bi-layer (Figure 1).[8] Coronavirus is further classified into four major subgroups namely alpha, beta, gamma, and delta. Out of these, beta coronavirus is further classified into 4 viral lineages from A-D. Approximately 30 different types of CoVs infect humans and mammals. Human coronaviruses (HCoVs) mainly belong to the alpha (HCoV-229E and NL63) and beta (MERS-CoV, SARS-CoV, HCoV-OC43 and HCoV-HKU1) genres of CoVs.[9]

The coronavirus genome size ranges from 20 to 32 kb, which is the largest among all the RNA viruses.[10] Both 5′ end and 3′ ends are flanked by untranslated regions (UTRs) containing 265 and 358 nucleotides respectively. The genetic structure of SARS-CoV-2 resembles the other CoVs and consists of not less than approximately 10 open reading frames (ORFs). First ORF (ORF 1a/b) which represents around two-thirds of the viral genome encodes 16 nonstructural proteins (nsps).[11] Two large polyproteins (pp), pp1a and pp1ab, are translated from ORF1a/b which are needed for viral replication and transcription.[12,13,] Other ORFs of SARS-CoV-2 encode accessory and basic structural proteins (Figure 2). The important structural proteins of coronavirus include spike (S), envelope (E), nucleocapsid (N), and membrane (M) proteins and are encoded by ORFs 10, 11 near 3′- terminus of the genome.[14,15]

Pathogenesis of Coronavirus Infection

Many facets of the pathogenesis and primary infection site of SARS-CoV-2 are still unknown. According to the available data, the primary site of infection might be the lungs presented with symptoms of respiratory problems. The maximum number of COVID-19 infected cases are asymptomatic (approximately 80-85%) and only 10-15% of cases show severe respiratory problems.[16,17] It has been seen that COVID-19 has less severity and fatality rate than SARS and MERS. Due to the similarity between SARS-CoV, MERS-CoV, and SARS-CoV-2, pathogenetic knowledge available on SARS-CoV and MERS-CoV might be useful to understand SARS-CoV-2 pathogenesis.

Viral Replication

Out of the four important structural proteins of SARS-CoV-2, M protein encircles the membrane three times, in a manner the short -NH2 terminal is towards the outside and the long -COOH terminal lies inside.[18] Virus intracellular structure is mainly formed by the M protein. The invasion of the host cell by the virus is mediated by the S protein of the virus.[4] S protein is a type I membrane glycoprotein forming peplomers. If the virus particle is grown in a certain medium that contains M protein but is devoid of S protein then the formed virus is spikeless and noninfectious.[18] Angiotensin-converting enzyme 2 (ACE2) receptor is utilized by both SARS-CoV-2 and SARS-CoV for entering the host cell,[12,19] whereas Dipeptidyl peptidase 4 (DPP4, also known as CD26) receptor is used in case of MERS-CoV for entering in host cell.[20] SARS-CoV-2 attaches to the ACE2 enzyme with the help of S protein thereby entering and infecting the host cells (Figure 3). However, the

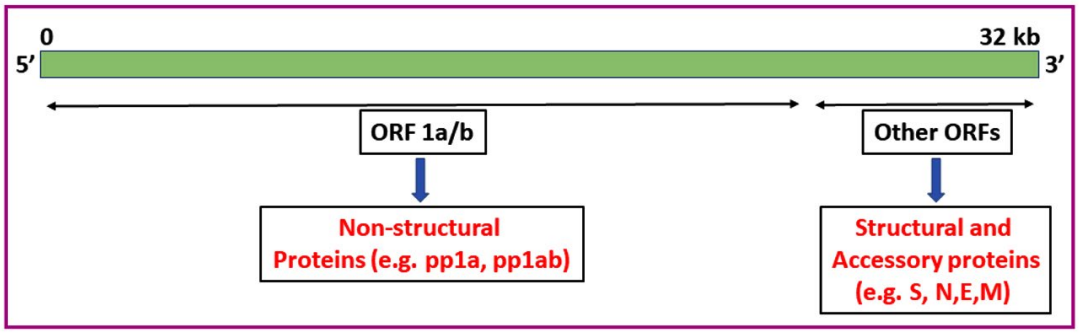

Figure 2. Schematic representation of SARS-CoV-2 viral genome showing ORF's and different proteins encoded

