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Epidemiology and diagnostic tools of the new virulent pathogen of COVID-19: Case of Saudi Arabia

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Abstract

The rapid spread of COVID-19 from Wuhan City of China to the whole world makes the necessity to adopt the latest technologies in epidemiology and diagnostics mandatory. In Saudi Arabia, the case of COVID-19 is moving more classically. It was proven that the death rate in Saudi Arabia can be the lowest across all countries in the world. A large number of companies and governmental laboratories started to develop new kits and tentative vaccines as for the diagnosis and treatment, while time is not permitting enough testing of either. One of the major factors in controlling the COVID-19 pandemic is the ability to identify all cases of acute respiratory coronavirus type II (SARS-CoV-2). Available robust technology is the main pillar of the success of the virus containment plan, which aims to provide widespread testing to the largest number of health service providers and rapid screening laboratories to isolate the progressive number of cases. In addition to the inaccuracy of the tests supplied to many countries including the Kingdom of Saudi Arabia due to their design based on isolated viruses from non-Saudi samples. The expensive Reverse Transcription Polymerase Chain Reaction (RT-qPCR) is currently considered the gold standard for SARS-CoV-2, however, laboratories equipped with this technique is still a stumbling block in examining large suspected cases and asymptomatic cases in the incubation or recovery period. On the other hand, the inexpensive and rapid immunological tests used to diagnose SARS-CoV-2 indirectly carries problems of inaccuracy, and for this reason, most ministries of health across the globe have prohibited their use in the diagnosis. Therefore, the use of the latest Next Generation Sequencing (NGS) technology became the perfect alternative to conducting genomic scans of some locally isolated viruses and using this information to design a diagnostic test with high-throughput and without the need for expensive laboratory equipment and dependent on the latest genetic information for the locally spreading virus..

Keywords: Covid-19, pathogen, coronavirus, genetic, SARS-CoV-2, Next Generation Sequencing (NGS).

INTRODUCTION

A virus of COVID-19 spread fast across the world emphasized the ability of most countries to respond to such a new virulent pathogen. SARS-CoV2, extreme acute respiratory syndrome coronavirus 2, is responsible for COVID-19 (coronavirus disease 2019), which has spread from Wuhan City in China to the whole world [1]. Up to date (1 March 2020), around 3.4 M cases of patients with COVID-19 and 239 thousand deaths have been reported (Coronavirus Outbreak. 2020, https://www.google.com/search?q=corona+map+google&rlz=1C1GCEA_enSA753SA753&oq=coro&aqs=chrome.1.69i57j35i39l2j0l5.9528j0j4&sourceid=chrome&ie=UTF-8). Of which, Saudi Arabia has reported over 25,000 cases and 176 deaths. Fortunately, so far, the percentage of affected children is low, but the future behaviour of COVID-19 is unpredictable.

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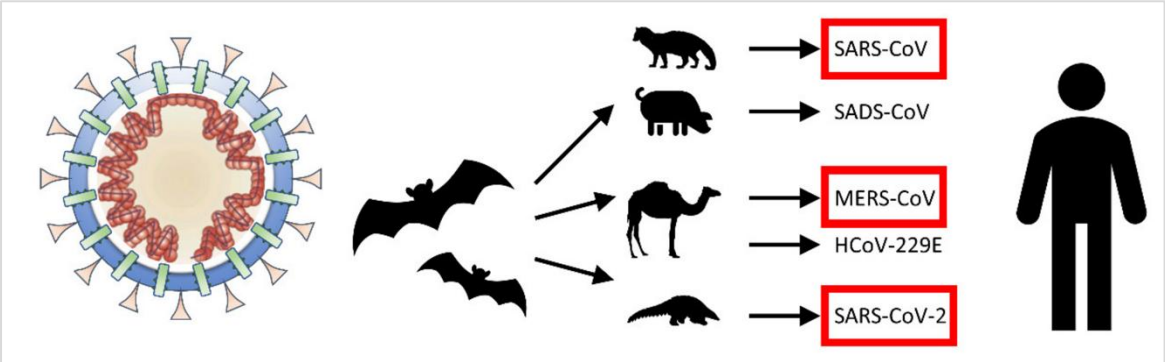
Coronaviruses (its name came from its surface that giving it crown-like appearance with spike) are RNA viruses with envelope ranging from 60-140 nm in diameter [2]. There are four coronaviruses (HCoVs), namely HCoVs-HKU1, HCoVs-NL63, HCoVs-229E, and HCoVs-OC43, discovered so far that can infect humans and cause mild to moderate respiratory diseases [3]. All coronaviruses that infect humans have a common feature of which they



are transmitted from animal origins either in bats or rodents [4]. SARS-CoV and MERS-CoV, for instance, was directly transmitted to humans by civet cats and dromedaries' camels, respectively (Figure 1).

