Asian Journal of Innovative Research. (2020): 5(2) 1-8.

ISSN 2455 7285

ASIAN JOURNAL OF INNOVATIVE RESEARCH

Available online at http://www.asianjir.com

Received 09 April 2020;
Accepted 05 May 2020
Online May 2020

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Review Article **Biochemistry and Biotechnology**

COVID-19- MOLECULAR TRANSMISSION AND DIAGNOSIS- REVIEW ARTICLE

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ABSTRACT

Coronaviruses are a group of related RNA viruses that cause diseases in mammals and birds. In humans, these viruses cause respiratory tract infections that can range from mild to lethal. Diagnostic testing to identify persons infected with severe acute respiratory syndrome–related coronavirus-2 (SARS–CoV-2) infection is central to control the global pandemic of COVID-19 that began in late 2019. In a few countries, the use of diagnostic testing on a massive scale has been a cornerstone of successful containment strategies. In contrast, the United States, hampered by limited testing capacity, has prioritized testing for specific groups of persons. Real-time reverse transcriptase polymerase chain reaction–based assays performed in a laboratory on respiratory specimens are the reference standard for COVID-19 diagnostics. However, point-of-care technologies and serologic immunoassays are rapidly emerging. Many affluent countries have encountered challenges in test delivery and specimen collection that have inhibited rapid increases in testing capacity. These challenges may be even greater in low-resource settings. Urgent clinical and public health needs currently drive an unprecedented global effort to increase testing capacity for SARS–CoV-2 infection. Here, the authors review the current array of tests for SARS–CoV-2, highlight gaps in current diagnostic capacity, and propose potential solutions.

Keywords: *Corona Virus, SARS, Diagnosis, Global, highlight, challenges.*

***Citation*:** K. Jeyaprakash, S. Velavan, S. Ganesan and P. Arjun. (2020) Covid-19- molecular transmission and diagnosis- Review article. *Asian Journal of Innovative Research*. 5(2): 1-8.

INTRODUCTION

In December 2019, a cluster of cases of unexplained viral pneumonia was identified in Wuhan, a metropolitan city in Hubei province, China. Initially, most of the confirmed cases were linked with the Huanan seafood market in Wuhan, where numerous types of live wild animals are sold, including poultry, bats, groundhogs, and snakes. To identify the causative agent of this disease, a large number of tests were conducted, which ruled out several etiological agents that may cause similar symptoms, including the severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome coronavirus (MERS-CoV), avian influenza virus, and other common respiratory pathogens. Finally, a new coronavirus, putatively named 2019-nCoV by the World Health Organization (WHO) on January 12, 2020, was identified as the causative pathogen of this outbreak. On January 20, after a visit to Wuhan, Professor Zhong Nanshan, a SARS intervention specialist, confirmed that 2019-nCoV was spreading between people [1], which led to increased vigilance by theChinese government and people. At 10:00 on January 23, Wuhan, the birthplace of the disease, declared a general closure to prevent its further spread.

However, Wuhan is a major transportation hub located in the central region of the People"s Republic of China with approximately 11 million inhabitants [2], and the period from the end of December 2019 to February 2020 was the time of the "Spring Festival travel rush". Although the Chinese government made great efforts to control the flow of people, the disease spread rapidly from Wuhan to other cities, as well as other countries, likely through asymptomatic carriers. On January 30, 2020, the WHO declared the outbreak of novel coronavirus a public healt emergency of international concern, the sixth public health emergency after H1N1 (2009), polio (2014), Ebola in West Africa (2014), zika (2016), and Ebola in the Democratic Republic of Congo (2019) [3]. The International Committee on Taxonomy of Viruses renamed 2019-nCoV as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and the related pneumonia as coronavirus disease 2019 (COVID-19) on February 12, 2020 [5]. As the disease is highly infectious, "the law of the People's Republic of China on the prevention and treatment of infectious diseases" lists it as a class B infectious disease, and recommends preventive and control measures similar to those against class A infectious diseases. The outbreak is ongoing, and poses a great global challenge. Health workers, governments, and the public need to co-operate globally to prevent its further spread.

