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CHAPTER-4

THE EMERGENCE OF COVID-19 AND ITS SPREAD ALONG WITH SYMPTOMS

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ABSTRACT

The recent emergence of the COVID-19 pandemic caused by the novel severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) has put extreme pressure on all sectors of life influencing society, medicine, and the economy around the globe. SARS-CoV-2 is the third zoonotic coronavirus (CoV) identified to cause pronounced disease in the human population, following SARS-CoV of 2003 and MERS-CoV of 2012. Its rate of transmission exceeds than that of SARS-CoV, and its fatality rate varies from 1.2-15.2%. CoV is a large enveloped virus with a linear positive-strand RNA genome. Owing to the fast mutation rate and recombination, CoVs are among the fastest evolving viruses, which might have also helped SARS-CoV-2 to attain novel infectious properties and adaptation to the human host. The genomic and evolutionary studies established so far have suggested that bats and pangolins serve as the natural reservoirs for SARS-CoV-2, eventually spilling over to humans. The transmission of this virus occurs by the inhalation of droplets from the respiratory tract of an infected person and thus strict quarantine measures are needed to prevent its spread. COVID-19 poses major

Keywords

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health risks in the elderly or people with underlying medical problems, while otherwise infecting all age groups. Disease severity may reach to acute respiratory distress syndrome or multi-organ failure. The asymptomatic prevalence is more of a concern. Due to the lack of any drug, vaccine, or existing immunity against this virus, there is an immediate need for the development of drugs and vaccines. Exploring the molecular biology of CoV, it's possible zoonotic mechanisms, and vaccine targets might help control COVID-19. This chapter presents the current understanding of CoVs, with an emphasis on SARS-CoV-2 causing COVID-19, its spread, and its symptoms.

1. Introduction

Viruses surround us, the number of viruses on the Earth are a hundred million times more than the number of stars in the universe [1]. Daily, we breathe in these viruses in large numbers, but most of them are harmless. Viruses not only infect animals and humans, but they are microbial predators as well. Viruses influence global biogeochemical cycles and play an essential role in the ecology of the Earth. Interestingly, human genome retains fragments of past viral infections, indicating that humans have faced several viral attacks and the viral DNA has remained with us. In the last twenty years, the world has seen SARS and MERS outbreaks from coronavirus (CoV), causing severe respiratory infections. The first human respiratory CoV study is from late 1960, when Almeida and Tyrrell published the CoV structure by using electron microscopy [2]. The CoV structure shows spikes or protrusions on the viral envelope, giving a crown-like appearance; hence, the name coronavirus. CoVs are present in birds and mammals (bats, cats, rats, and camels) [3]. Bats serve as the richest source of CoVs [4]. The past two CoV infections, SARS and MERS, revealed the involvement of intermediate hosts for virus transmission from a bat to a human(palm civets in SARS, and camels in MERS). Now a new virus called SARS-CoV-2 has caused the COVID-19 pandemic, leading to social and economic crises globally. The worrisome features are that the virus is extremely contagious and that it causes a severe disease in the elderly and people with

comorbidities. There are more than 8million people infected globally, and more than 440,000 people have died due to a lack of any drug or vaccine to control it (Figure 1). To stop the spread, we need to know the details of the disease emergence, spread and symptoms. This chapter highlights these aspects of SARS-CoV-2.

2. Human pathogenic coronaviruses

The CoVs are a large family of RNA viruses with a size of 60-120 nm in diameter. CoV has a linear positive-strand RNA genome that is 26000-32000 nucleotides long. The RNA is encased inside a lipid-bilayer membrane that is associated with proteins. Figure 2D shows SARS-CoV-2 with transmembrane protein (M), the spike glycoprotein (S), and envelope protein (E). Before2019, six humans pathogenic CoVs existed. Four of them (HCoV 229E, HKU1, NL63, and OC43) induce mild upper respiratory diseases in people with immuno competency. The other two (SARS-CoV and MERS-CoV) cause severe respiratory syndrome in humans. SARS-CoV caused Severe Acute Respiratory Syndrome (SARS), resulting in 8098 cases, with 774 deaths in 26 countries [5]. MERS-CoV caused the Middle East respiratory syndrome outbreak in 2012 infecting 2494 people across 27 countries. The case fatality rate was 34.4% with 858 deaths for MERS;[6]. We are now experiencing the seventh newly discovered CoV, named SARS-CoV-2 [7], which has caused Coronavirus Disease of 2019 (COVID-19), a pandemic.