Fig. 1: Candidate animals that can transmit coronaviruses to human.

SARS-CoV is introduced to humans by bats and civet animals, MERS-CoV by bats and camels and dromedaries to humans, SARS-CoV-2 is potentially transmitted to humans by bats and then by pangolins illegally shipped to Chinese markets [4, 5].

Spike proteins across both coronaviruses have variable receptor-binding (RBD) domains. Consequently, RBD binds to ACE-2 (Enzyme-2 receptor that translates angiotensin), which promotes viral entry into target cells. Receptors are found in different forms of cells, such as gastrointestinal tract, pulse, kidneys and, most notably, lung cells [5], Extreme acute respiratory syndrome coronavirus-2 (SARS-CoV-2) was also capable of infecting other species with descendants from bats and after the specific genetic alteration. However, this sequence of mutations not only improved the interaction of RBD with humans and ACE-2 receptors but also decreased the link with rodent and civet receptors, which is why Cyranoski and his colleagues conclude that pangolin is an intermediate host of SARS-CoV-2 [6].

This is only early speculation, and there are other suggestions that SARS-CoV-2 is created by men from an established coronavirus, but no evidence to confirm such a hypothesis until now; Extreme acute respiratory syndrome coronavirus-2 (SARS-CoV-2) was also capable of infecting other species with descendants from bats and after the specific genetic alteration. However, this sequence of mutations not only improved the interaction of RBD with humans and ACE-2 receptors but also decreased the link with rodent and civet receptors, which is why Cyranoski and his colleagues conclude that pangolin is an intermediate host of SARS-CoV-2 [6]. This is only early speculation, and there are other suggestions that SARS-CoV-2 is created by men from an established coronavirus, but no evidence to confirm such a hypothesis until now; except for the claim of recovering the new devastating version during manipulation a strain for vaccination purposes.[5]. stated the difficulty to prove or disprove theories of the SARS-CoV-2 origin.

To contain the infection, it is very important to determine the origin and transmission process to develop preventive strategies. In the case of SARS-CoV, only civet palm could be a secondary host on the basis of positive results for viral RNA in the food market [7]. It has been shown that SARS-CoV spread on humans prior to the 2003 outbreak [8]. through a seroprevalence survey of healthy persons from Hong Kong that showed that 2.5% had antibodies against SARS-coronavirus [8]. Additional signs that Rhinolophus bats have anti-SARS-CoV antibodies suggest that bats are a source of viral replication [9]. The second outbreak was the Middle East respiratory syndrome (MERS) caused by the MERS-CoV virus that emerged in Saudi Arabia in 2012 [10]. MERS-CoV also belongs to a beta-coronavirus family and its primary host is camels [11], but recently, MERS-coronavirus was also found in Perimyotis bats and Pipistrellus [12], withdrawing that the main transmitting key host of the virus is a bat [13] ; [14]. In the beginning, it was suggested that a possible host is snakes. Despite this, an intensive genomic study discovered novel SARS-like coronavirus in bats, not snakes (Table 1) [15] ; [16]. A recent study by [16]. using homologous recombination of a receptor that binds spike protein of SARS-CoV 2 was found to have been developed from a SARS-CoV and unknown Beta-CoV [16].

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Table 1. The main features of SARS-CoV, MERS-CoV, and SARS-CoV-2.

Features	SARS-CoV	MERS-CoV	SARS-CoV-2
Date of Emergence	Nov. 2002	Jun. 2012	December 2019
Country of emergence	Guangdong, China	Saudi Arabian	Wuhan, China
Date of fully controlled	Jul. 2003	Jan. 2014	Not controlled until the date of publication
Transmitted Host	Bat, palm civets, and Raccoon dogs	Camel, Bat	Bat
Number of countries infected	26	27	globally
Entry receptor in humans	ACE2 receptor	DPP4 receptor	ACE2 receptor
Sign and symptoms	fever, malaise, myalgia, headache, diarrhea, shivering, cough and shortness of breath	fever, cough, and shortness of breath. Pneumonia is common, but not always present.	Cough, fever, and shortness of breath
Disease name	ARDS	MERS	COVID-19
Total infected patients	8,098	2,494	1,468,833
Total recovered patients	7,322	1,636	316,482
Total died patients	776 (9.6% mortality rate)	858 (34.4% mortality rate)	85,445 (5.8% mortality rate)