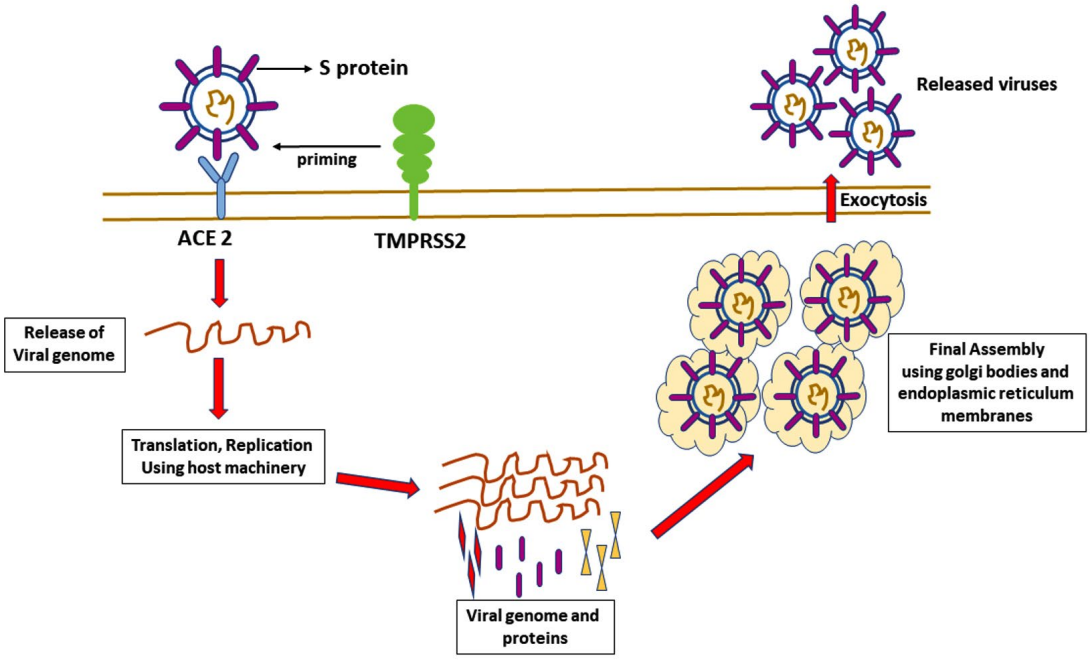

Figure 3. Schematic representation of SARS-CoV-2 virus replication and intermediary stages.

spike protein of SARS-CoV-2 shows a greater affinity towards ACE2 receptors in comparison to SARS-CoV.[21] For the complete invasion of the host cell by the virus after binding with the receptor, the spike needs to be primed by a transmembrane serine protease called TMPRSS2. The protease TMPRSS2 activates the S-protein of virus and cell contact site.[22] In SARS-CoV infection, first the virus and plasma membrane of the host fuse as a result of membrane fusion.[23] In SARS-CoV infection at the S20 position of the S protein proteolytic cleavage occurs, this event is important for the virus infectivity and cell membrane fusion.[24] The entry of SARS-CoV also occurs through a clathrin-dependent and independent pathway.[25,26] In MERS-CoV infection, furin activation occurs for virus infectivity and membrane fusion.[27] The viral genome undergoes replication and forms structural proteins and polyproteins after its entry into the host cell.[9] SARS-CoV-2, through replication and transcription forms viral proteins and genome which fuse with the membrane of the endoplasmic reticulum and Golgi bodies, as a result of the fusion between genomic RNA and nucleocapsid protein, the viral nucleocapsid is formed.[22] RNA polymerase, nsp12, plays an important role in the replication and transcription of viral RNA with help of cofactors nsp7 and nsp8.[28] In the end, the vesicles are formed having the virus particles which finally fuse with the cell membrane and release the virus via exocytosis.[22]

Viral Antigen Presentation

After entering the cell, the viral antigen is presented before antigen-presenting cells mostly in front of macrophages of the upper respiratory tract. These antigens presenting cells engulf the virus and are identified by major histocompatibility complex (MHC) or human leukocyte antigen (HLA) in humans. The presented antigen is recognized by the T-helper lymphocyte and initiates the immune response in the host cell and antibody production starts.[29] Information regarding antigen presentation is vital for understanding the infection process of SARS-CoV-2, but the immune mechanism and pathology of the virus have not yet been fully unraveled. SARS-CoV and MERS-CoV have been explored to a great extent for their pathogenesis. In both SARS-CoV and MERS-CoV infection MHC I and MHC II play the main role in the immune mechanism.[29-31] In MERS-CoV infection, MHC II mainly triggers the host immune

response, and molecules like HLA-DRB1*11:01 and HLA-DQB1*02:0 plays a vital role.[32] MBL (mannose-binding lectin) molecule plays a vital role in antigen presentation in SARS-CoV infection.[33] Owing to their similarities, the pathogenesis of SARS-CoV and MERS-CoV might aid in the understanding of SARS-CoV-2. This will also be helpful for planning the future treatment for COVID-19.

