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Review / Derleme

General View on the COVID-19 Infection Factor and Diagnosis

COVID-19 Enfeksiyon Etkenine ve Tanı Konulmasına Genel Bakış

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ABSTRACT

The coronavirus family is a large family that can be determined in humans or animals. They may cause both mild common colds in humans and severe infections such as Middle East Respiratory Syndrome (MERS) and Severe Acute Respiratory Syndrome (SARS). Coronavirus subtypes determined in humans are easily transmitted from person to person and usually cause mild colds. Many coronavirus subtypes detected in animals can cause severe illnesses in humans by transmitting from animals to humans. World Health Organization, China Country Office, reported cases of pneumonia of unknown etiology in Wuhan, Hubei Province, on December 31, 2019. On January 7, 2020, the agent causing pneumonia was identified as the new coronavirus (2019-nCoV) which has not been detected in humans before and because of its close similarity to SARS-CoV, SARS-CoV-2 and the infection it caused has been named as Acute Viral Respiratory Infection (COVID-19). The World Health Organization declared the COVID-19 infection caused by SARS-CoV-2, the 7th coronavirus known to infect humans, as a pandemic on March 11, 2020 because of its prevalance and severity. Today, the pandemic still continues and the number of deaths and cases is increasing day by day. The agent is needed to diagnose accurately and quickly to control the pandemic and to prevent its spread. The standard and reliable method for diagnosing the SARS-CoV-2 agent is the detection of viral genes by real-time reverse transcription (qRT) polymerase chain reaction (PCR), which is the nucleic acid amplification test (NAAT). In this study, it is aimed to give information about the structure of SARS-CoV-2 virus causing the pandemic today and the tests used to diagnose SARS-CoV-2.

Keywords: COVID-19, diagnosis, SARS-CoV-2

ÖZ

Koronavirus ailesi, insanlarda veya hayvanlarda tespit edilebilen geniş bir ailedir. İnsanlarda hafif soğuk algınlığı tablolarından, Orta Doğu Solunum Sendromu (MERS) ve Ağır Akut Solunum Sendromu (SARS) gibi ciddi enfeksiyon tablolarına kadar değişen hastalıklara neden olabilmektedirler. İnsanlarda tespit edilen koronavirus alt tipleri, insandan insana kolayca bulaşmakta ve genellikle hafif soğuk algınlığına sebep olmaktadırlar. Hayvanlarda tespit edilen pek çok koronavirus alt tipleri ise hayvanlardan insanlara geçerek insanlarda ağır hastalık tablolarına sebep olabilmektedirler. Dünya Sağlık Örgütü, Çin Ülke Ofisi, 31 Aralık 2019'da, Hubei Eyaleti, Wuhan kentinde etiyolojisi bilinmeyen pnömoni vakaları bildirmiştir. 7 Ocak 2020 tarihinde, pnömoniye sebep olan etken, daha önce insanlarda tespit edilmemiş yeni koronavirus (2019-nCoV) olarak tanımlanmış ve SARS- CoV' a yakın benzerliği sebebi ile SARS-CoV-2 ve sebep olduğu enfeksiyon Akut Viral Solunum Enfeksiyonu (COVID-19) olarak isimlendirilmiştir. Dünya Sağlık Örgütü, insanları enfekte ettiği bilinen 7. koronavirus olan SARS-CoV-2' nin sebep olduğu COVID-19 enfeksiyonunu, yayılma hızı ve ciddiyeti sebebi ile 11 Mart 2020 tarihinde pandemi olarak ilan etmiştir. Günümüzde, pandemi hala devam etmekte ve her geçen gün ölüm ve vaka sayıları artmaktadır. Pandeminin kontrol altına alınabilmesi ve yayılmasının önlenebilmesi için etkenin doğru ve hızlı teşhis edilmesi gerekmektedir. SARS-CoV-2 etkenine tanı konulmasında kullanılan standart ve güvenilir yöntem, nükleik asit amplifikasyon testi (NAAT) olan gerçek zamanlı ters transkripsiyon (qRT) polimeraz zincir reaksiyonu (PCR) ile viral genlerin tespit edilmesidir. Çalışmamızda, günümüzde pandemiye sebep olan SARS-CoV-2 virusunun yapısı ve teşhisinde kullanılan testler hakkında bilgi verilmesi amaçlanmıştır.