Entry mechanism of human coronaviruses

Each coronavirus has three proteins encoded in polycstrnic open read frame 1 (OPF1).: viral replication, nucleocapsid, and spikes formation [17]. Spikes placed on the surface of the virus are responsible for recognition and attachment to host cells through RBD, which is loosely attached among viruses; therefore, the virus is capable of infecting multiple hosts [18] ; [19]. As shown in Figure 2, the key factor of the coronavirus entry mechanism depends on the cellular proteases TMPRSS2 (cathepsins and transmembrane protease serine 2) that split the spike protein and causes changes in penetration [20] ; [21]. after initial recognition between viral RBD and angiotensin-converting enzyme 2 (ACE2) on the host cell [22].; [18]. SARS-CoV-2 has a typical coronavirus entry mechanism with spike protein [23] ; [24]. In addition, the 3D structure of the RBD protein of the SARS-CoV-2 spike maintains the affinity through van der Waals forces [25]. The glutamine residue in the RBD region of SARS-CoV-2 is recognized by the human ACE2 receptor [26].

SARS-CoV-2 virus disease and an outbreak of COVID-19

Many citizens of Wuhan, China were diagnosed with severe pneumonia of unknown causes in local hospitals in December 2019. Many of them were exposed to a local wholesale market for seafood, which also buys and sells live animals. Breathing samples were taken directly from patients and submitted to reference laboratories for etiological investigations. The outbreak was reported by the Chinese government reported to the World Health Organization (WHO) on 31 December 2019, and the Huanan seafood market was closed and on 1 January.

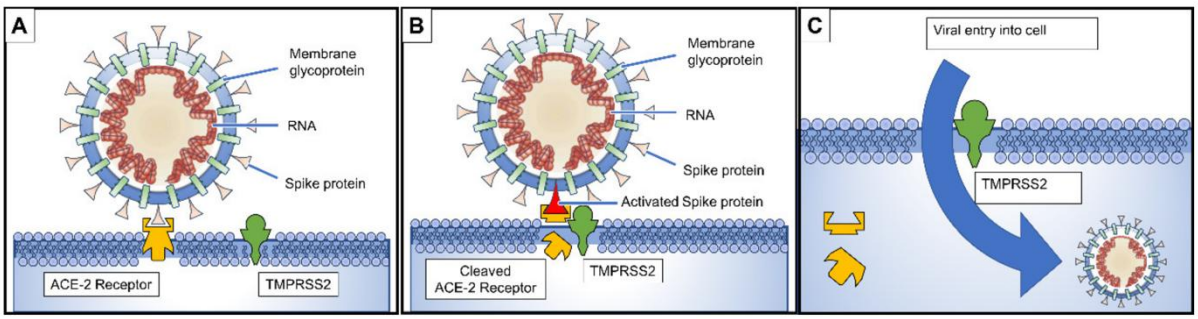

Fig.2: (A) Affinity between spike proteins on coronavirus and angiotensin-converting enzyme 2 (ACE-2) receptors on the surface of target cell; (B) TMPRSS2 (trans-membrane serine protease type II) cleaves ACE-2 receptor.

In the process, spike protein is activated; (C) Cleaved ACE-2 and activated spike protein facilitates viral entry. TMPRSS2 expression increases cellular uptake of coronavirus. (https://doi.org/10.3390/pathogens9030231). On 7 January, reference labs have identified the virus as a coronavirus and environmental samples were proven to be

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originated from this food market (http://www.xinhuanet.com/english/2020-01/27/c_138736278.htm). Because of the dramatic increase in cases, some of which have never been exposed to animals in the market supporting the hypothesis of virus transmission from humans to humans [27]. The intense Chinese tourist activity for the Chinese New Year vacation outside Wuhan Province has caused the epidemic, and cases have already been reported in other provinces as well as in other countries such as Thailand, Japan, South Korea, etc. On 20 January, several cases were discovered among health care workers. By 23 January, strict restrictions were imposed on entry and exit from Wuhan, and compulsory quarantine were imposed on 11 million citizens. Soon, the virus was spread to other cities in Hubei Province. A few days later, cases of COVID-19 have been reported in countries outside of China in countries with no history of travel to China indicating a local transition from one person to another in these countries [28]. Due to these exceptional circumstances, airports in various countries, including Saudi Arabia, have established mechanisms to screen the travelers returning from China who have symptoms and to be isolated and tested for COVID-19 and it soon became clear that the infection could be transmitted from people who do not appear symptoms and also before symptoms develop. As a result, countries including Saudi Arabia evacuated their citizens from Wuhan on special trips and put them or all people with symptoms in isolation for 14 days and tested them to check the presence of the virus. Cases continued to increase significantly doubling its time by 1.8 d [29]. As of 8 April, 1,468,833 cases worldwide existed and only 316,482 have been recovered, while 85,445 died thereafter (https://www.worldometers.info/coronavirus). In Saudi Arabia, only one case had been reported until 3 March, but within the two months, the number of cases increased to more than 25,000 (Figure 3).