Symptoms of COVID-19 Disease

Approximately a period of 5 to 6 days is needed for the symptoms of COVID-19 infection to present themselves.[34] The time gap between the first appearance of symptoms to the development of severe disease ranges from 6 to 41 days, various factors affect this gap period such as a person's immune response, age factor, comorbidities such as diabetes, and hypertension. In elderly people, this gap period becomes even less.[35] COVID-19 infection shows symptoms like chills, rigor, high-grade fever, dry cough, myalgia, shortness of breath, anorexia, loss of smell, loss of taste sensation, diarrhea, dyspnea, and fatigue.[36,16,35,37] CT scan of the chest shows symptoms like pneumonia and also shows some abnormal features such as the presence of ground-glass opacities.[16] In some cases, the subpleural area of the lungs shows a peripheral ground-glass appearance that induces an immune response leading to increased inflammation due to the release of various inflammatory mediators. To subside this inflammation some anti-inflammatory drugs were used but showed no clinical improvement.[38] COVID-19 infection also has symptoms of upper respiratory passage like running nose, sneezing, and sore throat. In comparison with SARS-CoV-2, SARS-CoV and MERS-CoV infections also presented symptoms such as chills, rigor, fever, cough, headache, dyspnea, myalgia, and less commonly gastrointestinal symptoms.[39,40] It is very important to find out possible routes for virus transmission to control the spreading of infection.

Immune Mechanism in SARS-CoV-2 Infection

During SARS-CoV-2, adaptive immunity provides protection in the form of humoral and cell-mediated immunity. Studies on COVID-19 cases suggest that both humoral and cell-mediated immunity play a protective role via T-cell and B-cell.[34,41] In SARS-CoV infection, an immune response occurs typically in the form of IgM and IgG antibodies production like other common acute viral infections. IgM antibodies appear early during the initial entry of the virus while IgG antibodies appear late and remain for a long time and play a protective role.[42] IgG antibodies mainly attack the S and N glycoprotein of COVID-19. Both S and N proteins are the most immunogenic and expressed proteins in SARS-CoV-2 infection. There is a vital role of innate immune response in the pathogenesis of SARS-CoV-2 infection through cytokines and interleukins.

Innate Immune Response

At present very little information is available about the innate immune response of the body against SARS-CoV-2 infection. Host innate immune response is represented by the total leucocyte, serum IL-6, C-reactive protein, and interferons (IFN) levels. COVID-19 infected patients may show leukopenia or normal leukocyte count, some cases show increased neutrophil count, increase C-reactive protein, increase the level of serum interleukins, and interferons. The severity and mortality of the disease are related to the increased neutrophil and decreased lymphocyte count.[1,43] Innate immune response against virus mainly depends upon IFN type I. ACE2 receptor, the entry receptor during both SARS-CoV and SARS-CoV-2, is mainly present in type 2 alveolar cells in the lungs which represent a very small subset of cells.[44] Lungs have an ample number of monocytes and macrophages but only a minimal amount of these cells in the lung express ACE2.[44] According to the previous study SARS CoV mainly infects macrophages and T cells which are important for the pathogenesis of the virus.[45]

The viral RNA after entering the host cell is recognized as a foreign particle and an innate immune response is initiated against it.[46] After the identification of the virus, a cascade of events starts, i.e., NF-κB and IRF3, leading to the viral nuclear translocation. These transcription factors induce type I IFN and proinflammatory cytokines such as IL-2, IL-6, IL-7, IL-10, G-CSF, IP-10, MCP-1, MIP-1A, TNFα.[4] JAK-STAT pathway is activated by Type I IFN through interferon-alpha/beta receptor (IFNAR). STAT, phosphorylated by JAK1 and TYK2, along with interferon regulatory factor 9 (IRF9) forms a complex and gets translocated

to the nucleus where the transcription of IFN-stimulated genes (ISGs) is initiated.[46] A strong type I IFN response will help in hampering the viral replication in the very initial stage of infection.

According to previous studies, SARS-CoV and MERS-CoV viruses lower the innate reaction for multiplication in the host. Coronavirus uses multiple pathways for downregulation of the signaling pathway of type I IFN production and/or the downstream signaling of IFNAR. This strategy of the virus to lower the innate immune response is related to the lethality of the disease.[47] Both the viruses inhibit the IFN signaling by decreasing STAT1 phosphorylation.[4] The viral structural proteins like M and N protein and non-structural proteins like ORF proteins are important for inhibiting the host type I IFN response. Since there are many similarities between the genomic sequences of SARS-CoV-2, SARS-CoV, and MERS-CoV, it can be speculated that SARS-CoV-2 uses the same strategy to deteriorate the host innate immunity, specifically type I IFN response. The exact mechanism of action of SARS-CoV-2 is still under investigation and will require extensive research to explore it fully.