EPIDEMIOLOGY

In the beginning, an association with a seafood market selling live animals in Wuhan, where most of the earlier patients having pneumonia had worked or visited, was recognized. However, as the epidemic disease grew, person-to-person transmission became the principal means of spread. COVID-19 infection is spread using large droplets produced during coughing and sneezing by symptomatic cases but may also happen from asymptomatic individuals before starting of their symptoms [4]. These infected droplets can travel 1–2 meters and later put down on surfaces. Droplets normally do not extend more than 2 meters and do not hang on in the air. The virus could stay viable on surfaces for days in desirable environmental conditions but are ruined in less than a minute by regular disinfectants, such as sodium hypochlorite and hydrogen peroxide (5). SARS-CoV-2 is obtained either by breathing of the droplets or touching surface tainted by them and then touching the nose, mouth and eyes. Cases may be contagious for as long as the symptoms continue and even after clinical improvement. Moreover, certain cases may behave as super-spreaders. As said by a joint WHO-China statement, the rate of secondary COVID-19 disease attack varied from 1 to 5% among tens of thousands of close contacts of verified cases in China. In the USA, the symptomatic secondary attack rate was 0.45% among 445 close contacts of 10 verified cases [6]. SARS-CoV-2 RNA has been demonstrated in sputum, blood and stool samples. However, fecaloral, as well as materno-fetal vertical transmission, have not been identified as an important element in the spread of infectivity

Molecular Basics of Transmission of Coronavirus

In case of SARS-CoV, transmission is through droplet infection (respiratory secretions) and lose person to person contact. It can also spread through sweat, stool, urine, respiratory secretions. When virus enters into the body, it binds to the primary target cells such as enterocytes and pneumocytes thereby establishing a cycle of infection and replication. Other target cells of CoV are epithelial renal tubules, tubular epithelial cells of kidney, immune cells, And cerebral neuronal cells. [7,8]

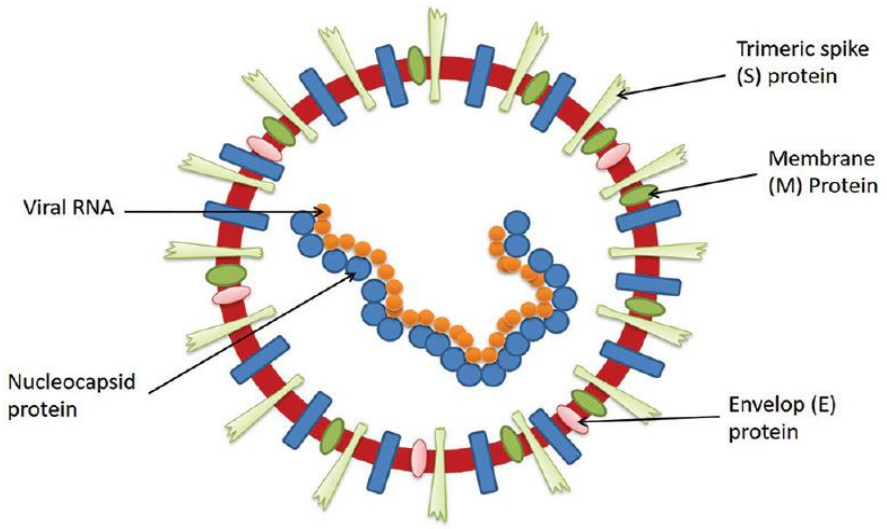
CoV attaches to the target cells with the help of spike protein–host cell protein interaction (angiotensin converting enzyme-2 [ACE-2] interaction in SARS-CoV and dipeptidyl peptidase-4 [DPP-4] in MERS-CoV. After the receptor recognition, the virus genome with its nucleocapsid is released into the cytoplasm of the host cells. The viral genome contains ORF1a and ORF1b genes, which produce two PPs that are pp1a and pp1b, which help to take command over host ribosomes for their own translation process. Both pp1a and pp1b take part in the formation of the replication transcription complex. [9] After processing of PP by protease, it produces 16 NSPs. All NSPs have their own specific functions such as suppression of host gene expression by NSP1 and NSP2, formation of a multidomain complex by NSP3, NSP5 which is a M protease which has role in replication, NSP4 and NSP6 which are transmembrane (TM) proteins, NSP7 and NSP8 which act as a primase, NSP9 – a RNA-binding protein, the dimeric form of which is important for viral infection. Induction of disturbance to the dimerization of NSP9 can be a way to overcome CoV infection.[10]P10 acts as a cofactor for the activation of the replicative enzyme. NSP12 shows RNA-dependent RNA polymerase activity, NSP13 shows helicase activity, NSP14 shows exoribonuclease activity, NSP15 shows endoribonuclease activity, and NSP16 has methyltransferase activity. All NSPs have an important role in replication and transcription [11].