Anahtar Kelimeler: COVID-19, tanı, SARS-CoV-2

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INTRODUCTION

In December 2019, cases of atypical viral pneumonia of an unknown cause were reported in Wuhan, Hubei, China (1). On January 12, 2020, the World Health Organization referred to the factor that causes very serious respiratory tract infection in humans as the novel coronavirus (2019-nCoV) (1). Until January 20, 2020, infections with 2019-nCoV were confirmed in 291 patients in China and 4 patients in Japan, South Korea and Thailand based on clinical diagnosis, epidemiological examinations and sequence analyses (1). The novel coronavirus was subsequently named as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) owing to its similarity with SARS-CoV and the infection caused by it was named coronavirus disease 2019 (COVID-19) (2). On March 11, 2020, the World Health Organization declared COVID-19 a pandemic because of its prevalence and severity (2). COVID-19 rapidly spread to Italy and other European countries, the United States of America, 5 continents and 200 countries after its outbreak in China (3). Currently, the highest number of cases and death rates are observed in the United States of America followed by India, Brazil and Russia (4). Using several sequencing methods, the genome structure of SARS-CoV-2 has been determined and three different strains have been identified. SARS-CoV-2 belongs to the *Betacoronavirus* type 2b ancestry within the family *Coronaviridae*. It is closely related to bat SARS-like coronavirus isolate bat-SL-CoVZC45 (2). The fact that SARS-CoV-2 is similar to bat coronaviruses indicates that bats are likely the reservoir hosts (5, 6). However, an intermediate host that causes SARS-CoV-2 to emerge in humans has not yet been identified (3). Evidence reveals that SARS-CoV-2 is zoonotic and was transmitted to humans from the Huanan Seafood Wholesale Market, an illegal wild animal sale market located in Wuhan (7). A great variety of animals were sold in this market. All animals that were sold in the market, notably bats, wild animals and farm animals, should be observed to trace the origin of SARS-CoV-2 transmission (1).

General Information

Coronaviruses were first discovered in 1920s. In 1960s, human coronaviruses were discovered. They got their name from the crown-like sharp spikes that project from their surface envelopes (8). These spikes contain proteins that play a role in connecting host cell receptors to the virus (9).

Endemic human coronaviruses (HCoV-229E, HCoV-HKU1, HCoV-NL63 and HCoV-OC43) are also known as cold viruses (10, 11). SARS in 2002-2003, Middle East respiratory syndrome (MERS) in 2012 and SARS-CoV-2 in 2019 were the coronaviruses that caused epidemics through animal-to-human and human-to-human transmission (1).

Mutations occur in ribonucleic acid (RNA) viruses at very high rates. New species are formed because of mutations and the virulence of the virus increases.

The interspecies infection mechanism of new coronaviruses has not been completely explained. It is likely that new coronavirus outbreaks will occur in future owing to the increasing interactions between humans and animals, global warming and ecological changes. Therefore, studies on developing effective treatments and vaccines against new coronaviruses should be concentrated on immediately (1).

Pathogenesis

The transmission of COVID-19 occurs when the virus in the respiratory system of an infected individual is transmitted to healthy individuals through respiratory droplets. Moreover, respiratory droplets contaminate the hands of those exposed and from there, the transmission occurs through the mouth, nose and eye mucosa (2). Apart from direct transmission, indirect transmission can occur through surfaces or objects contaminated by the patients (12). The incubation period of the infection is 2-14 days (2).

An infected person starts shedding the virus 1-2 days before the onset of symptoms and can continue shedding up to 2 weeks later (2). The detection of clinical symptoms occurs 3-6 days after presumed exposure to the virus within 11.5 days in the majority of patients, within 5 days in most patients and within maximum 14 days in some patients (13). The course of COVID-19 differs from patient to patient; the patients could be asymptomatic or present with mild flu or severe respiratory distress. Asymptomatic or mild-form, severe and critical prognosis rates have been reported differently in studies. An asymptomatic course or mild symptoms are observed in approximately 80%-90% of the patients. A serious course is observed in approximately 10% cases and a critical course is observed in approximately 5% cases (5).