Our first recorded encounter with any CoV occurred in 1930. In that year an acute respiratory infection occurred in only chickens, caused by a CoV known as avian infectious bronchitis virus (IBV). Phylogenetic dating of CoV RNA genomes (Figure 2A) using the sequence of viral RNA-dependent RNA polymerase (RdRp) estimates the most recent common ancestor (MRCA) of mammalian CoVs occurred ~ 300 million years ago [8]. Contemporary CoVs are well known for human respiratory tract infections and infect many mammalian and avian species [9].

3. Emergence of COVID-19

A disease with characteristics very similar to viral pneumonia appeared in China in December 2019. This disease arose in people who had visited the seafood market in Hunan of Wuhan city (the capital of Hubei province, China). This wet market sells different wildlife live animals, including bats, birds, frogs, hedgehog, marmots, snakes, and

rabbits [10,11] Approximately 50 patients reported the disease at first. The patients reported fever and cough and/or respiratory distress [12]. Experts determined that the pneumonia caused by a novel coronavirus that differed from SARS-CoV and MERS-CoV. The disease was named coronavirus disease 2019 (COVID-19; The World Health Organization (WHO). The International Committee on Taxonomy of Viruses named the causative virus severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The virus belongs to the β-coronavirus family (a naturally prevalent large class of viruses).

- **Geographic distribution**

COVID-19 rapidly spread through human to human contact to all over China and then around the world. According to WHO coronavirus disease (COVID-19) report 149, there are more than 8.38 million confirmed cases, with 449,695 deaths globally. Figure 1 shows the pattern of case comparison by different regions, with 38,99,859 confirmed cases in the Americas, 24,52,247 cases in Europe, 817,458 in Eastern Mediterranean, 503,034 in Asia, and 1,87,625 cases in Africa. The USA alone has reported the highest number of confirmed cases—21,64,497—followed by 955,377 cases in Brazil, 560,279 in Russia,366,946 in India, and 300,717 in the United Kingdom. The total number of deaths are 117,783 in the US, 46,510 in Brazil, 42,238 in the UK, 34,448 in Italy, 29,578 in France and 12,237 in India. The first COVID-19 infection was reported in China in December 2019. China has now 84,462 reported cases with 4638 deaths.