Synthesized proteins such as M, E, and S are entered into the endoplasmic reticulum (ER)-Golgi intermediate compartment (ERGIC) complex and make the structure of viral envelope. On the other hand, the replicated genome binds to N protein and forms the ribonucleoprotein (RNP) complex. The outer cover is formed by the M, E, and S proteins.[12] . Finally, the virus particle comes out of the ERGIC by making a bud-like structure. These mature virions form a vesicle, which fuses with the plasma membrane and releases the virus particles into the extracellular region. The detailed structure of CoV and its life cycle is depicted in [Figure 1] and [Figure 2]. On infection, the SARS-CoV and MERS-COV cause a surge of pro-inflammatory cytokines and chemokines, which cause damage to lung tissue, deterioration of lung function, and then finally lung failure in some cases. [13]

Currently, there is no specific antiviral drug for the treatment of CoV-associated pathologies. Most treatment strategies focus on symptomatic management and supportive therapy only. Some therapeutic agents that are under development or being used off-label are ribavirin, interferon (IFN)-α, and mycophenolic acid.[14] . There are many newspaper articles citing effectiveness of anti-HIV drugs: ritonavir, lopinavir, either alone or in combination with oseltamivir, remdesivir, and chloroquine; and among these, ritonavir, remdesivir, and chloroquine showed efficacy at cellular level which further need experimental support and validation.[15]

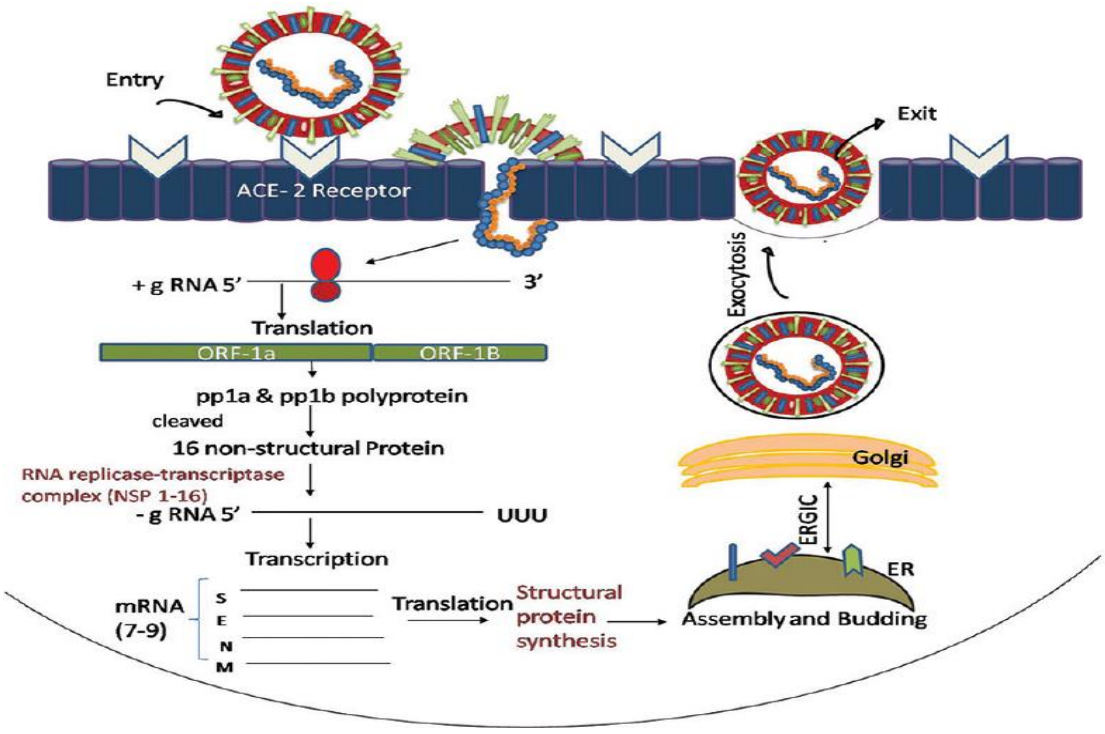
As there is no well-defined therapy available, which specifically targets CoV, in this article, we have reviewed the possible protein structures, which could be potential targets for the development of a therapeutic approach for the treatment of CoV.