SARS-CoV or MERS-CoV infection causes increased neutrophil and monocyte-macrophages influx in advanced stages of infection.[45,48] In SARS-CoV and MERS-CoV infection, there is a high monocyte-macrophage influx and release of proinflammatory cytokines also known as "cytokine storm" or cytokine release syndrome (CRS). This cytokine storm is the main cause of lethal pneumonia and massive lung injury. This "cytokine storm" may also play a very important part in the SARS-CoV-2 pathogenesis, due to its similarities to both SARS-CoV and MERS-CoV.[49-51] This cytokine storm causes acute respiratory distress syndrome (ARDS), pneumonia-like symptoms, shock, organ failure, respiratory failure, and lead to death. On the basis of the hospital history of admitted patients, ARDS is the major cause of death in the high number of SARS-CoV-2-infected patients.[16] COVID-19 infection affects the population of all age groups. From the above discussion, it might be concluded that innate immune mechanism plays a key role in the severe outcome of SARS-CoV-2 infection, and in the future, the type I IFN may be used as an important strategy for the treatment of COVID-19 infection.

Adaptive Immune Response of the Host

To prevent the severity of COVID-19 infection the adaptive immune response plays a vital part through the T and B cell activation. Monocytes and macrophages release the cytokines and act as the antigen-presenting cells (APCs). T-helper cells identify the viral antigen presented by the APCs, while cytotoxic T-cell directly kills the virally infected cells. T-cell antibody-mediated response prevents the infection in the acute phase and also forms memory cells to prevent the severity of infection in the future. According to the data available in both SARS-CoV and MERS-CoV infection, antibody titer appears after two to three weeks of infection. In both infections severity of the disease depends upon the low and delayed antibody titer.[52] Very little data is available about the serological response during SARS-CoV-2 infection. According to Zhou et al., there is some cross-reactivity between serum from confirmed SARS-CoV-2 infected cases with only SARS-CoV and neutralized the SARS-CoV-2 *in vitro*, demonstrating activation of the humoral response.

T-cell Role in Coronavirus Infection

According to many previous studies, lymphopenia is observed in most SARS-CoV infections in the acute phase. This reaches the lowest titer after a week of infection and then is gradually restored in the recovery phase.[53-55] In the acute phase of SARS infection, instigation of cytoplasmic caspase 3 in both CD4 and CD8 lymphocytes is attributed to enhanced cell death.[56] T cells including CD4+ and CD8+ are important in combating the progress of infection and preventing the development of autoimmunity and inflammation.[57] T cell immune response is extensively studied in SARS-CoV. T-helper cells mediate their response in the form of B-cells which produce the antibodies. Cytotoxic T-cells produce immune induced injury to the lung interstitium during SARS-CoV infection.[58] A high level of T-cells along with neutralizing antibodies and increased serum cytokines (IL-4, IL-5, IL-10) were seen in severe fatal cases.[59] In MERS-CoV infection, the fatality rate is decreased by the T cell response,[60] and it was found that CD4+T cells are more responsive in MERS-CoV infection. CD8+ T cells respond in the early phase of MERS-CoV infection and correlate with disease severity,

in later phases dominant-helper responses are observed.[61] A decreased CD4+T cells population decreases production of neutralizing antibodies and cytokine production, resulting in interstitial pneumonitis in SARS-CoV.[62,63] T cells' role in human coronavirus infections is still not completely known. CD8+T cells also play an important role in controlling the lethality of infection. As antibody response plays a major role in infectivity and fatal outcome in all coronavirus infections, a person who has been infected many times by the same or different strains of the coronavirus family has more immunity in the form of developed antibodies and shows no or very few symptoms of the disease.

B Cell and Antibody Role in Coronavirus Infection

The B cell and neutralizing antibodies, limit the fatality of infection in the late phases of infection and also minimizes the chances of re-infection in the future during viral infections as a part of the humoral immune response.[64] In any viral infection, the antigen is presented before the CD4+T cell which stimulates the B cell to start the synthesis of antibodies. By the use of immunofluorescence assays and ELISA, serum IgG against the N protein was detected four days after the infection.[65,66] In SARS-CoV infection presence of antibodies IgG, IgM, and IgA have been found in the serum of patients around the same time and also IgG-specific antibodies even after 2 years of maximum recovery of patients.[66,67] Follow-up of SARS-CoV patients showed specific IgG and neutralizing antibodies in the serum which reached a peak in 4 months and decreased after that.[65,68] In many studies, it has been observed that SARS infected patients' serum has antibodies that were capable of neutralizing other coronavirus bearing S protein, which belongs to SARS-CoV strains demonstrating the cross-reactivity of antibodies.[69] In the SARS family S protein is the only cell membrane protein which elicits antibody production in the host cell.[70] S protein amino acids 441 and 700 are major epitopes for antibody production.[71] A specific 9-mer peptide "CYSSLILDY" helped in the identification of the MERS-CoV infection antigen.[72,73] Recovered patient serum from MERS-CoV infection contains various antibodies strain like MCA1, CDC-C2, CSC-C5, CDC-A2, CDC-A10, MERS-GD27, and MERS-GD33.[74-76] Memory cells formed from IgG or human monoclonal antibodies produced by B cells immortalized with Epstein–Barr virus showed protection against SARS infection.[77] Analysis of some SARS patients who received plasma therapy in the form of passive immunization had a shorter recovery time without any significant adverse effects.[78,79] Human monoclonal antibody (m336) interacted with the receptor-binding region of the S protein of MERS coronavirus and was effective against MERS-CoV *in vitro.*[80] According to various previous studies, IgG production plays an important part in the humoral immune response. In both SARS-CoV and MERS-CoV infections, IgG production starts in the acute phase of infection and is also detected in later phases of the infection. These IgG present up for a very long time like 2 years after infection so provide protection from the infection of the same strain or genetically similar strain in the future.