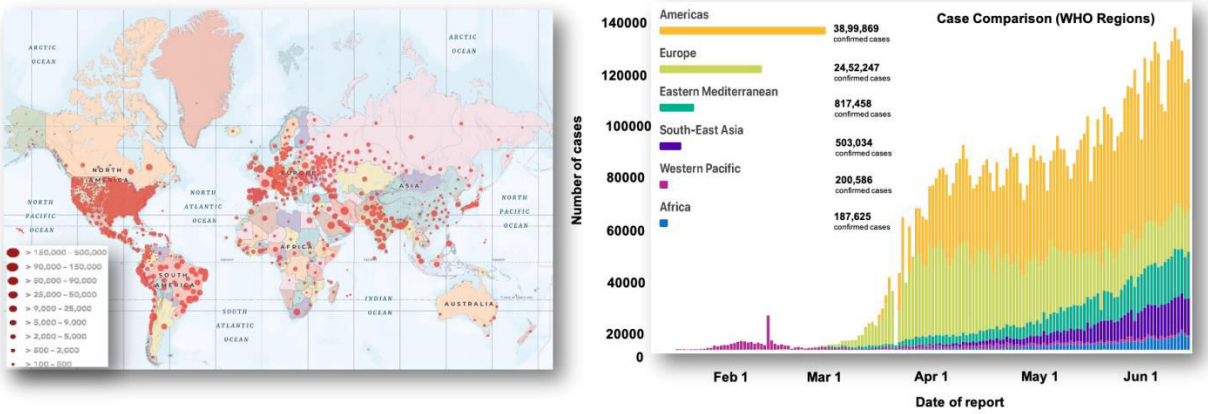

Figure 1. Geographic distribution of COVID-19 cases. (June 17th, 2020). Left: Cumulative confirmed cases globally. Red dots show the number of confirmed cases as per inset (Source: https://coronavirus.jhu.edu/map.html). Right: Number of confirmed COVID-19

cases, by date of report and WHO region, 30 December 2019 through 17 June 2020 (source who.int).

- **Origin of SARS-CoV-2**

COVID-19 is the third CoV attack after SARS and MERS CoVs. SARS-CoV and MERS-CoV cause severe to fatal respiratory tract infections. SARS-CoV-2 is different from SARS-CoV (79.5% identity only) but has a 96.2% identity to a bat CoV RaTG13 genome sequence [13]. Advanced protein amino acid alignment for 27 proteins showed differences in 2019-CoV and SARS-CoV [14]. In total, 380 amino acid substitutions exist in SARS-CoV-2 proteins. Some are in nsp2,nsp3, and spike protein, with 67, 103, and 27 substitutions, respectively. Six relevant mutations are in the receptor binding domain and six in the stalk. The receptor-binding subunit S1 domain has four substitutions in two peptides, and these peptides are antigens for SARS-CoV [15]. The virus uses angiotensin-converting enzyme 2 (ACE2) as its receptor for entry to the human cell, as did SARS-CoV. Although bat is the probable natural host of the SARS- CoV-2,it might rely on transmission from bats via some intermediate hosts to infect humans.

Wuhan is a business hub with elite status, fashionable lifestyle, and the popular game meat. It is a place where one can buy, sell, gift and eat wildlife animals. Game meat has a high possibility of microbiological contamination. The first 27 cases of COVID-19 originated in either the proprietor of shops or with people who had visited this market. It is a crowded market of 50,000 square meters that deals in sales of a wide range of live wild animals of different types, with species caged close for human consumption. SARS-CoV-2 most likely originated here.

Surprisingly after a long tight control over the virus spread in China, a cluster of new COVID-19 patients emerged from another wholesale food market. The Chinese capital reported 36 new cases in a single day, bringing total to 106 on June 12, 2020. These cases are linked to Xinfadi market, which supplies meat, seafood, fruit and vegetables. The outbreak has spread to Liaoning and Hebei provinces. It is still not clear how the virus is spreading. If the spread continues to be same, it may become explosive soon.

- **Source of SARS-CoV-2**

The wholesale wildlife animal market at Hunan was potentially closely linked to the virus source. Usually, viruses undergo recombination and mutation processes and reassortment processes to get equipoise in the final host. SARS-CoV had an in vivo mutation rate for nucleotide substitutions of ~ 5.7×10^{-6} per site per day.It is very similar to RNA viruses [16]. A high rate of mutations in these RNA viruses permits quick adaptations to a varying environment. The concurrent infection of the same tissue or animal by multiple viruses eventually results in new viral progeny having genome from different parents (a reassortment process). This process in RNA influenza A virus, which has eight single-stranded RNA segments, changed its the viral surface glycoprotein. The spike glycoprotein S of CoV is the critical component for their transmission. This S protein binds to a receptor called angiotensin-converting enzyme 2 (ACE2) to enter the host cell. This spike glycoprotein S already underwent significant evolution in CoVs [17]. These mutations accelerated intra and interspecies transmissions [18]. The spike protein from SARS-CoV can recognize ACE2 receptors from different animals—including bat, mouse, civet, and raccoon dog—boosting interspecies spread [19], [20]. On the other hand, bats, being as the host for many CoV, have incredible species diversity (~1240) and fly long distances ~ 2000 km. These features allow bats to get or disseminate viruses.