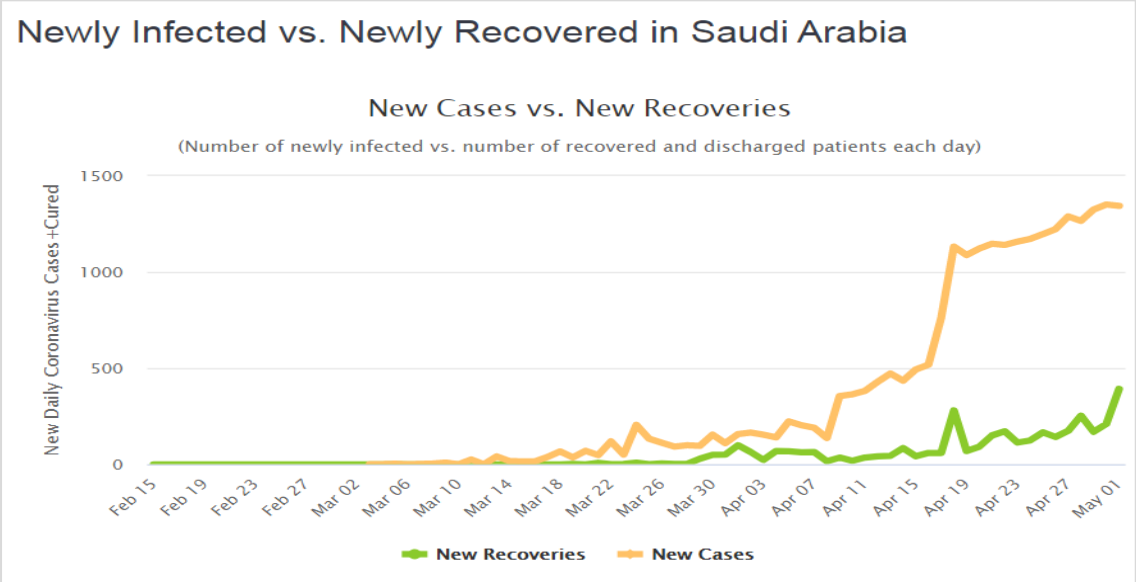

Fig. 3: Newly infected vs. newly recovered residents in Saudi Arabia from 15 February to 8 April (2020) (https://www.worldometers.info/coronavirus/country/saudi-arabia).

Epidemiology and Pathogenesis

The infection is spread by major declines that occur while coughing and sneezing in patients with symptoms but can also occur from people who do not have symptoms and before symptoms appear and all ages are at risk [28]. Regarding the viral load, Zou and his colleagues reported that the maximum viral loads present in the nasal cavity and less amount in the throat with no difference with symptomatic and asymptomatic people [30]. These infected droplets spread infection within 1-2 meters and precipitated on the surfaces and can remain viable for a few days in suitable temperatures but easily being disinfectant using common antiseptics such as sodium hypochlorite (NaOCl), 70% alcohol gel, H_2O_2, etc. [31]. As per WHO, an infection could be acquired either by inhalation of these droplets or by touching contaminated surfaces then touching eyes, mouth, or nose. As per current information, transplacental transmission from pregnant women to their fetus has not been described [32]. The incubation period of the virus ranged from 2 to 14 days (average 5 days).

Clinical Features

Clinical symptoms of COVID-19 coronavirus disease differ, varying from asymptomatic to sudden initiation of severe lung inflammation (acute respiratory distress syndrome) and multi-organ dysfunction. General health symptoms include (but not all) nausea, exhaustion, dry cough, headache, fatigue, body pain, and shallow breath. This illness cannot, therefore, be easily distinguished from other common respiratory infections. In severe cases,

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the disease may progress to pneumonia, breathing failure, and possibly death by the end of the first week. This progress is associated with a storm of inflammatory cytokines including the most frequent inflammatory factors [32]. The illness interval from the incubation time to early signs has been 5 days, accompanied by 7 days to 8 days of acute respiratory distress syndrome (ARDS). 25-30 per cent of patients with serious circumstances need emergency treatment to prevent problems such as acute respiratory disease, bleeding, and acute kidney failure. In the second or third week, the fortunate patients continue recovery. Unfortunately, at the late stage of the illness, mortality is normal (50-75 per cent). In fact, 4-11% of hospitalized patients can lose their lives, but just 2-3% of the overall amount of sick individuals will lose their lives (https://www.worldometers.info/coronavirus/). Ironically, the disease in patients outside Hubei Province was milder than in patients outside China [33]. Likewise, patients outside China reported mild seriousness and lower mortality rates (WHO), suspected to be attributed to higher levels of expression of ACE2 receptors on respiratory mucosa [34]. The incidence of COVID-19 in neonates, babies and children were slightly milder than adult counterparts [32,35].

