Novel Research in Microbiology Journal (2020), 4(3): 746-765
Review Article

(Print) (ISSN 2537-0286)
(Online) (ISSN 2537-0294)
https://nrmj.journals.ekb.eg/
DOI: 10.21608/nrmj.2020.95319

An overview of SARS-COV-2: Virology, Epidemiology, Pathogenesis and Treatment

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Received: 20 May, 2020; Accepted: 5 June, 2020; Published online: 12 June, 2020

Abstract

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is a causative agent of COVID-19 infections. In late December, 2019 emergence and pandemic outbreak of SARS-CoV-2 virus has created serious health threat globally, unlike emergence of SARS-CoV in 2002, and Middle East Respiratory Syndrome (MERS-CoV) in 2012. Globally, about 4,434,653 confirmed cases of COVID-19 infections are reported including 302,169 deaths. Wild animals' bat, snakes, and pangolins are potential sources of this virus, based on the sequence homology of these animals and the nucleic acid of SARS-CoV-2 virus isolated from infected persons. Human infection occurs due to the inhalation of respiratory droplets, which mainly infects the lower respiratory tract causing a mild flu like symptoms that may extend to severe pneumonia. Currently, there are no any vaccines or antiviral drugs against this virus. Treatment of patients is based mainly on symptomatic management. The aims of the present study were to summarize the information on the origin, evolution, structure and genomes, epidemiology, molecular immunopathogenesis, and diagnostic approaches of the SARS-CoV-2 coronavirus. Moreover, we discuss the current approaches, progress in vaccine development, and the antiviral therapies to cope with COVID-19 infection. Thus, the information and data gathered on coronavirus will be helpful in understanding all the aspects on SARS-CoV-2 virus, and helps to reduce the global health threat and economic impact.

Keywords: Coronavirus, SARS-CoV-2, MERS-CoV, SARS-CoV, Epidemiology

1. Introduction

Coronavirus is a positive-sense single-stranded RNA (+ssRNA) enveloped virus, with a size ranging from 65-125 nm in diameter. This virus belongs to the *Coronaviridae* family, *Coronvirinae* subfamily, and order Nidovirales. Numerous large prominent protrusions are formed on the outer surface of the envelope giving the virus a crown shape appearance (Corona in Latin means crown). A previous study conducted by Enjuanes *et al*., (2006) highlighted that coronaviruses are classified into four major genera Alpha (α), beta (β), gamma (γ) and delta (δ). Alpha (α) and beta (β) strains of coronavirus infect the

mammals causing mild to severe respiratory and gastrointestinal symptoms. Similarly, gamma (γ) coronavirus strains infect avian species, while the delta (δ) coronavirus strains infect both the mammals and avian species.

Cui *et al*., (2019) reported that seven strains of Coronavirus have been identified so far causing the human infections including; four human CoVs (HCoV-NL-63, HCoV-229E, HCoV-OC43 and HKU1), SARS-CoV-1, MERS and SARS-CoV-2 virus. The four human CoVs viruses are known to cause mild respiratory and gastrointestinal infections. On the other hand, SARS-CoV-1, MERS and SARS-CoV-2 were reported to cause severe lower respiratory tract infection leading to pneumonia. The spread of coronavirus at the different time intervals poses serious health threat to human, animals and leads to severe economic losses.

In late December, 2019, at Wuhan City, Hubei province, China, several cases of people suffering from severe pneumonia with unknown etiology were reported (Qun *et al*., 2020). In a previous study of Enjuanes *et al*., (2006), genomic sequencing of the unknown etiological pathogen isolated from patients with pneumonia revealed 79.5% identity to the previously identified SARS-CoV virus. Earlier Chinese's scientists named this virus as novel corona virus-2019 (nCoV-2019). In the 15th of January, 2020, the International Committee on Taxonomy of Viruses (ICTV) named SARS-CoV-2 as COVID-19 (Gorbalenya *et al*., 2020). Since, 31th of December, 2019 till 17th of May, 2020, World Health Organization (WHO) reported 4,434,653 confirmed cases of COVID-19 infection, including 302,169 deaths. An epidemic outbreak of SARS-CoV from Guangdong province of China has affected 32 countries and resulted in more than 8422 cases of SARS-CoV infection including 919 deaths (case fatality rate of 11%) (WHO. 2003). Recently, WHO. (2019); Shang *et al*., (2020) reported that in 2012, emergence and spread of MERS-CoV from Saudi Arabia to 27 nations resulted in 2449 cases of MERS-CoV infection including 858 death (case a mortality rate of 34.4%).

Similarly, Stevenson *et al*., (2013); Chen *et al*., (2014) reported that in 2013, emergence and spread porcine epidemic diarrhea coronavirus (PEDV) in the United States has killed more than 10% population of piglets of less than a year in age. This virus has a high fatality rate of almost 100%, and seriously impacted the pig farming in USA.

The objectives of the current review were to provide comprehensive information regarding the origin, structure and genome, epidemiology, molecular immunopathogenesis, diagnostic approaches and treatment of SARS-CoV-2 virus. Moreover, the current approaches for developing the vaccine and therapeutics regime to cope with this virus outbreak have been discussed. This study will be very fruitful in the control and treatment of COVD-19 infection.

2. Structure of the SARS-CoV-2 virus

Coronaviruses are spherical in shape with average diameter of 80-120 nm, and possess numerous number of club shaped (17-20 nm) glycoproteins spikes projecting from the surface of the viral envelope. The virus particle contains five major structural proteins, which are glycoprotein spikes (S), envelope protein (E), matrix protein (M) and nucleocapsid (N) protein. Wu *et al*., (2020); Chan *et al*., (2020) demonstrated that the glycoprotein spikes mediate the attachment of the virus to different host cell receptors, depending upon the receptor binding domain (RBD). Chan *et al*., (2020); Yang *et al*., (2020a) reported that on attachment to the host cell receptor, the glycoprotein spikes S protein cleavages into two subunits namely; N-terminal S1 and C-terminal S2 subunit regions, by the host proteases enzyme. S1 subunit contains signal peptide and a receptor binding domain (RBD), meanwhile S2 subunit contains conserved fusion peptide (FP), Heptad repeat (HR) peptides, trans membrane domain (TM) and cytoplasmic domain. The S1 subunit of SARS-CoV-2 showed 70 % identity to the S1 subunits of Beta coronavirus (SARS-CoV)

isolated from human and bats. Walls *et al*., (2020) added that the S1 subunit with RBD mediates the viral attachment to the human angiotensin converting enzyme 2 (hACE2), as the key receptor to infect the human cells. On the other hand, Xia *et al*., (2020) demonstrated that the S2 subunit plays an important role in mediating the virus fusion and entry into the host cell, in which heptad repeat 1 and 2 (HR1, HR2) can interact with six helical bundles, thereby bringing the viral and cellular membrane in close proximity for fusion. The ACE2-binding affinity of RBD in S1 subunit of SARS-CoV-2 is 10 to 20-fold higher, which might contribute to the higher infectivity and transmissibility of SARS-CoV-2 compared to SARS-CoV (Walls *et al*., 2020).