For both SARS and MERS, the virus transmission is zoonotic. Zoonotic diseases are ones that usually occur among animals but under specific conditions can also infect humans. Another term is zoonotic spillover, which refers to the transmission of a virus from a vertebrate animal to a human. Zoonotic transmission involved palm civets in SARS and camels in MERS [21], [22]. A virus can be transmitted easily from bats to animals or humans via bites and scratches. These animals can serve as an intermediate source and could give it to humans by direct contact (for example, rabies virus). The CoV from Malayan pangolin has a 99% similarity toSARS-CoV-2, but the bat RaTG13 CoV has a 96% identity toSARS-CoV-2 [23]. All this information indicates that the probable intermediate source is pangolin, and the primary source is bat (Figure 2B).

4. Spread of COVID-19

The first 27 COVID-19 patients were associated with the selling or buying of live animals. These patients had worked at or visited this place. With the progression of the disease, the primary mode of COVID-19 transmission remains person-to-person spread. Other factors are discussed below.

- **Spread among people**

Analogous to the spread of the influenza virus, SARS-CoV-2 spread mainly via respiratory droplets. Through droplet transmission virus discharged in the respiratory secretions can infect another person upon direct contact with their mucous membranes. Respiratory droplets are discharged from an infected person through coughing, sneezing, or talking. The large droplets fall on the ground, and the small droplets do not travel more than two meters (six feet) nor linger in the air. The maximum range of transmission is uncertain, but high-speed imaging study shows a 22 foot range for respiratory exhalations in a gas cloud after coughing or sneezing [24]. The viral RNA is also exists in air samples and the ventilation systems of hospital rooms occupied by patients of COVID-19 [25], [26]. How long SARS-CoV-2 remains viable is clear but also a big concern. It remains viable for three hours in experimentally generated aerosols [27]. SARS-CoV-2 also exists in non-respiratory fluids including blood, stool, and ocular [28-31]. The blood and fecal-oral transmission is not clinically described yet [32].

- **Spread from air and inanimate surfaces**

SARS-CoV-2 can also spread from fomites (contaminated surfaces) if susceptible individuals transfer infectious virus by touch from surfaces to their eyes, nose, or mouth. The CoV survives on non-living surfaces for up to six to nine days. SARS-CoV-2 can also persist on variable surfaces from hours to days [25,33,34]. However, various disinfectants, including ethanol (60-70 %), inactivated SARS-CoV within one minute and also work on SARS-CoV-2[35]. The virus can persist in the air without losing infectivity in unventilated buses for at least 30 min [36]. SARS-CoV-2-bearing aerosols could exist in hospital wards [37]. Experimentally, a reduction in infectious titer of SARS-CoV-2 with $TCID_{50}$ value (50% tissue culture infective dose per milliliter)is known for different surfaces. This value is 3 hours for aerosols ($10^{3.5}$ to $10^{2.7}$ $TCID_{50}$), 72 hours for plastic ($10^{3.7}$ to $10^{0.6}$ $TCID_{50}$),

and 48 hours for stainless steel ($10^{3.7}$ to $10^{0.6}$ $TCID_{50}$). On copper and cardboards, no viable SARS-CoV-2 survived 4 hours [27].