Genomic structure of SARS-coronaviruses

COVID-19 is more than 80% as the previous human coronavirus (SARS-like bat CoV) [36]. The genome of SARS-CoV-2 is encoded by the four structural genFes encoding spike (S), envelope (E), membrane (M), and nucleocapsid (N). The *orf1a* gene encodes pp1a protein, which also contains 10 SNPs [36] ; [15]. Although SARS-CoV-2 lies close to the group of SARS-coronaviruses (Li *et al.* 2020 [29]) as shown in the evolutionary tree of Figure 4, a remarkable variation in SARS-CoV and SARS-CoV-2 is detected in terms of an absence of fluctuation in the number of amino acids in 8b, 3c and 8a proteins in SARS-CoV-2 [36]. (Figure 4). Furthermore, the spike protein of coronavirus isolated from Wuhan is found to be mutated. The SARS-CoV-2 spike protein is a mixture of SARS-CoV bat and the not identified Beta-CoV [29]. It was confirmed that SARS-CoV-2 and SARS-CoV use the same ACE2 cell receptor to enter the host cell [37] ; [33], also a binding affinity for ACE2 may have been significantly enhanced by the single N501T mutation in SARS-CoV-2's Spike protein [26].

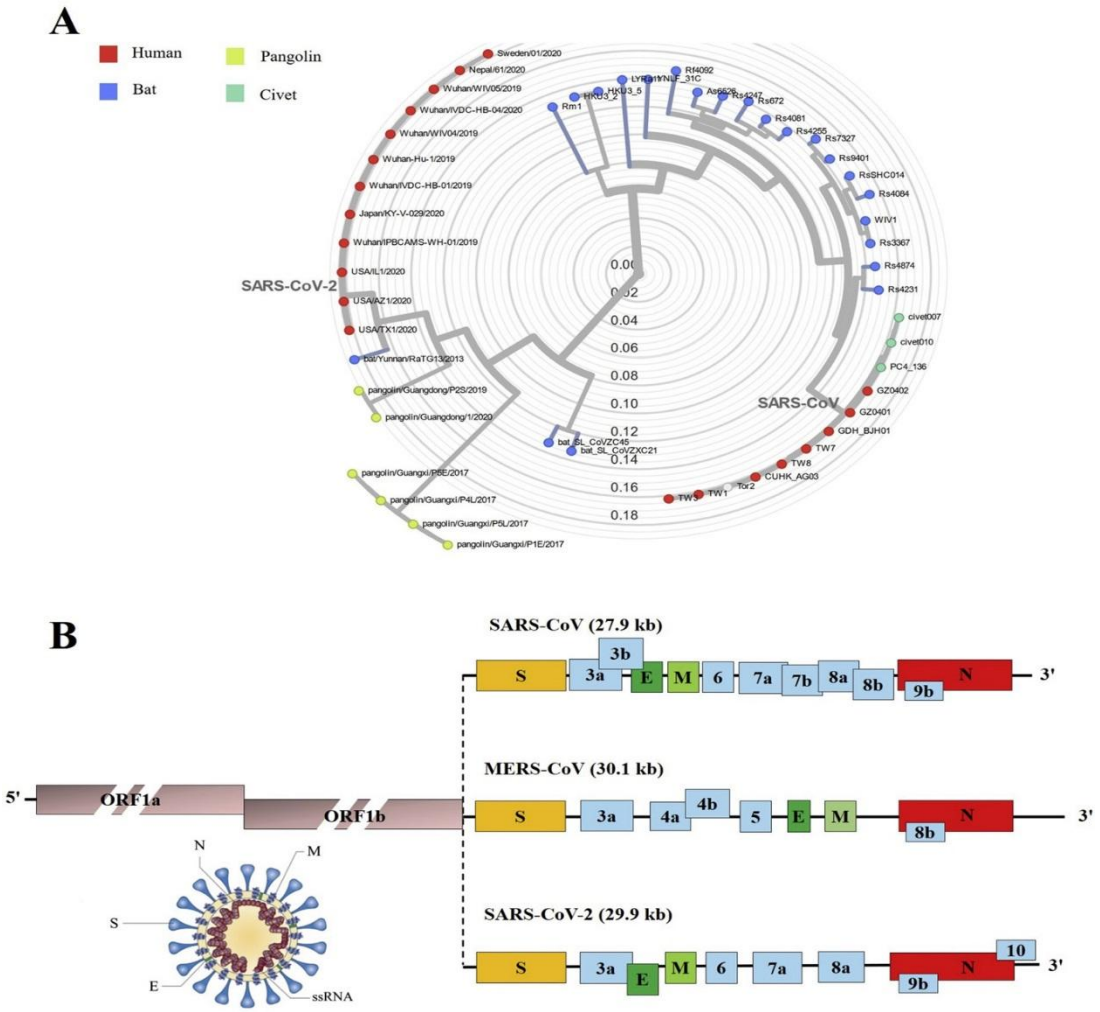

Fig. 4: (A) Respiratory tree and (B) genome organization of Betacoronaviruses; SARS-CoV-2, SARS-CoV, and MERS-CoV genomes (https://doi.org/10.1016/j.jpha.2020.03.001).

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Appearance of antigens in coronavirus infection

Once the virus reaches the cell that is the centre of the body's antiviral immunity, the antigen is introduced from the presentation cells (APC) of the antigen. This is linked to and recognized as antigenic peptides by a broad histocompatibility complex (MHC; or human leukocyte antigen (HLA) in CTLs (virus-specific cytotoxic T lymphocytes). Learning the presence of antigen SARS-CoV-2 is essential in our interpretation of the pathogenesis of COVID-19. The distribution of SARS-CoV antigens is mainly focused on MHCI molecules [38] but MHCII also contributes to its distribution. If the virus reaches the tissue, which is the nucleus of the anti-viral immunity of the body, the antigen is introduced from the presentation cells of antigen (APCs). CTLs (virus-specific cytotoxic T lymphocytes) introduce and identify antigenic peptides through a large complex of histocompatibility (MHC) or human leukocyte antigen (HLA) in humans. Comprehending the antigen appearance of SARS-CoV-2 is important to our knowledge of COVID-19 pathogenesis. The presentation of SARS-CoV antigens is mainly focused on MHCI molecules [38], but MHCII also contributes to their presentation.

PREVENTION

This segment offers useful information on COVID-19 detection, diagnosis, and process. Until now, COVI-19 has not been licensed, so protection is highly important. Previous research shows that several HLA polymorphisms are associated with SARS-CoV such as several, HLA-Belf0703, HLA-Belf460, HLA-DR B11202 [39] and HLA-Cw0801 [40], among others. It also suggests that allles HLA-DR0301, HLA-Cw1502, and HLA-Aelf0201 are concerned with SARS defense [41]. On the other hand, MHCII molecules are associated with the susceptibility to MERS-CoV infection [42]. Also, the gene polymorphism of mannose-binding lectin (MBL) associated with antigen presentation is linked to the risk of SARS-CoV infection [43]. Spreading rate of the virus makes the prevention of a complex process difficult