Diagnosis of COVID-19 Infection

COVID-19 infection is diagnosed on the basis of clinical history, symptoms, and techniques like molecular detection, CT scan, blood culture, and serological assays (Figure 4). For confirmation of the infection tests, nucleic acid detection and CT scan are necessary.

Clinical History and Clinical Examination

In the diagnosis of COVID-19 cases, travel history plays an important role. People who have had recent travel histories from the area where the active case or silent carriers of COVID-19 have been present are more prone to be infected with the disease. These people may act as the carriers hence careful monitoring and testing of these cases are of utmost importance to stop the disease spread.

COVID-19 infected patients show atypical signs and symptoms such as high-grade fever, dry cough, sputum production, running nose, headache, shortness of breath, dyspnea, loss of smell, loss of taste, and viral pneumonia. In the later phase of the disease, symptoms like persistent fever, malaise, myalgia, worsened cough, and anorexia are usually present. These all signs and symptoms are atypical because all of these might be present in any type of lower respiratory tract viral infection.

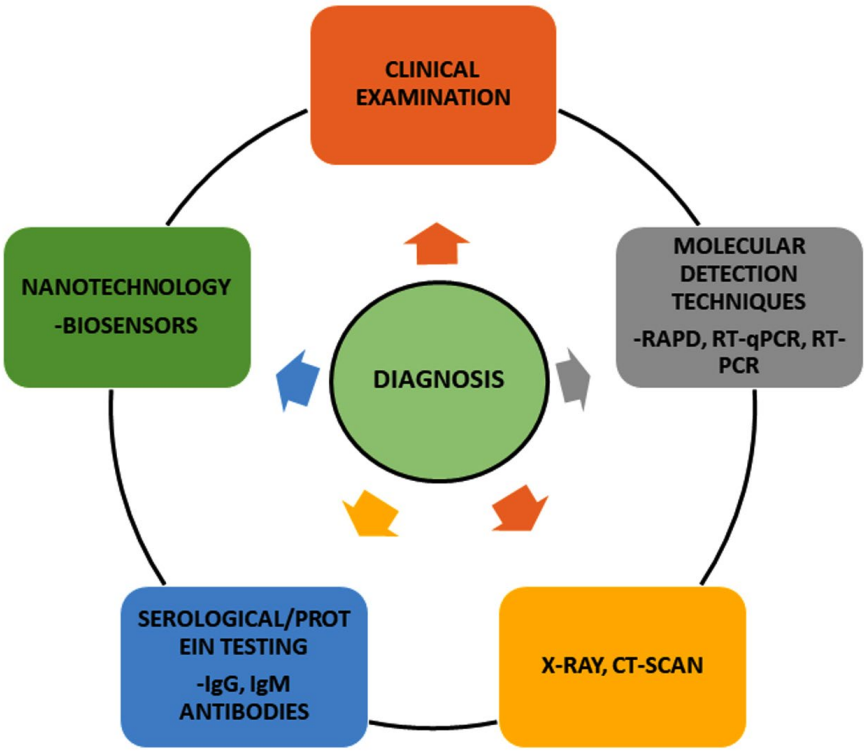

Figure 4. Different strategies used for diagnosis of SARS-CoV-2 infection.