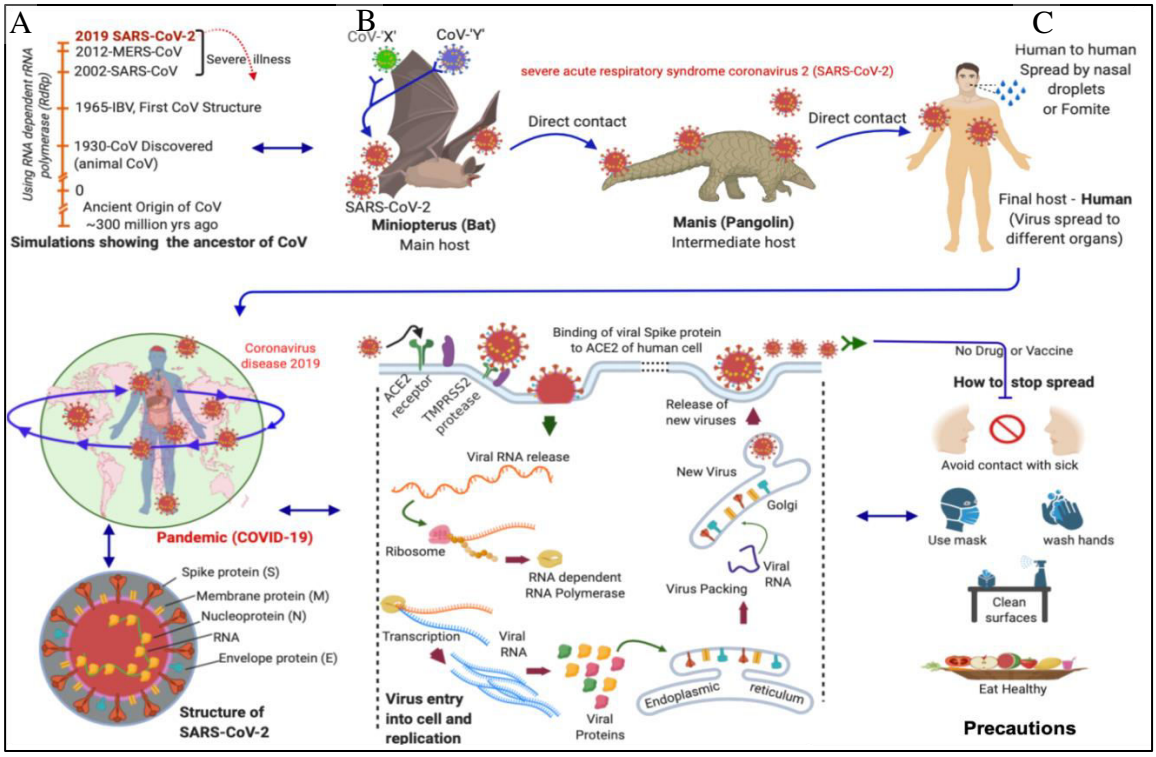

Figure 2. Spread of COVID-19. A: The most recent common ancestor (MRCA) of mammalian CoVs, and human severe pathogenic CoVs: simulations using RNA-Dependent RNA polymerase gene sequences date the probable origin of CoV to around 300 million years ago. Three severe CoV strains that are pathogenic to humans are listed with the date of origin. B: Transmission of SARS-CoV-2:The most probable origin of SARS-CoV-2 is from bats, as it has 96% identity to a virus found in bats and may have originated from recombination in two viruses. Malayan pangolin has a 99% similarity with SARS-CoV-2, identifying it as the probable intermediate host for spread to humans. C: CoV spread among people: SARS-CoV-2 spreads through direct human-human contact either by respiratory droplets or fomite. D: Structure of SARS-CoV-2: crown-like appearance of the CoV with highlighted essential proteins and RNA genome. E: Virus replication inside the cell: Virus Spike protein (a trimer) binds to the ACE2 receptor on the surface of the host cell and gets cleaved by protease calledTMPRSS2, leading to fusion of the membranes of the virus and the host cell. The viral RNA is released into the cytoplasm of the cell, where it gets translated directly. RdRp forms RNA copies that are translated. The viral proteins move to the endoplasmic reticulum (ER) and Golgi. From these compartments, new packed viruses bud into the membrane to form vesicles with the mature virus, which isthen released by exocytosis. F: Precautions for COVID-19 spread: social distancing, use of masks, washing hands with soap, and healthy eating to keep immune system robust. (Figure created with BioRender.com).