The M glycoprotein is pre-glycosylated M polypeptides with size range of 25-30 kDa (221-262 amino acids), and gives shape to the virus envelope (Armstrong *et al*., 1984). Envelope protein (E) is a small polypeptide with a size range of 8.4- 12 kDa (76-109 amino acids), and is the integral membrane protein. Godet *et al*., (1992) reported that this E protein facilities the assembly and release of the virus particle. A helical shape nucleocapsid protein (N) with a size of 43-50 kDa encloses long un-segmented negative-strand RNA (Chang *et al*., 2006). This nucleocapsid protein is most abundant and is a highly immunogenic phosphorous-protein in SARS-CoV-2, and is often used as marker in the diagnostic assays. A fifth structural protein called hemaglutinin-esterase (HE) projects from the surface of the viral envelope, and enhances S-protein mediated cell entry through the mucosa (Fehr and Perlman, 2015).

3. Genome Structure of SARS-CoV-2

The genome of coronaviruses ranges in size from (29.9 ~30 kb), is a long non-segmented single stranded positive sense (++ssRNA) with 5`cap structure and 3` polyadenylated -A tail, and encodes 9860 amino acids (Wu *et al*., 2020; Chan *et al*., 2020; Pachetti *et al*., 2020). The 5` and 3`-UTRs of SARS-COv-2 virus are 265 and 358 nucleotides long, respectively. The UTR sequence of 5` and 3` terminal of SARS-CoV-2 are >83.6 % identical to the other beta coronavirus nucleotides sequences.

Recent studies conducted by Wu *et al*., (2020); Chan *et al*., (2020) highlighted that SARS-CoV-2 virus genome contains 14-16 open reading frame shift (ORFs) [ORf1a/b, ORF3a, ORF6, ORF7, ORF8, and ORF9] encoding 27 proteins. ORF1a/b located in 5` terminus accounts for two-third of the whole length of the genome, and encodes for 15-16 non-structural protein form nsp-npsp10 and from nsp12- nsp16. Moreover, Wu *et al*., (2020) added that following the frame shift signals between ORF1a and ORF1b, leading to the production of polypeptides pp1a and pp1ab that are processed either by the viral encoded chymotrypsin-like proteases (3CLpro) and main protease (Mpro) in to 16 non-structural protein (nsp1), and helps in viral replication. The other ORFs account for one-third of the viral genome near the 3`-terminal, which encodes for 4 structural proteins [S, E, M and N proteins], and 5-8 accessory proteins (ORF3a, ORF3b, P6, ORF6, ORF7a/b, ORF8b, and ORF9b and ORF1).

According to Pachetti *et al*., (2020), the RNA dependent RNA polymerase (RdRps) named also as nsp12 is a key component of the virus replication/transcription machinery. SARS-CoV-2 shares a high homology for nsp12 compared to SARS-CoV, suggesting that its function and mechanism might be conversed. Xia *et al*., (2020) revealed that the viral genomic RNA is used as a template RNA to directly translate the poly-protein 1a/1ab (pp1a/pp1ab), which encodes for the non-structural protein (nsps) to form the replication-transcription complex.

4. Origin of Corona viruses along with SARS-CoV-2

Coronaviruses causing the human infections are originated from wild animals including bats, pangolins and camel, and transmission to the human occurred through intermediate hosts such as; civet cat, camel and pangolins. Cauchemez *et al*., (2013); Zhou *et al*., (2020); Wu *et al*., (2020) reported that SARS-CoV-1

and MERS-CoV were originated from bats, and are transmitted to human being through intimate contact with intermediate hosts including; civet cat and camels, respectively (Fig. 1). Meanwhile, the origin of SARS-CoV-2 is still unclear among the researchers. According to Andersen *et al*., (2020), two theories are speculating the origins of the SARS-CoV-2:

First theory: Few scientists believed that SARS-CoV-2 emerged through the laboratory manipulation of SARS-CoV like coronavirus, because RBD of SARS-CoV-2 is optimized for binding ACE-2 that is distinct from the other corona viruses (Zhang *et al*., 2020a; Astuti and Ysrafi, 2020). However, the genetic data of SARS-CoV-2 virus did not show any such evidence. **Second theory**: SARS-CoV-2 is thought to be originated from Wet seafood Market of Wuhan, China (Wu *et al*., 2020). It is possible that wild animals sold in Wet Sea food market may act like sources of infection. The genomic sequencing of bat-RaTG13 virus sample from *Rhinolophus affinis* bat showed 96% similarity with the genomic sequence of SARS-CoV-2. Similarly, genomic sequencing of the pangolins coronavirus obtained from illegally imported Malayan pangolins in province of Guangdong, China, has identical sequence to SARS-CoV-2. However, Zhang *et al*., (2020a) highlighted that the genomic sequence of bat-RaTG13 is more similar to SARS-CoV-2, compared to pangolins corona virus. The majority of researcher agreed that bat is the primary reservoir of corona virus, but human transmission of SARS-COV-2 from this primary reservoir animal is still under study.