Molecular Detection Technique

SARS-CoV-2 infections had been identified by Random -amplification deep (RAPD) sequencing methods initially.[81,36,1,82] RT-qPCR (Quantitative reverse transcription-polymerase chain reaction) is a commonly used nucleic acid identification technique and cost-effective method for the identification of SARS-CoV-2 infection.[83] RT-qPCR, a sensitive and specific technique, has been used for the diagnosis of SARS-CoV and MERS-CoV infections.[84] However, RT-qPCR can only achieve 50%-79% success in detection, depending on various factors such as sample collection site, and maintenance of cold chain for storage and transport.[85] Other molecular detection methods which are being used worldwide include loop-mediated isothermal amplification, multiplex isothermal amplification, microarray detection, and clustered regularly interspaced short palindromic repeats.[86] For molecular testing the real-time (RT-PCR) method is widely used.[87-90] RT-PCR has an advantage over normal PCR as it includes reverse transcription and PCR product amplification in one step. In RT-PCR firstly the genome of SARS-CoV-2 i.e., RNA is converted into the DNA and then cDNA is created for amplification of specific regions. RNA-dependent RNA polymerase gene (RdRP gene) and E protein of the SARS family have a high sensitivity for detection, and N protein has low sensitivity. China has approved various nucleic-acid-based methods and antibody detection kits by the National Medical Products Administration (NMPA) to detect SARS-CoV-2 infection.[91] RT-PCR has been used by the United States Centers for Disease Control and Prevention (CDC) for the diagnosis of SARS-CoV-2 infection. In SARS-CoV-2 infection, upper respiratory samples are mostly preferred for sampling, which includes oropharyngeal swabs, nasopharyngeal swabs, posterior pharyngeal wall swabs, oropharyngeal wash, nasopharyngeal wash, and nasal aspirates. A lower respiratory region sample including sputum collection, bronchoalveolar lavage (BAL), and tracheal aspirates are also taken but are less preferable.[92] A sampling of respiratory passage also depends on the time duration after the

infection. Throat swabs are unreliable after 8 days of infection and during the initial 10-14 days of infection, sputum samples may be most reliable for detection of SARS-CoV-2 infection.[93, 94] Three novel RT-PCR assays have been compared specifically for RdRp/Hel, S, and N genes; these assays were effective in the diagnosis of SARS-CoV-2 infection.[95]

CT Scan

Chest X-ray and CT-scan like radiological procedures are also practised to detect SARS-CoV-2 infection. Chest X-rays are highly nonspecific for diagnosis because it shows features of viral pneumonia. In many cases, RT-PCR is not able to diagnose the infection because of its false-negative rate. In SARS-CoV-2 infected patients, high-resolution CT (HRCT) chests are used for early diagnosis and to detect disease severity.[93] In chest CT scans, many X-rays are taken at various angles of the patient's chest for diagnosis of SARS-CoV-2 and are non-invasive.[96,97] A high-resolution chest CT scan of SARS-CoV-2 patients shows bilateral pulmonary parenchymal ground glass appearance, pulmonary consolidation, bilateral nodule, and interlobular septal thickening. Ground glass appearance in both lung parenchyma and parenchymal consolidations of both lungs (fluid incompressible lung tissue) is a characteristic feature of SARS-CoV-2 infection.[98,99,93] Chest CT scan in SARS-CoV and MERS-CoV infection also showed ground-glass opacities and consolidation of parenchyma.[100,101] In the SARS-CoV-2 infection chest, CT scan findings are normal in the initial 1-2 days of infection.[99] According to many retrospective studies, RT-PCR has lower sensitivity than CT scans.[86,102-104] The chest CT scan used for COVID-19 has lower specificity (25%) because radiological features overlap with other viral pneumonia symptoms.[86] In SARS-CoV-2 infection, an RT-PCR with chest CT scan was used for diagnosis and to rule out false-negative results with symptoms suggestive of COVID-19 infection. The most important advantage of a chest CT scan is that it is able to diagnose asymptomatic patients, who have recovered from SARS-CoV-2 which was not possible with RT-PCR. However, CT scans are expensive, need technical expertise, and are difficult to diagnose large populations.

Serological/Protein Testing

S,E,M and N viral proteins of SARS-CoV-2 act as the antigen, and antibodies are generated against them. Infected patients have a high viral load of saliva in the first week after the appearance of symptoms which decreases gradually.[105] S protein of SARS-CoV-2 has main antigenic properties. All known serological methods are mainly focused on detecting serum antibodies, such as IgG and IgM, against S -proteins of the virus.[106] ACE receptors are the binding sites for the S protein and are present in the respiratory epithelium cells, gastrointestinal cells, and renal cells.[107-109] The other viral protein with antigenic properties is N protein, which is present in viral nucleocapsid and plays a vital role in viral replication, RNA packaging, and pathogenesis.[108,110] IgG and IgM antibodies are detected by enzyme-linked immunosorbent assay (ELISA) in patient serum samples infected with COVID-19.[111] A study on many SARS-CoV-2 positive patients showed that antibodies titer increased in the initial 5-6 days of infection. On initial zero to one day of infection, IgM and IgG antibodies are detected in 50% and 81% of patients, respectively, but this reaches 81% and 100% in later phases of infection.[111,112] These antibodies can be detected in respiratory, blood, and faecal samples. The main challenge in serological testing is the cross-reactivity of antibodies to other SARS virus family groups.

