Journal of Multidisciplinary Dental Research

Review Article

Covid-19 – Clinical Presentation and Lab Investigations

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ARTICLE INFO

Article history:
Received 08.12.2021
Accepted 25.01.2022
Published 19.03.2022

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https://doi.org/10.38138/JMDR/v7i2.2021.19

ABSTRACT

Corona virus is a single stranded RNA virus that causes severe respiratory tract infection. In 2020, a novel form of Corona virus, 2019-nCoV has been isolated and is the cause of a worldwide pandemic with the number of cases ever rising and being the cause of morbidities. There is no specific presentation of this infection, with common presenting symptoms being fever, cough, breathing difficulties, throat irritation, sneezing, pneumonia and diarrhoea. It spreads through aerosols causing rapid spread. Hence, diagnosis of covid19 is challenging and the need of the hour, so that the infected people can be isolated and the spread can be prevented. The diagnosis of this infection is done by antibody detection test, antigen detection test and molecular methods. General precautions such as - wearing mask, avoiding contact with others, maintaining social distance, regular washing of hands with soap or sanitizing own hands and avoiding touching one's own face are the key to its prevention and spread.

Keywords: Antibody; Antigen; Covid19; Infection; Lab investigation; PCR; Molecular diagnosis

1 INTRODUCTION

The history of corona virus dates back to 1965, when the research on respiratory tract of an adult led to its identification and was first named as B814. Further research around the same time led to the finding of 229E virus and "OC" virus. Both 229E and OC virus had similar morphology. They showed pleomorphic cells with membranes coated with widely spaced club shaped projections. Hence, the group of viruses having similar crown like projections on their surface were categorized as corona (Corona in Latin means crown or halo) [(1)] virus belonging to Coronavirinae subfamily. [(2)]

The virus is currently named as SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) by the International Committee of Taxonomy of Viruses (ICTV) disease 2019. It is also commonly called as COVID-19, a term derived from "coronavirus 2019", WHO. This was first identified through an outbreak of respiratory illness cases in Wuhan City, China. Two strains, L and S types of SARS-CoV-2 have been identified. The L type was seen more in early stages of the outbreak and more recently S type have been isolated. [(3)]

Corona viruses are positive-sense, single-stranded medium sized RNA viruses. They are grouped into four genera; α, β, γ, and δ based on their genetic structure. α and β mainly infect mammals. Human coronaviruses - 229E and NL63 are α and SARS-CoV, MERS-CoV (Middle East Respiratory Syndrome), OC43, HKU1 and SARS-CoV-2 (the novel coronavirus that causes coronavirus disease 2019, or COVID-19) are β. [(4)] (Figure 1)

1.1 The route of transmission of coronavirus

This virus was originally found in animals and gained entry into humans via respiratory tract. It spreads to other humans through droplets. The acts of talking, coughing

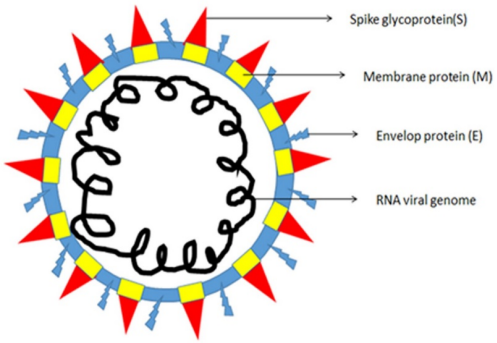

Fig. 1: Diagrammatic representation of the structure of corona virus

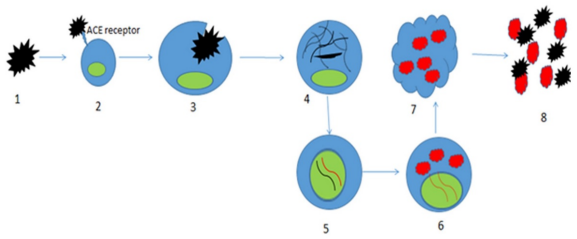

Fig. 2: Diagrammatic representation of pathogenesis covid19 infection.

and sneezing cause the virus to remain viable in air for about 1meter distance for 10 minutes. It is also known to stay on or adhere to the surface of objects for few hours or days depending on the material. Most commonly it is carried through contaminated hands of an individual to his own face.[5] Corona virus transmission to neonates occurs commonly through respiratory tracts through infected mother, housekeepers, and caretakers or through health workers. Vertical transmission through own mother is unclear.[4]

1.2 Pathogenesis of corona virus: [6]

Humans get infected when their respiratory apparatus gets in contact with the virus. (Figure 2.1) The virus first ATTACHES to the host cell receptors. (Figure 2.2) They then ENTER the host cells through endocytosis or membrane fusion (Figure 2.3) and release their contents inside the host cells (Figure 2.4), wherein the viral RNA undergoes replication. Viral mRNA produces viral proteins by BIOSYNTHESIS using the host replicative mechanisms.(Figure 2.5) The newly synthesised viral proteins undergo MATURATION (Figure 2.6) and the infected human cell undergoes autolysis (Figure 2.7) to RELEASE the new viral particles out of the host cell. (Figure 2.8)