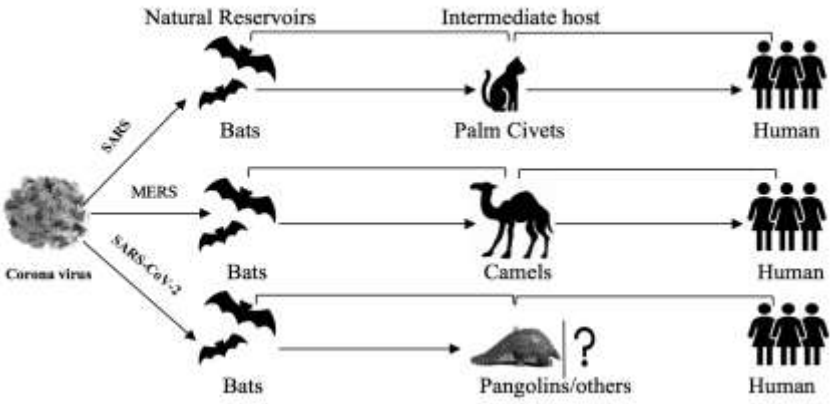

Fig. 1. Origins of SARS-CoV, MERS-CoV and SARS-CoV-2 viruses

5. Pathogenesis of SARS-CoV-2 virus

Coronaviruses infect the lower respiratory tract and other body organs with ACE-2 as a key cell receptor. Human infection occurs due to inhalation of the respiratory droplets produced by symptomatic and asymptomatic patients (Cauchemez *et al*., 2013; Astuti and Ysrafi, 2020). Moreover, Li *et al*., (2003); Shang *et al*., (2020) reported that on entry of the SARS-CoV-2, SARS-Cov-1, HCoV-NL63 viruses, they utilize the angiotensin-converting enzymes 2 (ACE2) as key receptors, meanwhile MERS-CoV-2 uses the di-peptidyl peptidase 4 (DPP4) as a key receptor for attachment to the host cell and establishing the infection. Skariyanchan *et al*., (2019); Li *et al*., (2019); Xia *et al*., (2020) added that entry of the coronavirus also depends upon the cellular proteases including; human airway trypsin-like protease (HAT), cathepsins and the trans membrane protease serine 2(TMPRSS2), which split

the viral spike protein and establish further penetration changes.

Mousavizadeh and Ghasemi, (2020); Shang *et al*., (2020) reported that entry and attachment of the S glycoprotein spikes of SARS-CoV-2 virus on the host cells receptors angiotensin converting enzymes (ACE-2), results in fusion of the viral envelope to the host cell membrane, and thus release of the viral RNA into the host cell cytoplasm. Moreover, Mousavizadeh and Ghasemi, (2020) added that viral genomic replication and transcription takes place in the cytoplasm, and involves a coordinated processes of RNA synthesis that are mediated by the viral replicate, which is a huge protein complex encoded by the 20-kb replicase gene.

The viral structural (S, M and E) and non-structural proteins (viral nucleic acid RNA, other enzymes), are processed in the host cell endoplasmic reticulum and Golgi apparatus. These proteins assemble at the host cell membrane to form mature viral daughter progeny particles, which are released by exocytosis through the secretory vesicles (Li *et al*., 2020), thus infecting the surrounding cells. Some of these viral progeny may get entry into the blood stream causing primary viremia. Recent study of Dhama *et al*., (2020) demonstrated that dissemination and lodgment of the virus into the different body organs (i.e. heart, kidney, intestine and liver) with the ACE-2 receptors, trigger strong host immune response thus causing the uncontrolled production of the pro-inflammatory cytokines [TNF-alpha, interleukin 2b(IL-1B), IL-6, GCSF, interferon gamma-induced protein-10, MCAP-1], macrophage inflammatory proteins 1-α in the patient's blood (IFN α, INF-γ, IL - 1β, IL-6, IL-12, IL18, IL-3, TNF-α, TGF-β, IL-2, IL-10, MCP1, IL-1RA), in addition to chemokine's (CCL2, CCL3, CXCL8, CXCL9, CXCL10). Prompetchara *et al*., (2020) added that this uncontrolled production of the strong chemokine's and cytokines causes pulmonary/alveolar tissue damage, leading to acute respiratory distress syndromes (ARDS) and ends with death of the patients.

6. Changes in host cells immunity caused by SARS-CoV2

The role of herd immunity, humoral and cell mediated response in COVID-19 infections is not clear. SARS-CoV-2 might induce the T-cell mediated protective immune response. Recent studies of Haveri *et al*., (2020); Diao *et al*., (2020); Andersen *et al*., (2020) highlighted that on entry and replication of the SARS-CoV-2 virus, uncontrolled stimulation of the $CD4^+$ and $CD8^+$ cells results in the production of huge amounts of pro-inflammatory cytokines (TNF-alpha, interleukin 2b(IL-1B), IL-6, GCSF, interferon gamma-induced protein-10, MCAP-1), macrophage inflammatory proteins 1-α in the patient's blood (IFN α, INF-γ, IL - 1β, IL-6, IL-12, IL18, IL-3, TNF-α, TGF-β, IL-2, IL-10, MCP1, IL-1RA), in addition to chemokine's (CCL2, CCL3, CXCL8, CXCL9, CXCL10), which might play an important role in the progression of COVID-19 disease. It is crucial to identify, study the functions of these cytokines and the different viral mechanisms that cause tissue damage, and contribute in the multiple organ failure in patients of sever COVID-19. Moreover, Qin *et al*., (2020); Diao *et al*., (2020) added that sustained and substantial reduction of the peripheral lymphocyte counts mainly; $CD4^+$, $CD8^+$ cells, NK cells, and B Cells in COVID-19 patients, suggest possible suppression of the cellular immune response with the progress of this disease. This is associated with a high risk of developing secondary bacterial infections. This condition is known as lymphopenia; however, the mechanism behind it is unknown.

Haveri *et al*., (2020) reported that humoral immune response also plays an important role in controlling COVID-19 infection. IgM antibody starts to appear within 7-14 days after the viral infection, and then its level gradually decreases with progress of the disease. Meanwhile, IgG antibody starts to appear in patient blood after 14 days, and remains for long period. Sustainable existence of the IgG antibody against the viral S-protein as a specific

neutralizing antibody response plays an important role in determining the disease outcomes.

7. Clinical manifestation

COVID-19 is a zoonotic disease and is probably transmitted from bats or pangolins. Infection by SARS-CoV-2 virus is called COVID-19 infection. The lower respiratory tract and other body organs including; kidney, heart, liver, intestinal tract with ACE-2 as receptors, are mainly affected.

7.1. Incubation period

The incubation period for SARS-CoV-2 virus is 5 -7 days; however it may extend up to 2 weeks (WHO. 2020). Meanwhile the incubation period of SARS-CoV is 2-7 days and may be up to 10 days (Leung *et al*., 2004), but it is 2-14 days for MERS-CoV.

7.2. Reproduction number and transmission rate

A virus reproduction rate (R0) is defined as the expected number of cases directly generated by one case in a population where all individuals are susceptible to infection (Fraser *et al*., 2009). Recently, Chen, (2020) demonstrated that viruses with (R0) > 1 cause serious epidemic or pandemic infections. In contrast, in a virus with R0 < 1, the transmission is guaranteed to fade away. SARS-CoV-2 has R0 greater than 1 ranging from 3.3- 5.5, which is slightly higher than R0 of SARS-CoV (R0: 2- 5) (Read *et al*., 2020; Liu *et al*., 2020a). R0 of MERS is 0.69 (95% CI 0.42-0.80) that is relatively very low, but still poses a risk of causing pandemic disease. Finally, the R0 of influenza virus is 1.1 to 2.3.