Nanotechnology in the Diagnosis of SARS-CoV-2

Technology-based on nanomaterials can be used as an alternative to RT-PCR for fast detection of SARS-CoV-2 infection. It is a biosensor based early detection technology for coronavirus infection. The combination of nanomaterial properties in virus detection techniques increases the detection efficacy of a particular test. In MERS-CoV infection paper-based colorimetric device based on silver nanoparticles is used for the diagnosis of infection. For the detection of coronavirus infection gold nanoparticle-based electrochemical immune sensor has been developed.[113] The nanoparticles that interact with the virus showed peroxidase-like properties and this property help in the detection of the virus. Recently magnetic nanoparticles are being used for the detection of coronavirus infection.

For detection of S gene-ACE2 receptor binding dynamics, the quantum dot technique can be used. S receptor binding site interacts with the fluorescent quantum dot to create an imaging probe quenched with ACE conjugated gold nanoparticles. Quantum dot probes are also used for the diagnosis of other viruses S-mediated cell entry.[114] Gold nanoparticles have the properties to absorb electromagnetic radiation of the visual spectrum due to their small size and photostability. By the use of this property of gold nanoparticles a colorimetric assay based on thiol-modified antisense oligonucleotides conjugated with gold nanoparticles has been established for the N-gene of SARS-CoV-2. The biosensor is a device developed to detect influenza, the human immunodeficiency virus and other viral infection in various body fluids.[115] By the use of biosensors and other material, science-based methods early and portable diagnosis of SARS-CoV-2 diagnosis can be enabled.

Emerging Variants of SARS-CoV-2

Various variants and subvariants of SARS CoV-2 have been associated with different waves of the pandemic. The first wave of COVID-19 started in September 2020 and lasted, up to January 2021 followed by the second wave which started in February 2021 and extend up to May 2021. The third wave started at the end of February 2022. The Alpha (α) (B.1.1.7), Beta (β) (B.1.351), Gamma (γ) (P.1), Delta (δ) (B.1.617.2), and Omicron (B.1.1.529) variants of SARS-CoV-2 have been identified worldwide. Different protein sequences were observed in the structure of SARS-CoV-2 variants. Four, six, eight, ten and thirty S proteins have been identified in the alpha, beta, gamma, delta and omicron variants respectively.[116] Maximum transmission and immune escape are seen in the omicron variant because of the triple mutation in the furin-protease cleavage site of the spike protein leading to more number of intact spike proteins which prevents the early splitting of the two subunits thus infecting more number of host cells (Figure 5a).[117] The genetic sequence of the omicron variant revealed that it belongs to Pango line B.1.1.529.

Initially detected in Botswana and South Africa, Omicron (B.1.1.529) was first reported by WHO on 24 November 2021. It was named