The symptoms of the infection are fever, dry cough, headache, throat ache, chest pain, difficulty in breathing, muscle and joint pain, diminished taste and smell perception, loss of speech and motion, fatigue, conjunctivitis, recurring gastrointestinal problems and pneumonia, especially in older adults (people over the age of 50 years) and people in the high-risk group (patients with cancer, hypertension, diabetes, kidney and heart diseases, dyspnea and multiple organ dysfunction) (1, 2, 5, 14).

SARS-CoV-2 infects people via the angiotensin converting enzyme (ACE-2) receptor that belongs to the

renin–angiotensin system (15). ACE-2 receptors are surface molecules situated mainly in the lungs, heart, kidneys, bowel, pancreas and buccal mucosa (16). Vasoconstriction, fibrosis and hypertrophy cannot be prevented because the virus uses ACE-2 receptors in the patients (17). As a result, pneumonia and organ damage occur and the metabolic balance is destroyed (15). The expression of ACE-2 receptors heals acute lung injury, represses hypertension and heart dysfunctions and prevents glomerular and biliary fibrosis (18).

The mortality rate of COVID-19 varies from approximately 2% to 5% (1). This variability can be affected by the places where the disease is more or less observed, the characteristics of patients, the prevalence rate of the infection and the relative number of tests (5).

According to the data of the World Health Organization, COVID-19 infection in the world has caused more than 107 252 000 cases and more than 2 355 000 deaths until February 12, 2021 (19).

Genome structure of the agent

Coronaviruses belong to the family *Coronaviridae* and subfamily *Coronavirinae* (1). This family encompasses the genera *Alphacoronavirus, Betacoronavirus, Gammacoronavirus* and *Deltacoronavirus*. The host tropism of coronaviruses is different among these four genera (1). The species of the genera *Alphacoronavirus* a nd *Betacoronavirus* infect mammals; most species of the genera *Gammacoronavirus* and *Deltacoronavirus* infect birds and fish and some infect mammals (1, 20, 21).

Genomic investigations suggest that SARS-CoV-2 is closely related to the species of the genus *Betacoronavirus*; however, on the basis of sequence, SARS-CoV-2 is 79% identical to SARS-CoV and 50% identical to MERS-CoV (22, 23). According to phylogenetic analyses, SARS-CoV-2 is taxonomically related to the subgenus *Sarbecovirus* along with SARS-CoV and bat SARS-like coronaviruses (bat-SL-CoVZC45 and bat-SL-CoVZXC21) (24). This reinforces that bats may be the natural reservoir of the virus (5).

Coronaviruses are the largest RNA viruses (3). According to genomic investigations, the new coronaviruses share the genomic structure of an ordinary coronavirus; they are RNA viruses that are spherical, approximately 30 kilobytes long, single stranded and positively oriented and usually have crown-shaped peplomers of 80-160 nanometer in size (1). The viral RNA works as a template to form the transcription complex (25, 26). The termination of transcription is followed by the formation of the leader RNA in the transcription regulatory domains in an open reading frame (ORF). There are at least 6 ORFs in the genome of coronaviruses and these ORFs constitute approximately two-thirds of the genome length. The ORFs located near the 3′-terminal region of the viral genome encode structural and non-structural proteins. The structural proteins include prominent envelope glycoproteins embedded in the envelope and envelope, nucleocapsid and membrane proteins. Envelope glycoproteins play a role in developing a connection to the host cell receptors, membrane proteins in shaping the virions, envelope proteins in the assembly and oscillation of the virus and in viral pathogenesis and nucleocapsid proteins in genome packaging. Apart from these structural proteins, ORFs encode auxiliary proteins and special proteins (1a, 1b, 3a, 3b, 4a, 4b, 6, 7a, 7b, 8 and hemagglutinin esterase proteins) that are structural in different coronaviruses. Most of the 16 non-structural proteins are responsible for replication; some prevent interferon induction and the roles of some are unknown (1).

There is 54% similarity among different coronaviruses on the basis of their genomes. Among different coronaviruses, structural protein-coding regions are 43% similar and non-structural protein-coding regions are 58% similar (1).