as a result of infection even before symptoms appear in the incubation period, and the transmission between people who do not show symptoms, in addition to the long incubation period, and its transmission even after clinical recovery (https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/infection-prevention-and-control). Among the most important recommendations that must be followed are: Separate positive cases of the virus such as COVID 19 or suspected cases. Patients and health care workers should wear clear surgical masks while they are in the same room and take care of hand hygiene every 15 to 20 minutes.

An important finding in the outbreak of SARS in 2002 is that 21% of the affected patients affected were health-care workers [44]. As a result, transmission to health-care workers is the highest risk in COVID-19, which is confirmed by infection of nearly 1500 health-care workers in China in addition to six deaths as well as doctors who first warned about the virus, realizing the importance of protecting the health-care workers and the continuity of care to protect the further transmission of infection to other patients.

Although the China National Health Commission classifies COVID-19, which is spread as a droplet pathogen, as class B of infectious agents (highly pathogenic H5N1 and SARS), it firmly advises prevention steps for Class A agents (including cholera and plague). There is often a need for more monitoring mechanisms, such as: putting patients together in different rooms or clusters, typically without the need for the harmful pressure rooms. Applying routine decontamination techniques to all spaces, structures and equipment ideally use sodium hypochlorite. They are providing health-care staff with fit-tested N95 respirators in addition to protective suits and goggles, monitoring all the contacts that including the health-care personnel for the development of any symptoms related to COVID-19. Patients may be discharged patients from isolation can take place once their body temperature has returned into normal for at least three days and have two repeated negative results tests at the molecular level at 1 d sampling interval. Note that these guidelines are different in case of pandemic flu where patients can return to their daily life activities once afebrile for 24 h or by day 7 of illness, and there is no need for a molecular test to confirm negative results. In terms of community, all people are strongly recommended to do the following; Avoid public and crowded places and stop any travels as much as possible that are not essential especially appropriate for places with the continuous transmission. Train how to cough effectively by coughing in gloves or cloth rather than the mouth, as well as lessons for the best way to wash hands by regularly washing every 15-20 min. Patients with respiratory problems are advised to use masks in public places emphasizing that the usage of masks for healthy persons is not suggested by the WHO because it has not been proven to shield them against respiratory virus infections. Given this, in China, the public was required to wear masks in traffic areas and ban large-scale gatherings (parks, cultural activities, etc). Moreover, China plans to take all possible control measures and considering introducing legislation to forbid selling and trading wild animals [44].