Fig. 1. Structure of Corona virus



The S proteins of CoV binds to cellular receptor angiotensin-converting enzyme 2 (ACE2) which is followed by entry of the viral RNA genome into the host cell and translation of structural and non-structural proteins (NSP) follows. ORF1a and ORF1ab are translated to produce pp1a and pp1ab polyproteins, which are cleaved by the proteases that are encoded by ORF1a to yield 16 non-structural proteins. This is followed by assembly and budding into the lumen of the ERGIC (Endoplasmic Reticulum Golgi Intermediate Compartment). Virions are then released from the infected cell through exocytosis. S: spike, E: envelope, M: membrane, N: nucleocapsid. PP: polyproteins, ORF: Open reading frame, CoV: coronavirus

Figure 2: The life cycle of CoV in host cells.



The incubation period of SARS-CoV-2 infection is assumed to be in 14 days succeeding exposure, with most patients taking place around four to five days (13). Individuals of all ages may acquire SARS-CoV-2 infection, although middle age and older individuals are the majority. In some cohorts of hospitalized cases with confirmed COVID-19 infection, the median age varied from 49 to 56 years [16].

The usual clinical characteristics involve fever, dry cough, fatigue, sore throat, rhinorrhea, conjunctivitis headache, myalgia, dyspnea, nausea, vomiting and diarrhea. Hence, there are no unique clinical features that yet dependably differentiate COVID-19 from other upper/lower airway viral infections. In a subgroup of cases, by the end of the first week, COVID-19 may develop to pneumonia, pulmonary failure and death [17]. Pneumonia seems to be the most common severe manifestation of COVID-19, distinguished mainly by fever, dry cough, dyspnea, and bilateral infiltrates on chest imaging. The median time from the beginning of symptoms to dyspnea was five days, hospitalization seven days and acute respiratory distress syndrome (ARDS) eight days. Recovery begins in the 2nd or 3rd week. According to the WHO, recovery time appears to be roughly two weeks for mild and three to six weeks for severe COVID-19 disease [18].The median period of hospitalization in recovered cases was 10 days. Poor outcomes and fatality are more common in the elderly than patients with co-morbidities (50–75% of a fatality).

Even asymptomatic cases may have an objective laboratory rather than clinical abnormalities. In a study enrolling 24 patients with asymptomatic COVID-19 infection, all of whom underwent thorax computed tomography (CT), 50% had typical ground-glass opacities or patchy infiltration, and another 20% had atypical lung imaging pathology. Five out of 24 cases had a low-grade fever, with or without other characteristic symptoms, a few days after diagnosis [19].The clinical picture of COVID-19 disease infection, is mostly not severe as follows:

- Asymptomatic (latent) infection: Cases positively tested for SARS-CoV-2, but

lacking clinical symptoms or pathologic lung imaging findings .

- Acute upper airway viral infection: Patients with only fever, dry cough, pharyngeal pain, nasal congestion/rhinorrhea, fatigue, headache, or myalgia, and devoid of findings of pneumonia by thorax imaging or sepsis.

The scale of COVID-19 disease is diverse, varying from clinically asymptomatic to ARDS and multiorgan failure. The authors of the Chinese CDC report categorized the clinical symptoms of the COVID-19 disease by the severity:

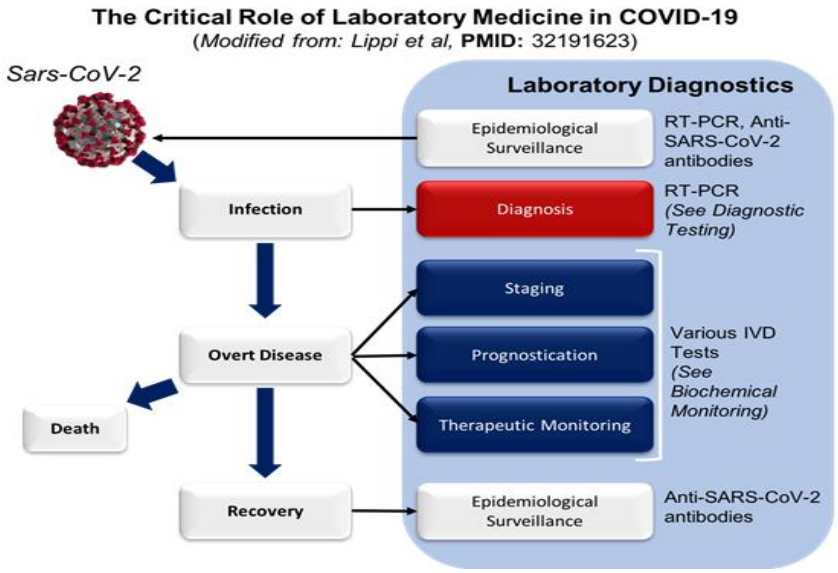
- Mild disease (e.g., with no/mild pneumonia) was described in81%.
- Severe disease (e.g., with dyspnea, tachypnea: ≥70 /min (<1 year), ≥50/min (≥1 year), hypoxia (oxygen saturation <92%), or >50% pulmonary involvement on imaging within 24 to 48 hours, disturbance of consciousness and feeding difficulty or food refusal, with signs of dehydration) was observed in 14%.
- Critical disease (e.g., with respiratory collapse, shock, or multiorgan failure) was reported in 5% (20).

In a study involving 138 patients, ARDS developed in 20% after a median of 8 days, and mechanical ventilation was required in 12.3%. Age higher than 65 years, diabetes mellitus and hypertension were each found to be related to ARDS [19]. The necessity for intensive care admittance was in 25–30% of affected cases in previous reported series . Complications comprised acute lung injury, ARDS, shock and acute kidney injury [20]. Others included arrhythmias and acute cardiac injury. In one study, these complications were reported in 16.7% and 7.2%, respectively [21].

The general fatality rate is expected to vary between 2% and 3% no deaths were observed among noncritical cases. The mortality rate in admitted adult patients varied from 4% to 11% [18, 21]. As said by a joint WHO-China fact-finding mission, the case-fatality rate ranged from 5.8% in Wuhan to 0.7% in the rest of China [22]. The proportion of fatal infections may vary by location, e.g.In Italy, the estimated fatality rate was 5.8% in March. On the contrary, the estimated case fatality rate in March in South Korea was 0.9% . Older age was also associated with higher fatality, with a fatality rate of 8% and 15% among those aged 70–79 and 80 years or older, respectively. Most of the mortal cases have happened in cases with advanced age or predisposing co-morbidities (such as cardiovascular disease - coronary heart diseases-, diabetes mellitus, chronic lung disease, hypertension and cancer) [23].

RT - PCR

Deng et al, (2020) conducted an observational study on ocular detection of SARS-CoV-2 in 114 cases of COVID-19 pneumonia in Wuhan, China. They investigated the possible transmission of SARS-CoV-2 through the ocular conjunctival pathway. This study was important to find out the possibility of ocular transmission. In this study, authors collected nasopharyngeal swabs and conjunctival swabs from 144 COVID-19 pneumonia diagnosed patients at Tongji Hospital in Wuhan, China. From electronic medical records, the demographic, epidemiological, clinical, laboratory and outcome data were obtained and laboratory detection of SARS-CoV was performed by real time RT-PCR. The swab samples were maintained in viral transport medium and further analyzed by real time RT-PCR. In the result, it was found that the novel corona pneumonia had been in the community epidemic stage and most of the infected patients were in three generations. Also found that, there was need for improvement in the rate of timely hospitalization and admission. Due to the delay of admission, patients with mild or normal disease may be aggravated to severe or critical disease. After testing nasopharyngeal swabs for SARS CoV-2 virus, 90% cases were positive. But when conjunctival swabs were tested, there was no viral nucleic acid could be detected in the samples of COVID-19 pneumonia patients.[24].