5. SARS-CoV-2 Period of infectivity

After getting infected, it is uncertain when the individual will be infectious to others. The uncertainty is because the COVID-19 RT PCR test detects virus RNA in the specimens collected from people but does not reveal whether it is infectious. It seems that the virus is not only transmitted throughout illness but also prior to the development of symptoms. A study of modeling the time of infection among infector and infected transmission pairs of COVID-19 has reported similar results. It suggested that infectiousness started about 2.3 days and peaked 0.7 days before symptom onset [38].

Asymptomatic individuals play a substantial role in virus transmission. It is tough to recognize any early signs and symptoms of the infections in these individuals. SARS-CoV-2 from upper respiratory tract specimens of these individuals (after testing positive by RTPCR test) has also been cultured. Surprisingly, asymptomatic individuals were found to be 24 out of 27 skilled workers in a nursing facility, with symptom onset at 7^{th} day after testing positive for SARS-CoV-2 [39]. Other similar reports also describe asymptomatic transmission [40-43]. The extent of the asymptomatic transmission and percent contribution to the pandemic remains unspecified. But it is an alarming way of spread as suggested by the simulation study. The emission rates simulated are> 100 quanta h^{-1} of infectious SARS-CoV-2 for a subject in just vocalization activity compared to emission rate of < 1 quantum h^{-1} for a symptomatic subject in resting condition. (Quantum is the dose of airborne droplets required to cause infection in 63% of susceptible persons [44] According to the CDC, 35% of the spread of COVID-19 is through asymptomatic people.

For symptomatic individuals, the period during which a person stays infectious can vary. It depends upon the severity of the illness, too. The virus-shedding ranges from one week to 6 weeks after onset of the symptoms in survivors or until death in non-survivors [45-48]. Notably, the virus load is higher in stool samples than respiratory samples [49]. However, fecal-oral transmission is not yet confirmed. Detection of viral RNA is different than detection of viral shedding, and how these processes correlate is uncertain. Understanding these dynamics in COVID-19 should be helpful in the clinical management of contagious people. It also suggests the need for prolonged observation, repeated testing, and new detection assays.

6. Cell-to-cell spread

One of the principal determinants of COVID-19 pathogenesis is the SARS-Co-V-2 entry into host cells. The surface spike protein mediates the CoV entry (Figure 2E). The spike protein is a trimer, and its receptor-binding S1 heads sit on the top of an S2 stalk [50]. The S1 head comprises a receptor-binding domain (RBD) that recognizes a receptor on human cells. The role of RBD is to continually switch between standing-up and lying-down positions for receptor binding and immune evasion, respectively. The receptor for the spike protein is an angiotensin-converting enzyme 2 (ACE2) on the host cell surface.

The spike protein needs proteolytic activation at the S1/S2 interface by TMPRSS2protease, which is present on the human cell surface. Proteolysis leads to the fusion of the membranes of the virus and the host cell. It releases the viral RNA into the host cell cytoplasm, where it is translated directly(Figure 2E). SARS-CoV-2 has 16 genes for nsp (nonstructural) proteins. Many of these nsps form the transcriptase complex which is helpful to provide the required milieu for the viral RNA and protein synthesis. Transcription of the sub-genomic RNAs is requisite for their service as mRNAs for the viral protein synthesis. The translation is necessary for making the viral proteins needed for the new virus assembly. The viral S protein contains an N-terminal signal sequence. This signal directs the spike protein into the endoplasmic reticulum (ER).This is the site of its N-linked glycosylation and proper folding. The three other most abundant viral proteins are M protein (which defines the shape of the envelope), E protein (which helps with the new virus assembly and also with virus budding), and N protein (which binds genome to help in viral assembly and in virus budding). These proteins move into the endoplasmic reticulum and Golgi. In these compartments, new packed viruses bud into the membrane to form vesicles with the mature virus. They are then released by exocytosisto infect host other cells, see also Figure 2E, [51, 52].