7.3. Clinical pictures

According to the Centre for Disease Prevention and Control (CDC), the clinical spectrum of COVID-19 varies from asymptomatic to symptomatic form. COVID-19 infection is classified into mild, moderate and severe cases based on the clinical spectrum. Patients with COVID-19 infection presents, high grade fever, dry cough, malaise and dyspnoea as major symptoms, and headache, nasal congestion, runny nose, sore throat, myalgia, vomiting and diarrhoea as minor symptoms. Yang *et al*., (2020b); Huang *et al*., (2020) studies showed that the occurrence and frequency of clinical manifestation of COVID-19 varied in different regions. Patients with severe COVID-19 infection develop pneumonia. Death of people occurred as a result of acute respiratory distress syndromes ARDS, septicemia and due to multiple organ failure (Huang *et al*., 2020).

8. Epidemiology

Earlier epidemiological evidence on the outbreak of SARS-COV-2 virus in Mainland, China, suggested that COVID-19 infection spread from local Huanan seafood market in Wuhan, Hubei province, China. Cluster cases of pneumonia with SARS-CoV-2 virus just reported in the last week of December, 2019, since then the virus spread rapidly within the different countries and outside China, affecting the millions of people. This drew the global health attention about the SARS-CoV-2 virus.

8.1. Patients

At the 16th of May, 2020, WHO reported about 4,434,653 of confirmed cases of COVID-19 infections, including 302169 deaths around the Globe (Fig. 2). Recent studies of Raoult *et al*., (2020); CDC. (2020) demonstrated that COVID-19 infection cases varied among the different nations from the different continents. American, European and Asian countries are more affected by COVID-19, compared to countries of Asian and African continents. The top five countries with the highest number of confirmed death cases due to COVID-19 infection are United States Brazil, Spain, Italy and United Kingdom (Table 1). The highest mortality rate due to COVID-19 infection was reported among people with distinct chronic diseases and weak immune systems.

Table 1. Distribution of COVID-19 cases in different countries of distinct continent. (16th May, 2020, ECDC)

Continent	Countries	Confirmed cases	Death
Asia	Turkey	146,457	4,005
	Iran	116,635	6,902
Confirmed case: 1,997,510	India	85,940	2,752
	China	84,038	4 637
Death case: 23,906	Saudi-Arabia	49,176	292
America	USA	1,443,397	87,568
	Brazil	218,183	14,817
Confirmed case: 1,997,510	Peru	84,495	2,393
	Canada	74,602	5,562
Death case: 120,406	Mexico	45,032	4,767
European	Russia	262,843	2418
Confirmed case: 1,666,892	United Kingdom	236,843	33,998
	Spain	230,183	27,459
Death case: 160,215	Italy	223,885	31,610
	Germany	173,772	7881
Oceania	Australia	7,019	98
	New Zealand	1 148	21
Confirmed case: 8444	Guam	154	5
	French Polynesia	60	0
Death case: 129	Northern Mariana Islands	19	2
Africana	South Africa	13,524	247
Confirmed case: 78,432	Egypt	11,228	594
	Morocco	6,652	190
	Algeria	11,228	536
Death Case: 2,635	Ghana	5,638	28

8.2. Geographical distribution

In late December, 2019 a new corona virus SARS-CoV-2 emerged from Wuhan City, province of Mainland, China. In mid-January, 2020 an epidemic outbreak and spread of SARS-CoV-2 virus stroke other cities of China, thus infecting thousands of people in Mainland, China (Broughton *et al*., 2020). In the 3rd week of February, 2020, there is a sudden raise in COVID-19 cases in South Korea and Italy, indicated the global spread of the epidemic (Zhang *et al*., 2020b). Currently, the virus spread in 215 countries across the world, and the number is in increasing order (Tian *et al*., 2020). Infection and mortality rates due to SARS-CoV-2 virus are increasing globally, and are varied among the different nations of different continents (Table. 1). There is no clear-cut evidence why pathogenicity and infectivity of COVID-19 infection is different in the distinct countries, but this may be attributed to a combination factors including; different national strategies adopted for people movements restriction, isolation and quarantine, and different genetic population herd immunity. Generally, the American and European continents countries are severely affected compared to the Asian and African continents countries. Meanwhile, in 2002-2003 SARS-COV-1 viruses emerged in Guangdong, China, which spread in 29

countries including Canada, Hong Kong, Chinese Taipei, Singapore, and Vietnam, infecting about 8098 people including 774(9.6%) deaths (Ding *et al*., 2003).

8.3. Age distribution and sex ratio

According to Verity *et al*., (2020); CDC. (2020), hospitalization of patients due to COVID-19 increase with age, with a 1% for those of 20-29 years, 4 % rate among 50-59 years, and 18 % for older than 80 years. The age group of populations from 30-79, are more vulnerable to COVID-19 infection. Males are more susceptible to COVID-19 infection compared to females. Raoult *et al*., (2020) revealed that the male to female ratio of COVID-19 infection was 0.99:1 in Wuhan, 1.041 in China overall. Onder *et al*., (2020) added that the highest mortality rate due to COVID-19 infection was in population ages between of 70 to 80 years or above.

8.4. Deaths, case fatality rates, and mortality

From the period of 31th December, 2019, to 16th May-2020, WHO COVID-19 dash board reported 4 098 018 confirmed cases of COVID-19 infection, including 283,271 (6.9 %) deaths. The highest mortality cases have been reported from the American continents countries (USA and Brazil), and the European continents countries (United Kingdom, Italy and Spain). China reported 4,440 death cases, which is the highest death number among the Asian continent countries. No cases/mortality of cases was reported from the Antarctica continent (Table. 1). Highest mortality rate of 14.8% was reported in patients with ≥ 80 age. Recently, CCDC. (2020); Raoult *et al*., (2020) reported 2.8 % case fatality rate in males, compared to 1.7% in females. Epidemiological analysis of new corona pneumonia cases by CCDC. (2020) in Mainland, China, reported that higher fatality rate is more common in people with distinct underlying preconditions; 10.5% for those with cardiovascular disease, 7.3 % with diabetes, 6.3 % with chronic respiratory diseases, 6% in hypertension and 5.6 % in patients with cancer. As per the USA, CDC reported that 80 % of deaths associated with COVID-19 are among adults older than 65 years. The fatality rate due SARS-CoV-2 infection is low compared to MERS-CoV (34 %) and SARS-CoV (9.6 %), but the confirmed cases of COVID-19 are very high and are increasing, which raise the red epidemiological flag.