Lippi G, Plebani M. Laboratory abnormalities in patients with COVID-2019 infection. Clinical Chemistry and Laboratory Medicine (CCLM). 2020 Mar 3. PMID:32119647

BIOCHEMICAL PARAMETERS

A study mainly focused over the predictive factors of progression to severe disease to facilitate proper allocation of COVID-19 patients to different levels of medical facilities. For the study, 49 COVID-19 patients were collected, which were divided in two groups: stable non-severe and progressive to severe diseases. Among them 34 (69.4%) had stable non-severe disease and 15 (30.6%) progressed to severe disease. The study mainly exposed the risk factors which are responsible for progressive to severe COVID-19 group, these are comorbidity, age >50, absolute lymphocyte counts <1500 /μL and serum ferritin >400 ng/mL. Researchers also mentioned that during their study only a patient had died who had all the risk factors.[25]

Tian et al, (2020) showed in a study compared the characteristics between severe and common confirmed cases which including mild cases, no-pneumonia cases and asymptomatic cases, the features between COVID-19 and 2003 SARS. 46 (17.6%) of severe cases, 216 (82.4%) of common cases, which including 192 (73.3%) mild cases, 11 (4.2%) non-pneumonia cases and 13 (5.0%) asymptomatic cases were found respectively. Fever (82.1%), cough (45.8%), fatigue (26.3%), dyspnea (6.9%) and headache (6.5%) are the common symptoms of COVID-19. The fatality of COVID-19 infected patients in Beijing was 0.9%, significantly lower than the whole national average level while the rate of discharged patients of Beijing was significantly higher than the whole national average level. Among the top 10 provinces and cities with confirmed cases of COVID-19, Beijing was successful in preventing and controlling on the COVID-19 infection. This success comes from the correct leadership and experience of SARS in 2003.[26]

In another study, 27 patients were initially affected, later the number rose to 41 and one death was noted. In February 3, 2020, at least 117,496 cases with death of 362 have been reported. Peak travel season was probably an important factor for the global transmission of this infection. Each patient can spread the infection to more than two healthy persons. Mild clinical presentations, lack of infrastructure in resource-limited countries are hurdles to control this infection. Plenty of morbid conditions present in hospital population which can worsen the condition. Mostly infected people were aged between 49 – 61 years. Presence of non-specific symptoms such as malaise, fever 98%, cough 76%, dyspnea 55%, and myalgia / fatigue 44% were seen. Other biochemical parameters include – elevated ALT / AST level (41%), high serum ferritin (63%) and high C reactive proteins (86%) were also observed.[27]

ANTIBODY TEST

Researchers developed a serum-based testing method which detects the SARS-CoV-2-specific immunoglobulin M from patients' sera. SARS-CoV-2 is spreading faster and initially COVID- 19 may present without symptoms, or sometimes including fever, coughing, shortness of breath, pain in the muscles, and tiredness. COVID-

19 may also develop into pneumonia and acute respiratory distress syndrome (ARDS), as SARS outbreak in 2003. The method which has been used to define cases in Hubei province having epidemiology history, clinical features including fever and/or respiratory symptoms; early onset of normal/decreased white blood cell count or decreased lymphocyte count along with sign of pneumonia.[28]

CT SCAN

COVID-19 can be confirmed by Reverse Transcription Polymerase Chain Reaction (RT-PCR) of blood or respiratory specimens. The detection rate is low by this process in early stages of disease and takes 1-3 days for confirmation of positive result. On the other hand, through the process of Chest Computed Tomography (CT) in diagnosis of COVID-19 shows high sensitivity. The speed of acquisition of CT and timely reporting can make diagnosis of COVID-19 within minutes. It can also monitor the improvement and disease progression in patients with COVID-19. In another study, 27 patients were initially affected, later the number rose to 41 and one death was noted. Upper respiratory tract sign and symptoms, chest X-ray and CT findings showed bilateral lung involvement in 114 of 140 confirmed patients.[29,30].