The infected patients vary from being asymptomatic, to having very few symptoms to severe respiratory distress with multiple organ failure. Angiotensin converting enzyme 2 (ACE2) are the functional receptors for SARS-CoV. Apical side of lung epithelial cells in the alveolar space have high ACE2, and hence the virus can enter and destroy the lung epithelia easily. Reports of early lung injury in the distal airway has been noted.[6]

1.3 Symptoms of corona virus infection

Symptoms such as fever, dry cough, tiredness, body aches and pain, sore throat, diarrhoea, conjunctivitis, loss of taste or smell, difficulty in breathing or shortness of breath, chest pain or pressure, loss of speech or movement, are all seen in covid19 disease and such clinically symptomatic patients are grouped as suspected cases.Persons showing the clinical symptoms and having an epidemiological link are grouped as probable cases. Both these group of patients need to be confirmed with further laboratory investigations. If the persons are confirmed as positive based on laboratory criteria, they are grouped as confirmed cases.[7]

Oral manifestations of Covid -19 are non specific and they may include red and white patches, apthous like lesions, vesicles, pustlues, ulcers, mucositis, petechaie and necrotizing periodontal disease.[8]

1.4 Types of laboratory testing

The covid 19 testing in laboratories can detect either the antigen (the virus itself) or the antibodies (IgA or IgG) elicited against the antigen. The antigen can be detected by rapid method that takes only 15 minutes or by molecular methods such as polymerase chain reactions which takes few hours, to declare the results. The samples for antigen detection are the swabs from oropharynx and nasopharynx. Testing for antibodies against covid 19 is not specific and hence the results are only indicative of a probable covid 19 infection, but needs further confirmation. The blood sample from finger pricking is used as the sample for antibody testing.[9]

1.5 Sample collection & transportation for testing

Dacron or polyester flocked swabs are used to collect the nasopharyngeal and oropharyngeal samples. Once collected both are placed in the same tube to obtain increased viral load. The samples from broncho alveolar lavage, tracheal aspirate, nasopharyngeal aspirate, nasal wash material or sputum can also be tested. The samples should be collected and transported in sterile containers to the testing laboratory at 4°C. It can be stored upto 4days at this temperature or at -70°C for longer duration. Tissue biopsy or autopsy from the lungs can also be collected in sterile containers containing

saline at 4°C and stored for up to 24 hours or greater than 24 hours if stored at -70°C. Serum samples of acute (one week of illness) or convalescent (2 to 3 weeks of illness) at 4°C should be collected in anticoagulant and stored for up to 5 days or longer if stored at -70°C.Currently saliva samples are collected for testing. [10]

Swabs like synthetic fiber swabs with plastic or wire shafts are used. Calcium alginate swabs or swabs with wooden shafts can inactivate some viruses and hence not useful in PCR test. [3] After collection, the swabs are immediately placed in a sterile transport tube containing 2-3mL viral transport medium (VTM), Amies transport medium, or sterile saline, unless the test is designed to analyse a specimen immediately. If VTM is not available, the viral transport medium in accordance with CDC's protocol needs to be prepared in accordance with the standard operating procedure for public health laboratories. [9]

2 LABORATORY TESTS

2.1 Rapid antigen detection

Probable cases are tested. It is an immunoassay test that detects the viral antigen. In India, the ICMR has proposed to use the antigen detection kits called as Standard Q COVID-19 Ag detection kit (developed by the South Korean company S D Biosensor). The kit consists of covid antigen test device, viral extraction tube with viral lysis buffer and sterile swab for sample collection. [11] Samples are collected from the respiratory tract using the sterile swab provided in the kit. If the antigen is present in the sample, it will bind to the paper strip containing specific antibodies covered with plastic casing and produces visually detectable signals within 15 minutes. If the result is negative, then further confirmation to be done by RT-PCR (Reverse Transcription Polymerase Chain Reaction). The rapid antigen detection testing is useful in early detection of covid19, as it shows positivity only when the antigen is replicating. False positive results are reported with this test, as the antibodies on the strip can detect other antigens too. Hence this test is not preferred commonly and the results from this testing needs further validation. [12]

2.2 Rapid antibody detection

In this test the antibodies produced against corona virus infection is detected. But this test is not specific to covid19 and detects antibodies produced against any corona virus. In addition if the test is carried out too early in the disease process after the exposure to the virus, the antibodies would not have formed and hence not detected. Therefore, it is not used nowadays. [13] (Figure 3)