8.5. Epidemiological curve

From December, 2019 to 16th of May, 2020, COVID-19 infection cases are increasing across the globes with different epicenters. In earlier outbreak of COVID-19 infection, Wuhan city was the outbreak epicenter, which then shifted to South Korea, Italy, Spain and USA. Now, countries like China, Italy and South Korea are able to control the spread of COVID-19 infection. Recently, the Chinese government has successfully controlled the infection rate of COVID-19, and now becomes below 100 (Fig. 2). Currently, the Americans and European continent countries are severely affected with a highest number of COVID-19 and death rate (Fig. 3-4).

9. SARS-CoV-2 diagnosis

The CDC recommends collection of upper respiratory tract specimens (nasal swab, nasopharyngeal swab, oropharyngeal swabs), and lower respiratory samples such as; expectorated productive cough, broncho-alveolar lavages, from patients who are intubated. However, collection of these clinical specimens from the patients requires a direct contact with them, and possesses a high risk of transmission of COVID-19 infection from the patient to the healthcare workers.

9.1. Laboratory findings

9.1.1. Blood and biochemical tests

A recent study of Bingwen *et al*., (2020) highlighted that hematological examination of COVID-19 patients showed lymphopenia with the decrease in $CD4^+$ and $CD8^+$, eosinopenia, in addition to an increase in the erythrocytes sedimentation rate (ESR). Similarly, Mardani *et al*., (2020) added that biochemical analysis of the patient's serum showed an

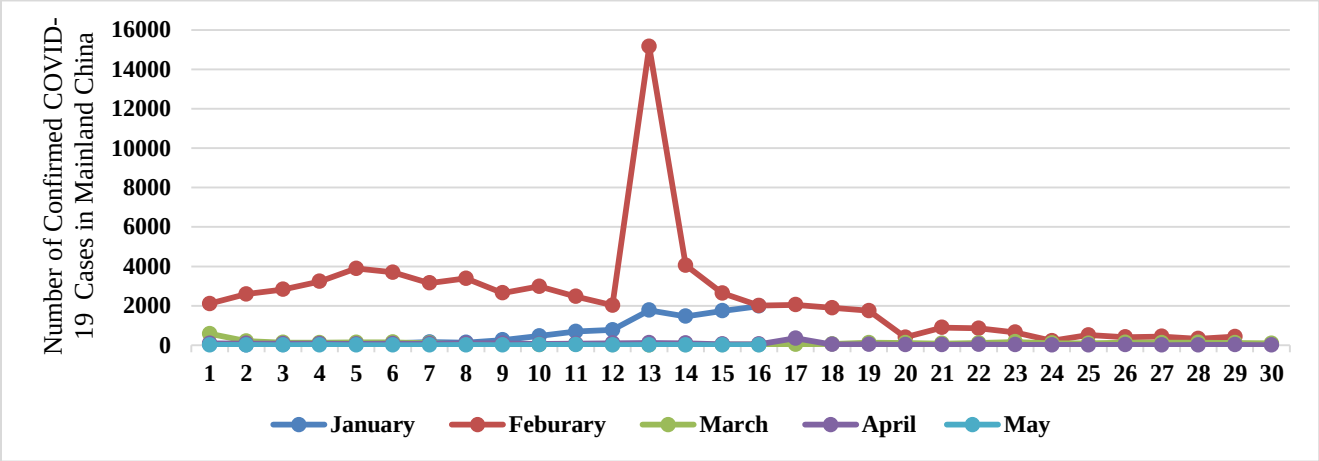

Fig 2. COVID-19 cases in Mainland, China, from 15th January, 2020 to 16th May, 2020. (WHO Corona disease COVID-19 Dash board)

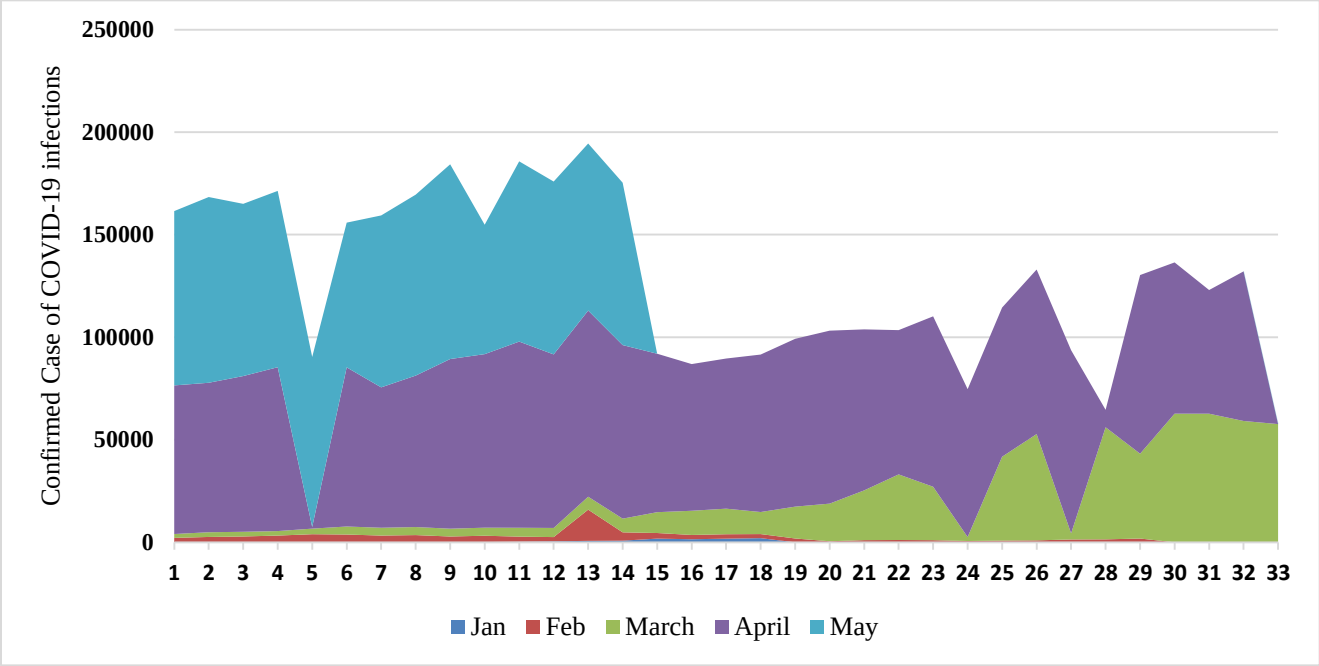

Fig 3. Globally confirmed cases of COVID-19 infection from 15th January, 2020 to 16th May, 2020 (WHO Corona disease COVID-19 Dash board)