Wang et al, (2020) assayed the clinical and imaging evidence of Wuhan-viral pneumonia. They aimed to analyze and explain the clinical and imaging evidence of the Wuhan-viral pneumonia from a large scale prospective cohort study in order to enhance public emergency preparedness and response. In that study, from the 1st until the 22nd of January 2020, 887 patients were identified as having Wuhan-viral pneumonia. They prospectively collected and analyzed clinical and imaging data of patients with viral pneumonia, determined by the both clinical and CT imaging scan at Wuhan's largest hospital. There are 5 cases which described the whole clinical and imaging spectrum of the Wuhan-viral Pneumonia. In the result, 96.40% were outpatients, 3.60% were inpatients and 0.56% was admitted to ICU. The patients had various underlying diseases. Common symptoms of the patients were fever, cough, and discomfort. Less common symptoms were dizziness, fatigue, hemoptysis, chest pain, abdominal pain, dyspnea, back pain, headache, and palpitation. Infected patients had complications included respiratory failure, heart failure, shock and enteritis. Abnormalities in chest CT image were detected among all patients. None of them were died so mortality rate is zero. Among 5 cases, 1st patient had fever, blood pressure, palpitation, respiratory problem, decreased WBC, neutrophil, lymphocytes count. CT imaging scan also showed abnormalities. They didn't show any outer significant symptoms. But they had decreased WBC, neutrophil and lymphocyte count. They also showed CT imaging scan abnormalities. All of them were improved after treatment and discharged from hospitals. So, this study showed clinical evidence that Wuhan-viral pneumonia had a low fatality rate. Also, CT imaging plays a pivotal role in the screening, diagnosis, isolation plan, treatment, management or prognosis of patients with Wuhan-viral pneumonia. This study was helpful and important to understand the patient's condition, common problems, susceptibility, level of sickness, severity, cure time, etc. specifically.[31]

CONCLUSION

Diagnosis of COVID-19 is an important issue because proper diagnosis can lead to treatment. As this corona virus shows flue like symptoms, it is indispensable to diagnose properly. RT - PCR is a widely used technique for the diagnosis of COVID-19. Moreover, CT scan is also used to diagnose this disease. Both methods have some advantages and disadvantages. In this review, we summarized the review on laboratory test used in RT–PCR, CT scan and other diagnostic analysis.

Real-time RT-PCR is widely deployed in diagnostic virology. In addition to information on reagents, oligonucleotides and positive controls, laboratories working under quality control programmes need to rely on documentation of technical qualification of the assay formulation as well as data from external clinical evaluation tests.

REFERENCES

1.Benvenuto D, Giovannetti M, Ciccozzi A, Spoto S, Angeletti S, Ciccozzi M. The 2019-new coronavirus epidemic: evidence for virus evolution. 2020, J Med Virol, 20; 89-92.

2. Sun P, Lu X, Xu C, Sun W, Pan B. Understanding of COVID-19 based on current evidence. J Med Virol, 22; 102-104.

3. Cleemput S, Dumon W, Fonseca V, Karim WA, Giovanetti M, Alcantara LC, Deforche K, de Oliveira T. Genome Detective Coronavirus Typing Tool for rapid identification and characterization of novel coronavirus genomes. Bioinformatics, 28 Feb 2020.32;12-16.

4. Kampf G, Todt D, Pfaender S, Steinmann E.Persistence of coronaviruses on inanimate surface and their inactivation with biocidal agents. J Hosp Infect 2020; 104(3): 246–51.

5. European Centre for Disease Prevention and Control data.Geographical distribution of 2019-nCov cases. Available online:(https://www.ecdc.europa.eu/en/geographical-distribution