7. Symptoms

The SARS-CoV-2 incubation period is 14 days, with a mean incubation time of 5.2 days following exposure[53,54].More than 95 % of infected individuals show symptoms within 12 days. Most of the infections are not severe [55,56]. When pneumonia occurs,

it is characterized primarily by fever, cough, dyspnea, and bilateral infiltrates on chest imaging. COVID-19 spread occurs in both symptomatic and non-symptomatic carriers. The scale of symptomatic infection among patients ranges from mild to critical. Initial significant symptoms of COVID-19 include cough, fever, muscular soreness, and difficulty in breathing (dyspnea). In some patients, the patient exhibits fever (not very responsive to antipyretics) associated with a dry cough only. In older patients with already impaired lung function, infection triggers shortness of breath, unease, and increased respiratory rate leading to dyspnea. Symptoms may appear later in younger patients who do not have a primary respiratory impairment or any other comorbidities. These patients experience the worsening condition of inflammatory-induced lung injury, which causes a decrease in oxygen saturation levels (<93%). There may be a rapid relapse of respiratory functions. Some atypical symptoms are diarrhea and vomiting. But some of the patients also had one or more underlying medical conditions[45, 57, 58]

The percentage of symptoms varies: cough (59–82%), fatigue (38–70%), sputum production (28–56%), myalgia (11–35%), dyspnea (3–55%), and headache (6–34%) [59-62] Sometimes, the above symptoms accompany sore throat, chest pain, hemoptysis, conjunctivitis, and rhinorrhea [59–61]. The severity of infection is exacerbated by pre-existing comorbidities like cardiovascular disease, hypertension, diabetes, chronic lung disease, cancer, and obesity. Other less common symptoms are anosmia and dysgeusia (smell and taste disorders, respectively) in 34 % of patients of total COVID-19 patients. In mild COVID-19 patients, the percentage went up to 64% with age range, 20-89 years [63–66]. The nasal cavity olfactory epithelium is the probable site of SARS-CoV-2 binding, as its non-neuronal cell types express both the required receptors, ACE2 and TMPRSS2 proteases, used for the CoV binding. This may explain the loss of smell and taste. This feature is still uncertain for a distinguishing feature of COVID-19. COVID-19 patients have anorexia and diarrhea. Other GI symptoms are nausea, abdominal pain or discomfort, vomiting, and hematochezia. More indicators of COVID-19 include gastrointestinal (GI) symptoms, such as nausea and diarrhea [67], GI symptoms are uncertain as they vary from 10-35% [68]. Sars-CoV-2 enters the GI system via the ACE2

receptor, which is expressed on cells called enter ocytes of the GI tract in ileum and colon.

COVID-19 infection may result in dermatological manifestations, but the dermatological findings in patients are not well characterized. Skin symptoms like urticaria (red itchy welts) and maculopapular (raised red bump) appear. Sometimes transient live do reticularis (reddish-blue skin) and vesicular eruptions occur [69–71]. Some patients also noted a burning sensation on their skin. Another symptom that looks similar to pernio (chilblains, skin sores) occurs. In this case, reddish-purple nodules appear on the toe in children and adults [72]. Some are calling this finding "COVID toes." The three CoVs that cause human infections--SARS-CoV, MERS-CoV, and SARS-CoV-2--share many similar symptoms, including cough, fever, myalgia, dyspnea, and bilateral ground-glass opacities on chest CT scans. However, GI infection was more common in patients with SARS and MERS than SARS-CoV-2. Also, MERS infection showed a high incidence of renal failure, which is not often present in other human CoV infections [73].

8. Summary

Viruses have always surrounded the human population. But now it seems that human actions have worsened the problem by disturbing the ecological niche (of CoV). The COVID-19 epidemic has driven a global crisis. Within recent years three virus spillovers from wild animals have occurred, without any clear view of the possible animal reservoir. Perhaps the safest way to protect the human population from these CoVs is to keep a barrier between civilization and the natural pools. The SARS-CoV-2 virus is spreading everywhere, and whether it will appear again and again like a flu virus or die out is not clear. We need to explore the fundamental adaptation-evolution mechanism in order to design the best strategies that will ensure human safety. Investigating the molecular biology of the infection to get transparency on transmission will be helpful for such outbreaks in the future.

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