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Review Article

Facts and Figures On Covid-19 Pandemic Outbreak

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ABSTRACT

A variety of infectious public health problems are prevailing in the world. Among these, epidemics of Severe Acute Respiratory Syndrome (SARS), H1N1 influenza and Middle East Respiratory Syndrome corona virus (MERS-Co V) emerged as very important issues during last three decades as these infections caused quite large number of human deaths worldwide. Coronaviruses are single-stranded positive sense RNA viruses which mainly in past were considered responsible for high percentage of (around 30%) of common cold/flu cases. Viruses causing SARS, MERS and COVID-19 are members of family *Coronavirdae*. World Health Organization (WHO) reported that the novel Cov-19 virus infection was first diagnosed in Wuhan, Hubei Province, China during December 2019. Initially the virus was named as nCoV-19 and later disease due to this virus was named as COVID-19, and recently named as Severe Acute Respiratory Syndrome Corona virus 2 (SARS-CoV-2) by the International Committee on Taxonomy of Viruses. This new coronavirus was found to have 86.9 % homology to a bat corona virus and hence it was suspected to have been originated from bats. Till 15th January 2021, the COVID-19 infection has been reported from 219 countries. It has caused over 20 million deaths in humans around the globe. Countries reporting very high death/infection rates include USA (393,948/23,617,815), Brazil (206,009/5,257,459), Mexico (136,917/1,571,901), India (153,000/10,596,442), UK (84,767/3,211,576), France (69,031/2,830,842), Russia (63,940/3,495,816) and Italy (80,326/2,319,036). Worldwide a total of over 96,750,700 COVID-19 cases have so far been reported. As reported earlier this pandemic has hit almost every country worldwide causing exceptionally high morbidity and mortality. Amongst the South Asian countries India is worst hit by this deadly COVID-19. Pakistan's neighboring Iran is also very badly infected and reported 57,057 deaths of 1,348,316 infected people. In Pakistan 52,411,930 confirm cases of COVID-19 and around 11,000 deaths are reported in various region of the country.

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Key words
Corona, COVID-19, Pandemic, MERS, SARS

INTRODUCTION

A highly fatal infection caused by novel coronavirus (2019-nCoV) in humans was first diagnosed in Wuhan (China) during December 2019. The initial epidemiological investigation linked majority of suspected cases with their origin at Huanan Seafood and the Live Wild animal markets. The isolation of the virus from environmental samples collected from this market suggested the possibility of this virus crossing the species barrier from animal(s) to humans. In late January 2020, the WHO (World Health Organization) declared the coronavirus (COVs) outbreak as "Public Health Emergency of International Concern" and the resulting disease was designated as COVID-19 and the causal virus re-named as "SARS-CoV-2". Presently the disease has been reported from all the continents of the world. As of today, the CoVID 19 has been reported from 219 countries and this disease has been declared as PANDEMIC.

Till 21st January 2021, the COVID-19 infection has been reported from 219 countries. It has caused over 200 million deaths in humans around the globe. Countries with very high death/infection rates include USA, Brazil, Mexico, India, UK, France, Russia and Italy. Worldwide a total of over 96750700 COVID-19 cases have so far been reported. As reported earlier this pandemic has hit almost every country worldwide causing exceptionally high morbidity and mortality. Amongst the South Asian countries India is very badly hit by COVID-19. Pakistan's neighboring Iran is also very badly infected and reported 57057 deaths of 1348316 infected people. In Pakistan round 11000 deaths are reported.

Presently all 219 countries, infected with COVID-19 around the globe are reporting daily progressive increase in infected/death cases. Actual number of cases (confirmed

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and reported versus unreported vs untested asymptomatic infectious cases) may probably be quite higher as there is always limitation of either the unavailability or limited availability of COVID-19 diagnostic testing kits especially in the developing and under developed countries.

The rapid spreading highly contagious SARS-CoV-2 infection now occurring almost worldwide has serious implications on human health, restriction on international travelling, quarantine at entry points in various countries, trade and economy, business shutdowns, lock downs have all badly affected the livability of humans in many countries; especially the poor ones. Presently, over two thirds of the world population is under orders either to stay-home and/ or under lockdown condition. In several countries, a ban on social gathering and movement of people was imposed to limit the interaction of sick or asymptomatic corona infected populations. However, despite adaptation such preventive and hygienic measures, public awareness and scientific guidance; this COVID-19 continues to spread. WHO also recommends above measures at the global levels especially under a situation when there is no effective antiviral therapy, non-availability highly efficacious anti-coronavirus vaccine. Human efforts to contain the spread of this disease are so far not successful. A few reports also indicate that this virus is affecting animals such as dogs, cats, bats etc. Although many pharmaceutical and research organizations in various countries are trying hard to develop vaccine against this virus entity; their efforts are not so far fruit bearing for the people. The vaccine production and antiviral development for COVID-19 may take almost a year of research and its safety studies before being made available for commercial use. It is argued by some that the vaccine for this disease may not be available to the population facing the present pandemic. It is suggested that using one health forum, coordination among, medical, veterinary and public health services be established to properly combat against this malady.