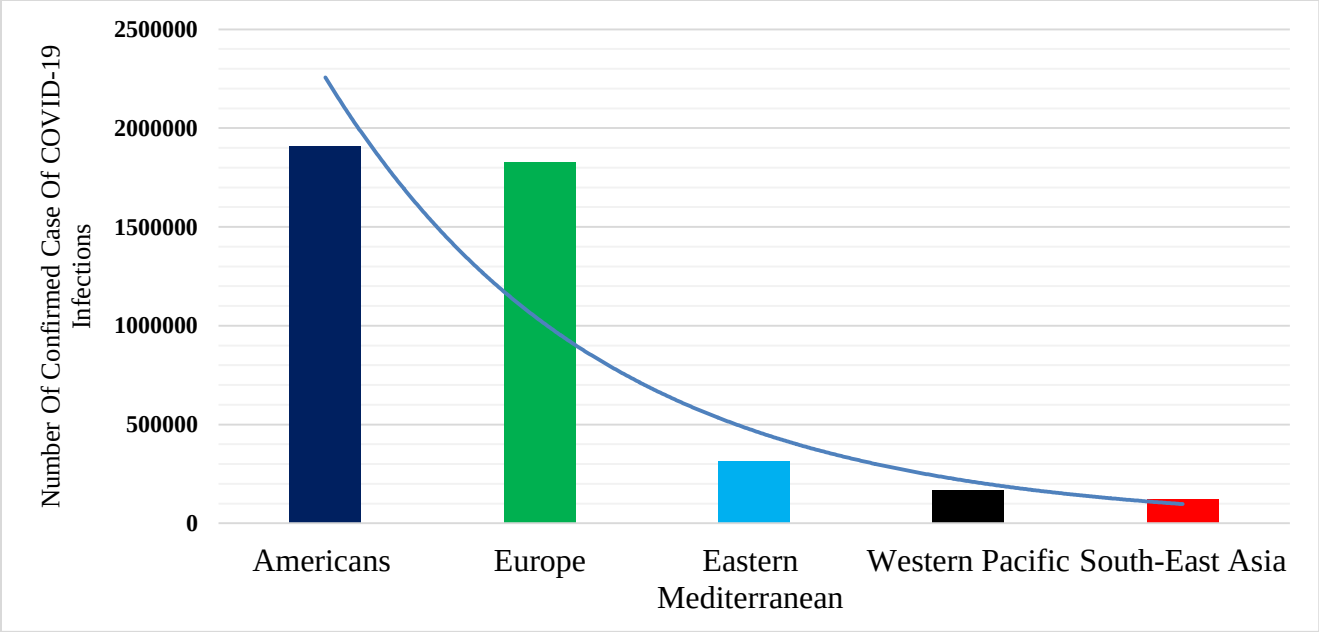

Fig 4. Distribution and comparison of COVID-19 cases among different continents (WHO Corona disease COVID-19 Dash board)

increase in the aspartate serum amino transaminase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), bilirubin, serum prolactin, and an elevation of the inflammatory markers such as C-reactive protein (CRP) and ferritin.

9.1.2. The molecular and serological tests

Recently, Yaun *et al*., (2020); CDC. (2020), recommended the use of a molecular assay qRT-PCR for the detection and quantitative analysis of the viral nucleic acid from distinct clinical specimens, and can produce the report within 4-6 hours. It is a more sensitive and widely used method in the distinct laboratories around the world. The serological diagnostic tests such as Enzyme linked immunosorbent assay (ELISA) and the rapid diagnostic test (RDT) are used to detect the presence of anti-SARS-COV-2 antibodies and\or antigens in the patient's blood. The ELISA kit can be used for detection of the N and S antigen of SARS-CoV-2. It is based on sandwich ELISA, which allows rapid quantification of the N and S antigen. Kits of the Rapid diagnostic test (RDT) are used for detection of IgM and IgG antibodies against SARS-CoV-2 virus in patient's serum. It is a rapid method that can produce a report within 10-15 min. and requires limited instrument; however its use may be limited for the diagnosis of acute SARS-CoV-2 infection. The CoV-2 rapid diagnostic kit has 89 % sensitivity and 91 % specificity, in reference to Li *et al*., (2020). Currently, WHO and CDC do not recommend the use of RDT kits for diagnosis of the COVID-19 infection.

A recent study conducted by Broughton *et al*., (2020) highlighted that Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR-Cas12a) based assay is used for detection of SARS-CoV-2 from extracted samples of the patients within 30-40 min. It is carried out using Reverse transcription-loop-mediated isothermal amplification (Rt-LAMP) for pre-amplification of the viral or control RNA targets, and Cas12a is used for the trans-cleavage assay. Accuracy of the near real-time

deforestation detection system (DETER) is comparable to the RT-qPCR, and does not require expensive laboratory instrumentation. It uses similar sample collection techniques and RNA extraction methods as those of RT-qPCR.

CRISPR-based Specific High Sensitivity Enzymatic Reporter unlocking technique (SHERLOCK) is used for detection of SARS-CoV-2, and is able to detect the SARS-CoV-2 target sequence in a range between 20-200 aM (10-100 copies per microliter input). This test is carried out using purified patients RNA samples as used in qRT-PCR, and can read out the test results using a dipstick in less than 1 hour, without using expensive instrument. In general, SHERLOCK technique is more accurate and takes less time compared to RT-qPCR (Joung *et al*., 2020).

9.1.3. Histological examinations

The histopathological Hematoxylin and Eosin (H,E) staining of lungs biopsy tissue of patients died due to COVID-19 infection showed pulmonary oedema, with bilateral diffuse alveolar damage and fibromyxoid exudates, interstitial mononuclear inflammatory infiltrates, dominated by lymphocytes in both lungs (Zhang *et al*., 2020b). Moreover, Tian *et al*., (2020) reported desquamation of pneumocytes with hyaline membrane formation, indicating acute respiratory distress syndromes (ARDS). Multinucleated syncytial cells with atypical enlarged pneumocytes, nuclei, prominent nucleolus, along with the presence of amphophilic cytoplasmic and nuclear granules, suggest the presence of intra-cytoplasmic and intranuclear viral inclusion bodies. The pathological characteristics of COVID-19 are similar to those reported in SARS-CoV and MERS-CoV viral infections (Ding *et al*., 2003, Alsaad *et al*., 2017).

9.2. Radiological findings

In early stages of COVID-19 infection CT scan of patients chest showed multiple ground-glass patchy (GGO) shadows in the periphery of lungs, along with interstitial changes (Shi *et al*., 2020; Xu *et al*., 2020). However, Rodrigues *et al*., (2020) added that with the progress of diseases CT scan revealed infiltration of both lungs and multiple bilateral diffuse patches, with a ground glass-like opacifications, pulmonary consolidation and nodules. Other abnormalities were noted including; linear opacities, opacities with rounded morphology, opacities with a “reverse halo” sign, opacities with a “crazy-paving” pattern and opacities with intralesional cavitation. A previous work of Muller *et al*., (2003) documented that these patterns of radiological findings of the disease, were somewhat similar to those described in earlier corona virus outbreaks such as SARS-CoV and MERS-CoV. Other uncommon radiological imaging findings involve pneumothorax, cavitation and lymphadenopathy.