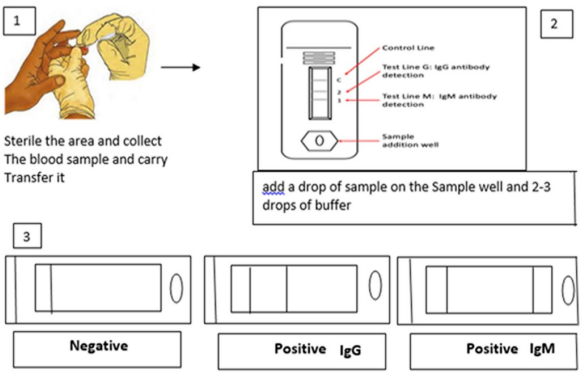

Fig. 3: Diagrammatic representation of rapid host antibody test procedure:

2.3 Antigen detection test

The confirmatory test for covid 19 is by antigen detection tests, which could be Polymerase Chain Reaction (PCR takes 2-3 hours), Reverse Transcription Polymerase Chain Reaction (RT-PCR takes 3-4 hours), Reverse transcription Loop-Mediated Isothermal Amplification (RT- LAMP takes), Recombinase Polymerase Amplification (RPA takes) or CRISPR-based diagnostics that takes 15-60 minutes. [9]

PCR was introduced by Kary Mullis in the 1980, where in billions of copies of a specific DNA sample of interest can be produced and used for analysis. The major limitation of this test is that the target sequence should be known before adding the primer. [14]

The basic components and reagents for PCR are - DNA template (DNA target region to amplify), DNA polymerase (enzyme that polymerizes new DNA strands by binding and elongation to form double-stranded DNA), heat-resistant Taq polymerase (remains intact during the high-temperature DNA denaturation process), DNA primers (each of the sense and anti-sense strands of the DNA), Deoxynucleoside triphosphates (the building blocks from which the DNA polymerase synthesizes a new DNA strand), buffer solution and the bivalent cations, typically magnesium and potassium ions. [14] (Figure 4)

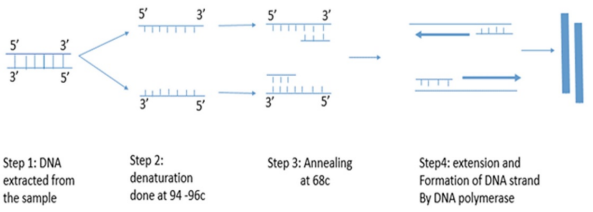

Fig. 4: 4: Representation of Polymerase chain reaction process:

2.4 *Procedure* [15]

PCR consists of a 20–40 repeated thermal cycles involving temperature changes.

Initialization: It is the first step and is required for activation of DNA polymerases by hot-start PCR.[12] It consists of heating the reaction chamber for 1–10 minutes, to a temperature of 94–96°C (201–205 F), or 98°C (208 F), if extremely thermo stable polymerases are used.

Denaturation: It is the process of DNA melting or denaturation, by breaking the hydrogen bonds of the double-stranded DNA between complementary bases into two single-stranded DNA strands. This is carried out at 94–98°C (201–208 F) for 20–30 seconds is the first cycle. Annealing: The next step is annealing at 50–65°C (122–149 F) for 20–40 seconds where primers get annealed to each of the single-stranded DNA templates at the 3' end of each strand.

Extension/Elongation: 75°–80°C (167–176 F), wherein the temperature depends on the DNA polymerase. New DNA strand is formed by adding free dNTPs to the DNA template in the 5'-to 3' direction. Millions of DNA copies can be obtained by following these steps.

To calculate the number of DNA copies per cycle is 2^n (where n is the number of cycles). Thus, a reaction set for 30 cycles results in 2^{30}, or 1,073,741,824, copies of the original double-stranded DNA target region. Then chamber is cooled to 4–15°C (39–59 F).

Gene target for SARS-CoV2 are RdRP- RNA dependent RNA polymerase, ORF (open reading frames ORF1a/b) and N2 nucleocapsid. It takes 5-6 hours to get the result.[15]

2.5 *Reverse transcriptase polymerase chain reaction: (RT-PCR)*

In this procedure, first RNA primers and reverse transcriptase enzyme are added for DNA formation. Then the PCR procedure is continued. Nowadays automatic machines are used for the entire laboratory test, so is the case for molecular confirmation test for Covid19. Only the test sample need to be added and the results will be out in 80 minutes.[15]

Recently researchers have developed CRISPR gene-editing technology which helps in detection of coronavirus in just 5 minutes. It is inexpensive lab equipment. The principal of this test identifies the sequence of coronavirus RNA by creating a "guide" RNA (complementary to the target RNA sequence) which binds to it in solution. When this happens CRISPR tool's Cas13 "scissors" enzyme cuts single-stranded RNA apart. These release fluorescent particles into the solution. When the laser light is passed it releases fluorescent light which identifies the presence of corona virus. However it is not yet commercially available for use.[16]