6. Gralinski L, Menachery V. Return of the

Coronavirus: 2019-nCoV, Viruses 2020;12:135.
7. Report of the WHO-China Joint Mission on Coronavirus Disease 2019 (COVID-2019). Feb 16-24, 2020. http://www.who.int/docs/default-source/coronaviruse/who-china-joint-mission-on-covid-19-finalreport.pdf. Accessed, March 4, 2020.
8. Burke RM, Midgley CM, Dratch A, Fenstersheib M, Haupt T, Holshue M, Active Monitoring of Persons Exposed to Patients with Confirmed COVID-19 – U.S, Jan 2020. 69(9): 245–6.
9. Stobart CC, Sexton NR, Munjal H, Lu X, Molland KL, Tomar S, et al. Chimeric exchange of coronavirus nsp5 proteases (3CLpro) identifies common and divergent regulatory determinants of protease activity. J Virol 2013;87:12611-8.
10.Hu T, Chen C, Li H, Dou Y, Zhou M, Lu D, et al. Structural basis for dimerization and RNA binding of avian infectious bronchitis virus nsp9. Protein Sci 2017;26:1037-48.
11.Wang H, Xue S, Yang H, Chen C. Recent progress in the discovery of inhibitors targeting coronavirus proteases. Virol Sin 2016; 31: 24-30.
12.Narayanan K, Maeda A, Maeda J, Makino S. Characterization of the coronavirus M protein and nucleocapsid interaction in infected cells. J Virol 2000; 74: 8127-34.
13. Du L, He Y, Zhou Y, Liu S, Zheng BJ, Jiang S. The spike protein of SARS-CoV--a target for vaccine and therapeutic development. Nat Rev Microbiol 2009 ;7:226-36.
14. Lin SY, Liu CL, Chang YM, Zhao J, Perlman S, Structural basis for the identification of the N-terminal domain of coronavirus nucleocapsid protein as an antiviral target. J Med Chem 2014 57:2247-57.
15. Shimamoto Y, Hattori Y, Kobayashi K, Teruya K, Sanjoh A, Nakagawa A, et al. Fused-ring structure of decahydroisoquinolin as a novel scaffold for SARS 3CLprotease inhibitors. Bioorg. Med Chem 2015;23:876-90.
16. Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet 2020; 395(10223): 507–13
17. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J et al Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. JAMA 2020; 323(11): 1061–9 .
18. World Health Organization. WHO Director-General's opening remarks at the media briefing on COVID-19 - 24 February 2020.Accessed, February 26, 2020.22;87-92.
19. Hu Z, Song C, Xu C, Jin G, Chen Y, Xu X, et al. Clinical characteristics of 24 asymptomatic infections with COVID-19 screened among close contacts in Nanjing, China. Sci China Life Sci. 2020 Mar 4.221;17-22.
20.Wu Z, McGoogan JM. Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in China: Summary of a report of 72314 cases from the Chinese Center for Disease Control and Prevention. JAMA 2020; 323(13): 1239–42
21. Zhou M, Zhang X, Qu J. Coronavirus disease 2019 (COVID-19): a clinical update. Front Med. 2020 Apr 2. 62;52-55.
22.Grasselli G, Pesenti A, Cecconi M. Critical Care Utilization for the COVID-19 Outbreak in Lombardy, Italy: Early Experience and Forecast During an Emergency Response. JAMA. 2020 Mar 13.
23. Zu F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet 2020; 395 (10229): 1054–62.
24. Deng C, Yang Y, Chen H, Chen W, Chen Z, Ma K and Wang J. Ocular detection of SARS CoV-2 in 144 cases of COVID-19 pneumonia in Wuhan, China: an observational study.
25. Ji D, Zhang D, Chen Z, Xu Z, Zhao P, Zhang M, Zhang L, Cheng G, Wang Y, Yang G, Liu H, Li B, Ji J, Lau G and Qin E. Clinical characteristics predicting progression of COVID-19. SSRN 2020, ssrn.3539674.
26. Tian S, Hu N, Lou J, Chen K, Kang X, Xiang Z, Chen H, Wang D, Liu N, Liu D, Chen G, Zhang Y, Li D, Li J, Lian H, Niu S, Zhang L, Zhang J.. Characteristics of COVID-19 infection in Beijing. Journal of Infection, 2020 Apr; 80(4): 401-406.
27. Habibzadeh P, Stoneman EK. The Novel Coronavirus: A Bird's Eye View. Int J Occup Env. Med, 2020 Feb 5; 11(2): 65-71.
28. Wang Y, Kang H, Liu X, Tong Z. Combination of RT-qPCR testing and clinical features for diagnosis of COVID-19 facilitates management of SARS-CoV-2 outbreak. J Med Virol. Feb 25, 2020.
29. Habibzadeh P, Stoneman EK. The Novel Coronavirus: A Bird's Eye View. Int J Occup Env. Med, 2020 Feb 5; 11(2): 65-71.
30. Patlas MN. COVID-19: What Can We Learn From Stories From the Trenches? Can Assoc Radiol J., 2020 Mar 4: 846537120913497
31. Wang L and Li Q. Clinical and imaging evidence of Wuhan-Viral pneumonia: a large scale prospective cohort study; The Lancet, 2020; THELANCET-D-20-01217.

Conflict of interest: None declared