Among all RNA viruses, only coronaviruses contain 3′-5′ exoribonucleases, which perform the proofreading function in the replication complex (27-29).

Diagnosis

Specimen collection, transportation and storage

Combined nasopharyngeal and oropharyngeal swabs are primarily used for the diagnosis and screening of infected patients (30-33). Performing tests with lower respiratory tract samples (sputum, bronchoalveolar lavage and endotracheal aspirate) is more reliable (34-36). Moreover, the swabs must not be contaminated (1). After collecting the samples, they must be packed properly, stored at 2-8°C and delivered to a laboratory within the shortest time (37). Nasopharyngeal or oropharyngeal washes, sputum, endotracheal aspirate and bronchoalveolar lavage can be stored for maximum 2 days and nasopharyngeal and oropharyngeal swabs, serum and whole blood for maximum 5 days (38). Samples that need to be stored longer than this duration should be stored at -70°C (34).

Sufficient safety procedures should be implemented in due course of sample collection, packaging, storage and transportation (34).

When samples arrive at a medical microbiology laboratory, they must be readily subjected to polymerase chain reaction (PCR) under biosafety level 2 conditions, using masks, goggles, face shields, gloves and disposable lab coats (14, 39-41).

Nucleic acid amplification test

The diagnosis of COVID-19 is based on the principle that the presence of SARS-CoV-2 is detected using molecu-

lar methods (22, 42). The standard verification of acute SARS-CoV-2 infections is based on the determination of unique viral sequences by NAATs such as qRT-PCR (43).

Since amplification and analysis are performed simultaneously and in a closed system in the qRT-PCR method, contamination does not occur and instances of false-positive results are decreased considerably (34). For detecting the presence of SARS-CoV-2 via qRT-PCR, genes that encode some structural proteins (envelope glycoprotein; envelope, membrane and nucleocapsid proteins; and helicase) (44, 45) and type-specific target molecules are used (46-49). The Centers for Disease Control and Prevention recommends that two nucleocapsid proteins (N1 and N2) should be used as targets (47), while the World Health Organization recommends that a person should be diagnosed based on the presence of the envelope gene followed by validation of the test using the RNA polymerase enzyme (36).

If the diagnosis is not performed using nasopharyngeal swabs in those exposed to the virus at high levels, the test should be repeated or lower respiratory tract samples should be preferred (1). In addition, if the test results of samples from suspected patients are negative, the test should be repeated after 24-48 hours (2).

In order to end the isolation of a patient, the virus should not be detected in two successive qRT-PCRs with an interval of at least 24 hours and the patient should not have clinical symptoms and epidemiological criteria (1).

Serological tests

Serologic assays are based on the detection of antibodies produced in those infected with SARS-CoV-2. Total immunoglobulins (Ig), IgM, IgG and/or IgA, formed in the body against SARS-CoV-2 factor can be detected using enzyme-linked immunosorbent assay and chemiluminescence immunoassay. The performance of serological tests depends on different patient populations, the age of patients, the time of the test and the target viral protein. Coronavirus antibody determination tests can cross-react with different pathogens, including other human coronaviruses or with pre-existing conditions (e.g., pregnancy and autoimmune diseases) and resulting in false-positive conclusions (43).

Virus neutralization tests are the gold standard tests for detecting antibodies formed against SARS-CoV-2 factor. However, these tests are not available for routine diagnosis because they require well-educated and qualified staff and biosafety level 3 laboratory facilities (43).

Rapid diagnostic tests based on antigen detection

These tests are based on the detection of proteins (antigens) that belong to SARS-CoV-2 agent in respiratory tract samples. They include lateral flow immunoassays that provide results in a short time. Since there is no target amplification, their sensitivity is low. In addition, false-positive results can occur in cases of other human coronaviruses. At present, the parameters of antigen performance in clinical settings are limited (43).

Viral isolation

Virus isolation is not recommended as routine diagnostic procedure because it requires experienced and qualified staff (43). Cell cultures are used in virus isolation and vaccine and treatment studies (50).

CONCLUSION

Both symptomatic and asymptomatic patients should be diagnosed and treated to prevent the spread of COVID-19 pandemic that still affects the world. Laboratory diagnosis and vaccine studies for SARS-CoV-2, which causes the infection, should be focused on.

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