2.6 *Chest CT and Covid-19*

Chest CT is an important and fast imaging tool used in the diagnosis of Covid-19-infected patients. The CT severity score index is used to assess the lung changes in patients involved by Covid-19. It can also predict the disease severity by showing the percentage of lung involvement and hence can also be useful in predicting the disease prognosis. It is based on approximate estimation of pulmonary involved areas.[17,18]

Each of the five lung lobes are visually scored and given a score from 1 to 5:

1. Representing less than 5% lobar involvement
2. 5–25% lobar involvement
3. 26–50% lobar involvement
4. 51–75% lobar involvement
5. > 75% lobar involvement

Then, the final score will be the summation of individual lobar scores and will be out of 25 (total score); the total lung involvement is then obtained by multiplying the total score times 4.[17,18]

2.7 *Salivary diagnosis and Covid -19*

Saliva is one of the non invasive tool that is been used as a diagnostic test in many diseases. RTPCR of the throat and nasopharyngeal swab (NPS) is the most commonly used test for the diagnosis of covid -19. However, NPS necessitates the availability of skilled technical staff, causes significant patient discomfort during test sample collection, and is associated with high risk of infection to healthcare workers involved. Several studies have investigated the presence of SARS-CoV-2 RNA in saliva. Several of those studies confirmed reliable detection of SARS-CoV-2 in the saliva of patients with COVID-19. Saliva offered sensitivity and specificity for SARS-CoV-2 detection comparable to that of the current standard of nasopharyngeal and throat swabs. However, the utility of saliva in diagnosing COVID-19 infection remains understudied. Clinical studies with larger patient populations that measure recordings at different stages during the disease are still necessary to confirm the accuracy of COVID-19 diagnosis with saliva. Nevertheless, the utility of saliva as a diagnostic tool opens the possibility of using rapid and less invasive diagnostic strategies by targeting bioanalytes rather than the pathogen. The literature supports that saliva offers a simple sample collection method compared to technique sensitive NPS and has the advantage of point-of-care testing for initial screening in community or hospital-based set-up.[19,20]

Viruses undergo constant change over time with most changes having no impact , however, sometimes there may be severe impact on the property of the virus, the disease process, its severity, or the performance of vaccines, therapeutic medicines, diagnostic tools, or other public

health and social measures. SARS CoV-2 too has undergone various genomic changes since its identification for the first time. These variants include : Variant being manitered (**VBM**)- Alpha (B.1.1.7 and Q lineages), Beta (B.1.351 and descendent lineages), Gamma (P.1 and descendent lineages), Epsilon (B.1.427 and B.1.429), Eta (B.1.525), Iota (B.1.526), Kappa (B.1.617.1), 1.617.3, Mu (B.1.621, B.1.621.1) andZeta (P.2); Variants of concern (**VOC)** - Delta (B.1.617.2 and AY lineages) and Omicron (B.1.1.529). The variant currently posing serious threat to the world is Omicron.[21,22] Due to the constant genomic evolution of the virus, the current diagnostics and even the efficiency of vaccines stands questionable.

3 CONCLUSION

Throughout history, nothing has killed more human beings than infectious disease. As human civilizations flourish, so do the infectious diseases. Large numbers of people living in close proximity to each other and to animals, often with poor sanitation and nutrition, provide fertile breeding grounds for disease. And new overseas trading routes spread the novel infections far and wide, creating the global pandemics. Covid 19 has been one such viral pandemic that has caused a lot of concern in the past months. Trying to understand the nature of this infection, its clinical presentations, early diagnosis and treatment of the affected individuals, has received lot of priority and has been ever evolving. Until such the times that a successful vaccine is generated against this infection, we need to rely on controlling the spread and mortality rates due to this disease.

4 ABBREVIATIONS

RNA: Ribonucleic acid, PCR: Polymerase chain reaction, OC virus: Organ culture virus, SARS- Cov: Severe acute respiratory syndrome – coronavirus 2, ICTV: International committee of taxonomy of viruses, COVID 19: coronavirus, WHO: World health organisation, ACE: Angiotensin coverting enzyme, Ig:Immunoglobulin, CDC protocol:Center for disease control and prevention, VTM: Viral transport medium, ICMR: Indian council of medical research, RT- PCR:Reverse transcription- polymerase chain reaction, MERS-COV:Middle East respiratory syndrome- corovirus, RPA:Recombinase polymerase amplification, CRISPR: Clustered Regularly Interspaced Short Palindromic Repeats, DNA: Deoxyribonucleic acid, dNTPs: Deoxynucleotide triphosphates, RdRP-RNA: RNA dependent RNA polymerase, ORF: Open reading frames, N2: Nucleocapsid 2

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