MOLECULAR DETECTION OF THE DISEASE

Detection of immunoassay

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Immunoassays have been widely used as a diagnostic tool. They recognize antigen-antibody reactions to identify viral antigens in samples or vice versa using viral antigens to detect patient antibodies in the presence of colouring indicator. The same principals, a rapid test was developed using lateral flow assay format encased in a cassette containing the necessary capture reagents immobilized at a specific position on a nitrocellulose membrane, as well as labeled detector monoclonal antibody (mAb) that recognizes the same target. A positive result is the coloured line due to the binding between the capture mAb and binding by the detector mAb. Only two drops of blood are enough to diagnose a virus. They are as same as home pregnancy kits.
Immunofluorescence assays (IFA) is commonly used in some countries as a valuable tool that directly detects viral antigens in tissue parts or infected cells. It uses a fluorescent label conjugated with antiviral antibodies and the amount of the fluorescence produced is associated with the binding between the viral antigens and antibody [45] ; [46] ; [47]. Rapid identification of both IgM and IgG antibodies enhances the diagnosis and treatment of COVID-19 disease, providing information on the time course of infection. Detection of IgM antibodies suggests a recent exposure to SARS-CoV-2, whereas IgG antibodies specify the previous exposure [46]. Serum IgM and IgG antibodies against SARS-CoV-2 can also be detected by IFA, where their presence indicates the humoral immune response to the viral infection [45]. However, serum antibodies take a few weeks to develop, and therefore it is not useful in the fast responding to the pandemic. It is only useful in providing a historical image of the viral infection. IgM and IgG titers were found to be undetectable in earlier infection [47]. The timeline for the first identification of anti-SARS-CoV antibodies ranged from day 3 to day 42 for IgM and day 5 to day 47 for IgG antibodies [48] ; [45]. Recently, a point-of-care side flow immunoassay (LFIA) test kit has been developed after the spread of the pandemic that can detect combined IgM and IgG antibodies in blood samples within 15 minutes. It is fast, easy, and has a high sensitivity and specificity to the diagnosis of COVID-19 [46].

A further biochemical serological detection approach based on serological enzyme-linked immunosorbent assays (ELISA) is used to confirm infection with SARS-CoV-2 [49]. Both viral antigens and defenses antibodies may be detected in the sample [50]. Several antibodies of SARS-CoV-2 may be used to detect ELISA including nucleocapsid, spike, and envelope antibodies. The study confirmed that combined nucleocapsid and spike protein-based ELISAs for antibodies detection against SARS-CoV-2 is an effective and sensitive platform for large-scale screening of COVID-19 especially 10 days of post-viral infection [38]. A simple and quick serological platform has been reported to detect seroconversion upon SARS-CoV-2 infection using ELISA to show the kinetics of humoral immunity reactions. The approach is based on recombinant antigens derived from spike proteins that doesn't require life virus. Therefore, It can be conducted at biosafety level 2 laboratories [51]. IFA and ELISA were used to detect serum IgM and IgG antibodies against acute respiratory syndrome coronavirus (SARS-CoV), which are closely related to SARS-Co-2, and the results were useful for clinical diagnosis. Serological research tools are useful in following of infection. Nevertheless, they are not important in the early detecting of viral infection [52].