10. Challenges for control of COVID-19 infection

A pandemic outbreak of new members of coronavirus SARS-CoV-2 around the globe poses serious threat in controlling the rapid spread of COVID-19 infection. The lack of antiviral drugs, vaccines, proper health care setup with ventilation, appropriate diagnostics tools, shortage of supply of medical and protective instrument, and limited skilled health care workers manpower in various nations, cause serious challenges in controlling of COVID-19 infection involving:

a. Strict implementation of infectious disease control measures within hospitals and community is very crucial, to protect all health care workers and peoples in a community.

b. Epidemiological health expertise should conduct training and providing important suggestion guidelines about the treatment and management of COVID-19 infected patients during being hospitalized.

c. Most of the SARS-CoV-2 patients do not require therapy, and over treatment of patients without current or future medical need should be completely stopped.

d. Trust between people and institutions, and between local people and government should be strongly

maintained, so that the local communities' people can adhere to medical advice, by respecting the temporally individual restrictive measures.

e. Antagonisms between the different countries, their federal government and between the local governments should be immediately stopped. Antagonism and lack of trust during this crucial period will affect the scientific collaboration and will affect the control of SARS-CoV-2. Thus, different countries should share their experiences and carry distinct scientific activities regarding the development of a vaccine and a medicine for treatment of COVID-19 infection.

f. Scientific researches on preventive measures and treatment of COVID-19 should be accelerated in every country, and their experience about treatment and management of patient's infection should be shared.

11. Treatment and management

Currently, antiviral drugs and vaccines against SARS-CoV-2 are not commercially available. Treatment of patients with a COVID-19 is directed towards relieving the symptoms and management of severely ills patients, through using supportive therapy including; artificial oxygen therapy and hemodynamic supports. However, the world is now desperate to find ways to slow the spread of corona virus, and to find effective treatments.

11.1. Use of drugs (Antiviral, antimalarial and rheumatoid drugs)

At present, there is no high-level evidence which favors the use of a single antiviral drug for treatment of patients with suspected or confirmed COVID-19 infection. Thus several drugs such as; chloroquine, arbidol, remdesivir and favipiravir are currently undergoing clinical studies, to test their efficiency and safety in the treatments of corona virus disease (COVID-19).

11.1.1. Chloroquine and Hydroxychloroquine

Chloroquine is a 4-aminoquinoline drug while hydroxychloroquine is an analog of chloroquine drug, are used for malarial and autoimmune diseases treatments, respectively. Vincent *et al*., (2005); Biot *et al*., (2006) documented that the efficacy of chloroquine and hydroxychloroquine in treatment of SARS-CoV infection was studied during the epidemic outbreak of SARS in 2002-2003. Recently, Colson *et al*., (2020) reported that clinical trials on both drugs in China, South Korea and France showed that they possess potential pharmacological activities the treatment of COVID-19 infection.

Al-Bari, (2015); Liu *et al*., (2020b) demonstrated that the molecular mechanism of action of Chloroquine and hydroxychloroquine drugs is similar, as they interfere with the virus replication in distinct ways. They inhibit binding of the S glycoproteins spikes on the host cell ACE-2 receptors, by inhibiting the terminal glycosylation. Moreover, they increase the pH in the intracellular compartments, ultimately inhibiting the nucleic acid replication, glycosylation of viral protein, virus assembly and the process of exocytosis.

11.1.2. Lopinavir/Ritonavir

Lopinavir/Ritonavir are protease inhibitor drugs used in effective treatments during the outbreak of SARS-CoV and MERS-CoV. They could be potential drugs in treatment of patients with COVID-19 infection. A clinical trial of Lopinavir drug in Hong Kong University showed its in vitro anti-SARS-CoV action, at a concentration of 4 mg/ ml (Chu *et al*., 2004). On the other hand, Ritonavir is used as a boosting agent. According to Stower *et al*., (2020), current treatment regime guidelines recommended the use of Lopinavir in combination with Ritonavir for treatment of the COVID-19 infection. The use of Lopinavir/Ritonavir in treatment of COVID-19 patients' has reduced the mortality rate by 2.3 vs 11 %.

11.1.3. Ribavirin

A recent work of Khalili *et al*., (2020) highlighted that Ribavirin is a nucleoside analogue antiviral drug targeting the RNA-dependent polymerase enzyme of SARS-CoV, MERS, and interferes with replication of the viral RNA and DNA. It is used in combination with the corticosteroids for treatment of patients with SARS-CoV, Hepatitis virus, and viral haemorrhagic fever including MERS. However, in-vitro and in-vivo activity of ribavirin drug in treatment of SARS-Cov-2 infected patients did not show significant reduction/clearance of the SARS-CoV virus. Excessive use of this drug produces distinct side effects such as; haemolytic anaemia, hypocalcaemia, and hypomagnesaemia. Currently, it is not recommended in treatment of patients with COVID-19 infection.

11.1.4. Remdesivir

Remdesivir is a nucleoside/tide analogue with a broad-spectrum antiviral activity (Goldman *et al*., 2020). Recent study of Beigel *et al*., (2020) reported that this drug blocks the corona virus RNA polymerase enzymes required to replicate its genetic material (RNS), and thus inhibit viral proliferation in the human bodies. During a clinical trial of drugs in mouse model for the pathogenesis of SARS-CoV, remdesivir drug improved the mouse pulmonary function, reduced the viral loads and severe lungs pathology. The National Institutes of Health (NIH) reported that early treatment with remdesivir as an antiviral drug significantly reduced the clinical disease, and reduced the damage of the lungs of rhesus macaques infected with SARS-CoV-2. On 1st May, 2020, the US Food and Drugs Administration (FDA) made remdesivir drug available for emergence use for treatment of severe COVID-19 infection in hospitalized adults and children. Moreover, Dolin and Hirsch, (2020) reported that the use of remdesivir antiviral drug in treatment of patients with server COVID-19 infection reduced the time of recovery from median of 15 days among placebo recipients to 11 days.