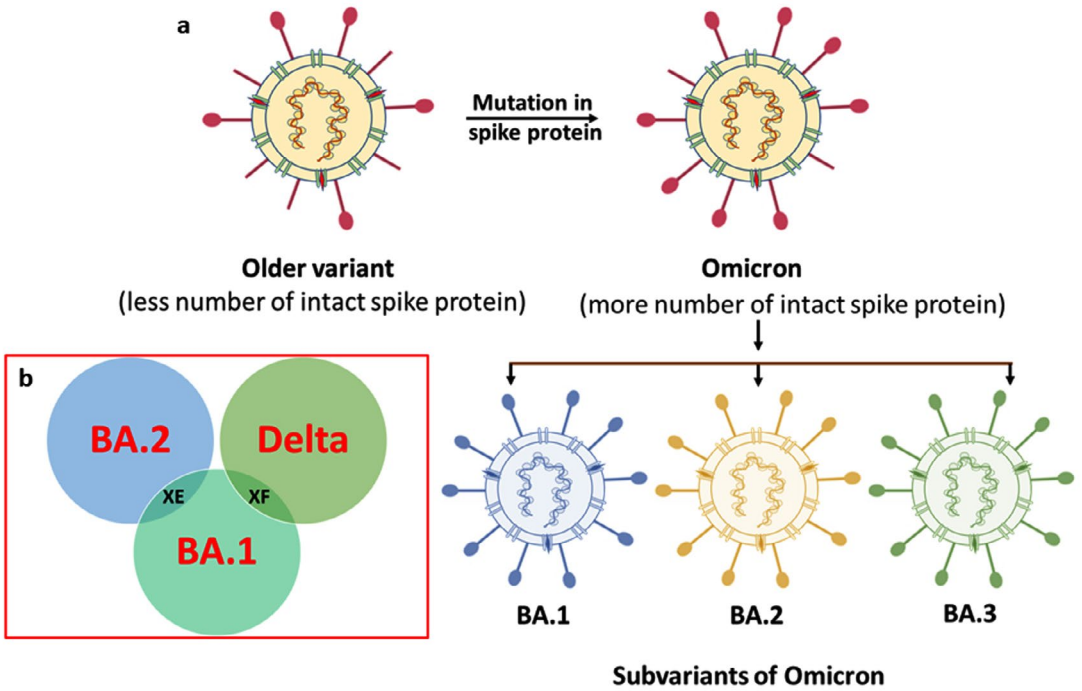

Figure 5. a) Omicron and its subvariants showing mutations in spike protein. b) Formation of XE through combination of BA.1 and BA.2 variant of Omicron; formation of XF through conbination of Delta and BA.1 variants.

omicron and defined as the fifth variant of concern by WHO on 26 November 2021. Omicron is the most mutated and rapidly spreading as compared to other variants.[118] Omicron consists of the three sublineages: BA.1, BA.2, and BA.3 lineage. Among the three strains of omicron, BA.1 is the most widely distributed strain of omicron and BA3 is the least prevalent. The maximum number of mutations observed in the BA.1 lineage among the three lineages. Presently 60 mutations have been observed in the BA.1 and 51 mutations in the BA2 lineage out of which the maximum number of mutations are seen in the S-protein of both the lineage.[119] Twelve mutations in the receptor-binding domain are common in the BA1 and BA2 lineage. BA.2 shares two mutations T376A and D405N with BA.3. There are various facts which are unclear about the omicron variant. According to the studies omicron can infect an individual who was recovering from the infection with previously virulent variants.[120]

Recently omicron variant has raised concern because of its co-infection with the Delta variant and is recognised as Deltacron or Delmicron. The recombinant or hybrid variant occurs due to the infection of two strains of a virus infecting the same cell at a time. There are various studies which describe the co-infection of COVID-19 with other viruses such as Flurona due to co-infection of flu virus with the SARS-CoV-2. It is believed that Deltacron is not a new variant but seems to be the combination of twin viral spike proteins. Since January 2022 recombinant virus has been continuously spreading in various regions of the world. In India, as the virus was spreading national health agencies were concerned about the spread and took precautions for avoiding the infection. The main findings of the co-infection are pneumonia, damage to the lung and heart, inflammatory changes to the brain and sepsis.[121] The second recombinant virus XE was identified recently by WHO, which is the combination of BA.1 and BA.2 sub-lineage of the Omicron variant of SARS-CoV-2 (Figure 5b). The recombinant virus XE is highly transmissible and was first time detected in the United Kingdom in the last of January 2022, whereas in India first case was identified on 6 April 2022 in Mumbai. XE is 10 times more transmissible than parent BA.2. XE shares similar features as parent BA.2 including spike protein features so vaccination may offer a similar level of protection for XE as for BA.2. Recently some more recombinant viruses are identified by the WHO such as XD and XF in Europe. The recombinant virus XF is the combination of the Delta variant of the United Kingdom and BA.1 (Figure 5b). It contains the genome of the Delta variant and structural and spike protein of BA.1. XD recombinant virus is BA.1 and French delta variant combination and contains S-protein of BA.1 and genome of delta variant. As the virus changes, there is always worry about the protection offered by the available vaccine against the previously available virus strain. Due to limited restrictions, there are always possibilities of development of new strains and recombinant or hybrid viruses.[117, 122] As of now, it is difficult to say whether the global threat is over or some new form of the virus awaits in the future.

CONCLUSION

COVID-19 is a pandemic that has shaken the world vigorously. It will not be incorrect to say that every part of the globe has been affected by this pandemic. Various studies and trials are going on worldwide for controlling the infection and the development of successful treatment. This review discusses virus infectivity, host immune response, presenting symptoms, available diagnostics tests and emerging new variants of SARS-CoV-2. In SARS-CoV-2 infection, immunity plays a vital role in controlling the infection which may be attributed to both humoral and innate immunity. SARS-CoV-2 is an RNA virus that reports frequent mutation and genetic recombinations. Spike protein and N protein play the main role in infectivity and pathogenicity. As disease symptoms are nonspecific hence confirmatory diagnosis is made by RT-PCR-based gene detection. Nanoparticles based diagnosis is also used for detection of SARS-CoV-2 infection, rapid detection and ease is the advantage of the use of this method. RT-PCR based results take 4 to 6 hours. The use of nanotechnology makes it simple and rapid. This study lays major emphasis on the importance of immunity, and early diagnosis techniques of SARS-CoV-2 infection.

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DATA AVAILABILITY

Not applicable.

ETHICS STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors.

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