RT-QPCR TEST

A test to be conducted for reported cases of the symptoms or travel to China or other places exposed to COVID-19 or socially contacting patients who have traveled before or those who have already been diagnosed with COVID-19. However, in some cases, there were no symptoms (asymptomatic) or even no fever. Thus, any suspected case will be reported as infected only if the infection-has been positive via molecular tests [53]. As per recommendations, a confirmed diagnosis will be made by molecular genetics examinations of respiratory samples of nasopharyngeal swab or throat swab or endotracheal aspirates or sputum or bronchoalveolar lavage. In addition, virus could also be detected in the stool as well and in severe cases, the blood [53]. Starting from the time Covid-19 was proclaimed a pandemic in China by the World Health Organization, a private and non-profit company quickly established and distributed therapeutic diagnostic test kits focused on RT-qPCR. China CDC (Chinese Center for Disease Control) suggested the discovery of two areas, ORF1ab and N, using RT-qPCR technique for SARS-CoV-2 genes. Patients are not considered to be contaminated if all the results are optimistic and not just optimistic (http://ivdc.chinacdc.cn/kyjz/202001/t20200121_211337.html). The Chu and his team developed a 1-step RT-qPCR assay based on TaqMan to detect ORF1b separately and N viral genome regions [54]. On the other side, To and his team established additional economic non-sample SYBR-based RT-qPCR test showed that the positive result of SARS-CoV-2 with a successful detection rate of 91.7% (11/12) in self-collected saliva suggested that saliva may be used as a promising non-invasive tool for the treatment, tracking and management of infections in SARS-CoV-2 patients [55]. SARS-CoV and MERS-CoV infections were identified using RT-qPCR detection tool [56]. However, Xie et al. [57] suggested that five patients with negative results of RT-qPCR for SARS-CoV-2 were shown to be positive in chest CT scan implying that repeated swab tests (RT-qPCR) should be mandatory to confirm the positive results [57]. According to the previous report on the identification of confirmed SARS-CoV (2003) outbreaks in China using RT-qPCR, only 50%–79% were positive indicating that sensitivities were correct. Varies in the detection based on the sample type and protocol used in addition to way of specimen collection [58]. Therefore, it is so important to learn a lesson from previous

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experiences and improve the sensitivity and detection rate of RT-qPCR for SARS-CoV-2 in addition to the method of sample collection and type of sample itself [59] ; [60]. Nevertheless, we still consider RT-qPCR to be the first step in the diagnosing COVID-19.

RT-qPCR methods mainly target the mixture of two regions of the SARS-CoV 2 genome, such as the RNA-dependent RNA polymerase (RdRp) genes open reading frame (ORF), spike (S), envelope (E), and nucleocapsid (N), depending on the package or country protocol [54, 16]. Currently, the emphasis in SARS-CoV-2 diagnostic tests is on early, fast, and accurate testing to monitor and prevent further spread of SARS-CoV-2 [61].

LAMP

Due to the continuous and steady increase in the suspected cases, some barriers have begun to emerge in the use of RT-qPCR technology in terms of speed, scale, the need for advanced equipment, isolated laboratories, and highly trained staff, and it more timely (about 1.5-2 h). Hence, it needed more sophisticated techniques. Jiang et al. [62] presented a rapid RT-LAMP (Reverse transcription Loop-mediated isothermal amplification) test that could expand laboratory ability to process twice as many clinical samples as qRT-PCR and could theoretically be used for high- throughput screening purposes when demand is increasing at critical situations [63]. Also developed similar methods named iLACO, which is an isothermal based method (isothermal LAMP-based method for COVID-19) for detecting an ORF1ab region. The primers have been tested against the sequences of 11 relative viruses. The turn-around time is about 15-40 minutes, depending on the virus loud in the collected samples. The sensitivity was compared to RT-qPCR and was the same. The new place developed methods can be applied to detect COVID 19 due to its precision, simplicity, and versatility, even in cases where specialized molecular biology equipment is not available.

CRISPR-Cas12

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) based SHERLOCK (Specific High Sensitivity Enzymatic Reporter UnLOCKing) is a fast and sensitive molecular-based platform recently developed for the detection of COVID-19 using synthetic COVID-19 RNA fragments. Viral RNA is removed from samples, then isothermal amplification (RT-RPA) is applied. In the visual lateral flow dipstick, findings can be read out instantly within 60 minutes without any instrument being involved. This method is focused on the identification of two conserved sequences in the genome COVID-19 which are genes S (spike) and Orf1ab.

Precise selection of amplification primers and CRISPR gRNAs is required to minimize off-targets that match other respiratory or viral genomes or coronavirus strains [64]. Another new molecular detector assay based on 'off-the-shelf' CRISPR-Cas12 has been developed for the rapid diagnosis of SARS-CoV-2 called the DETECTOR platform. DETECTOR is based on simultaneous, reverse transcription and isothermal amplification utilizing loop-mediated amplification (RT-LAMP) combined with CRISPR technology utilizing Cas12 enzyme and many highly specific gRNAs to preserve sequences in the SARS-CoV-2 genes N (nucleoprotein) and E (envelope) [64]. Having a visual lateral flow strip, which is considered an advantage over other molecular-based detection systems, including SARS-CoV-20 SHERLOCK and qRT-PCR, tests can be read back within 30 minutes. In addition to the molecular detection assays, biochemical, serological assays are important for the qualitative and quantitative analysis of the humoral immune responses to SARS-CoV-2. These assays assist in the precise determination of the risk of infection in an infected area which can help enforce disease control strategies [51] ; [45].

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