11.2. Use of immunomodulating drugs, proteins and vaccines

11.2.1. Corticosteroids

A recent study conducted by Zha *et al*., (2020) revealed that corticosteroids are immunomodulating drugs, which are used in combination with other antiviral drugs. Systemic administration of corticosteroid drugs has suppressed the lungs inflammation in 18.6- 44.9 % of COVID-19 patients. However, Russell *et al*., (2020) reported that its use in critically ill MERS-CoV and SARS-CoV patients result in delayed MERS-CoV and SARS-COV RNA clearance, required mechanical vasopressor and renal replacement. The study of Brown *et al*., (2020) demonstrated that excessive usage of corticosteroids drugs is associated with psychosis, diabetes and avascular necrosis. Thus, the use of corticosteroids drugs in COVID-19 patients' treatment is usually not recommended except in special circumstances.

11.2.2. Interferon's

Interferon's (IFNs) possess a broad-spectrum antiviral activity. Thus, several candidates of interferons including type 1 alpha interferon's (INF-1) are already investigated for their use in treatment of COVID-19 infection. Several *in-vitro* and *in vivo* experiments of Interferon alphacon-1 were performed in combination\ or not with Lopinavir/Ritonavir, Ribavirin, Remdesivir and Corticosteroids for their efficacy in treatment of SARS-CoV and MERS-CoV (Loutfy *et al*., 2003; Sallard *et al*, 2020). The use of INF-α and INF-β alone were effective in treating animals, but they failed to improve the diseases in humans. The combined use of Lopinavir/Ritonavir with Interferon-β have improved the lungs functions, but failed to reduce the viral load; however, Ribavirin in combination with IFNα2α significantly delayed the mortality rate. Thus, INF-1 can be used as a prophylaxis against SARS-CoV-2. Moreover, Sallard *et al*., (2020), Medhi *et al*., (2020) added that administration of 5 million units of IFNα in combination with Ribavirin twice a day by the

nebulization method, can significantly improve the health of the patients.

11.2.3. Convalescent plasma therapy

Convalescent plasma (CP) therapy is a classic adaptive immunotherapy used in prevention and treatment of infectious diseases. Previous studies of Luke *et al*., (2006); Arabi *et al*., (2015) revealed that over the past two decades, the use of convalescent plasma with a systematic corticosteroids therapy in critically ill SARS-CoV, MERS-CoV, H1N1 and Ebola virus patients, reduced the infections more efficiently, compared to placebo or no therapy. An in-vitro neutralization test performed on bronchoalveolar lavage sample obtained from critically COVID-19 patients, using convalescent sera of COVID-19 infection recovered patients, showed significant neutralization effect (Duan *et al*., 2020). The use of 200 ml of convalescent plasma containing higher neutralizing antibodies titer more than 1: 640, showed optimal results in treatment of critically ill COVID-19 patients. Thus, CP therapy can be an alternative treatment of critically COVID-19 ill patients until the antivirals and vaccines are developed.

11.2.4. Corona vaccine

The pandemic outbreak of SARS-CoV-2 across the globes created serious health problem. Full control of this virus is almost impossible until an effective vaccine is developed. In general, COVID-19 infection results in the appearance in the human body of virus neutralizing antibodies against the glycoprotein S Spike protein, and the receptor binding domain (RBD). Many researchers are focusing in their investigation on the utility of S protein or RBD as vaccine targets. About 78 companies across the world have already started their research work on vaccine development. Thanh *et al*., (2020) reported that a diversity of platforms including; nucleic acid (DNA and RNA) vaccine, virus-based subunit vaccine, mRNA vaccine, live attenuated and inactivated killed virus are being approached. The most advanced companies that have recently moved into clinical trials include; mRNA-1273 from Moderna, Ad5- nCoV from CanSino Biologicals, INO-4800 from Inovio, LV- SMENP- DC and pathogen-specific aAPC from Shenzhen Geno-Immune Medical institute. Recently, China`s CanSino Biologics, Institute of Biotechnology of the Academy of Military Medical Sciences used recombinant adenovirus types 5 vector has successfully, completed phase I trial period and currently running through in-phase II trial. Meanwhile, Moderna Therapeutics of US National Institute of Allergy and Infectious Diseases used lipid nanoparticle dispersion containing messenger RNA (mRNA-1273). It began in Phase I clinical trial in March, 2020, completed it successfully, and will enter in Phase II. This vaccine encourages the formation of antibodies through the injection of messenger RNA (mRNA) with the genetic code for the spike protein found on the virus surface. Sinovac Biotech used inactivated SARS-CoV-2 virus, has successfully completed Phase I trial and now is in phase II trial. Inactivated SARS-CoV-2 is easy to prepare compared to the other vaccines. Currently, all the vaccines development is in its trial phases, and may take 1- 2 or more years to complete.

Conclusion

Emergence and spread of new strains of corona virus SARS-CoV-2 over 215 countries across the globe has created a serious global health threat. Millions of people around the globe have been already become victims of SARS-CoV-2 virus, and thousands of people have already lost their life. Origin of SARS-CoV-2 virus is controversial, but the majority of researchers believed it is likely to be originated from wild bats. The whole genomic sequencing analysis of the bat and pangolins virus showed 95% identity to the genomic sequence of SARS-CoV-2 virus. Bat and pangolins are considered as primary natural reservoirs of SARS-CoV-2. Human to human transmission of this virus occurs due to inhalation of respiratory droplets produced by asymptomatic and symptomatic COVID-19 patients. SARS-CoV-1, MERS-CoV and SARS-CoV-2 viruses are known to cause mild to severe lower respiratory tract infections leading to pneumonia. Identification of corona virus plays a

crucial role in proper treatment and management of the COVID-19 patients, control of pandemic outbreak of this disease, and reduce the economic impact of this virus in China and all over the world.

Currently there are no compelling antiviral drugs or vaccines against the SARS-CoV-2 virus. Antiviral drugs such as ritonavir, arbidol, remdesivir and favipiravir, which were previously used in treatment of patients with SARS-COV, MERS-CoV and Ebola, are now used for treatment of patients with COVID-19. Complete control of the COVID-19 infection is only possible after the successful vaccination only. However, currently there is no vaccine against the SARS-CoV-2 virus, and is under the developmental trial phase. Strict implementation of preventive and control measures such as wearing masks, washing hands, maintaining the social distancing, isolation and quarantine, and disinfecting the surfaces, could help to reduce the risk of infection, and control the spread of COVID-19 infection.

Further researches ought to be coordinated toward the investigation of SARS-CoV-2 in reasonable creature models, for dissecting its replication, transmission and pathogenesis.

Acknowledgement

We are extremely thankful to Rajiv Bhandari, Sadikchha Chapagain, Liu Ya, Gui Feng for collecting and providing the valuable information, and helping in this manuscript preparation.

Conflict of interest

There is no any conflict of interests between the authors.

Funding of study

The current work did not receive any funding.

Ethical approval

Non-applicable.

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