This review focuses on the comparative biology of animal and human coronavirus types, its origin, transmission, zoonotic and reverse zoonotic potential of SARS-CoV-2, COVID-19 pathogenesis, diagnosis, and hopes for vaccine and antiviral development.

CORONAVIRUS-19 CHARACTERISTICS

Corona viruses are large, enveloped, positive sense single stranded ribonucleic acid (RNA) viruses, named for the presence of spike (S) proteins surrounding the lipid envelope, giving them a “crown-like” appearance. CoVs have adapted to infect a large numbers of animal species that range from bats to camels. These viruses cause respiratory, reproductive, gastrointestinal, hepatic, neurological and/or other systemic diseases in a wide range of animal species and birds (Mesel-Lemoine *et al*., 2012).

Being RNA viruses, CoVs are prone to spontaneous mutations, although they do have some proof-reading activity due to their large genome. Typically, unlike DNA viruses, RNA viruses lack proof-reading enzymes, which lead to random spontaneous mutations during replication. The mutation rate in SARS-CoV-2 is, however, less than 25 mutations per year compared to influenza A virus with almost 50 mutations per year. Transmissible gastroenteritis virus (TGEV) causes gastrointestinal disease and porcine respiratory coronavirus (PRCV) infects the respiratory tract in pigs. Phylogenetic analyses of these two CoVs have revealed that PRCV originated from TGEV through a deletion in a part of the S protein, which altered the tissue tropism of the virus from the gastrointestinal tract to the respiratory tract. Feline infectious peritonitis in cats, and sporadic cases of vomiting and diarrhea and canine infectious respiratory disease complex in dogs and respiratory and enteric infections in cattle are some of examples of diseases caused by animal CoVs. The first CoV, avian infectious bronchitis virus (IBV), discovered in chickens in 1937, causes an economically significant disease that affects the respiratory, renal and reproductive system of the chickens (Beaudette and Hudson, 1937).

Human coronaviruses (HCoVs) were first identified in 1960s in the nasal samples of humans who suffered from “common cold”. *Coronaviridae* family classifies into alpha CoV, beta CoV, deltaCoV, and gamma CoV (Chan *et al*., 2013). At present, seven types of CoVs can infect humans. Four of these, 229E, OC43, NL63 and HKU1 CoVs, are endemic, and have been estimated to cause 15 to 30 % cases of common cold (Liu *et al*., 2020; Lim *et al*., 2016). Most common cold cases occur during the winter and early spring months. Comparative phylogenetic analyses of human and animal CoVs suggest that 229E and OC43 might have jumped their animal reservoirs (bats and cattle, respectively) to infect humans within the last 200 years. HKU1 is believed to have originated from mice. Within the last two decades, three CoVs have jumped animal reservoirs directly or through an intermediate host to cause severe disease in humans; these are Severe Acute Respiratory Syndrome (SARS) coronavirus (SARS-CoV-1), Middle East Respiratory Syndrome (MERS) coronavirus (MERS-CoV) and most recently SARS-CoV-2 responsible for causing COVID-19 in millions of humans (Smith, 2006; Ren *et al*., 2020). SARS-CoV-1, MERS-CoV and SARS-CoV-2 belong to β-CoVs (Smith, 2006). SARS, caused by SARS-CoV-1, emerged in 2002-2003 in China and caused infections in 8,098 cases with

774 deaths globally in humans; the epidemic ended in 2003-2004. MERS, first reported in Saudi Arabia, caused only 2521 cases with 866 deaths indicating that MERS-CoV is fatal than SARS-CoV-1 as evident by higher case fatality rate (CFR) of 34% (Munster *et al*., 2020). MERS cases still occur periodically, most likely from occasional spill-over from intermediate host. SARS-CoV-1 is more infectious than MERS-CoV; MERS-CoV transmits poorly from human to human.

Although bats are considered natural reservoirs of probably more than 5,000 diverse CoVs, only about 500 bat CoVs have so far been identified. Based on comparative phylogenetic analyses, bats are considered the most likely natural reservoirs of SARS-CoV-1 and MERS-CoV. SARS-CoV-1 evolved to jump species barrier through intermediate hosts "masked palm civets and raccoon dogs" to cause a global epidemic, and MERS-CoV jumped through intermediate host "dromedary camels" to infect humans.

ORIGIN OF SARS-COV-2 (COVID-19)

The variability in CoV S-protein receptor-binding domain (RBD), receptors on host cells for binding to the S protein, and the ability of host cells to provide pre-requisites for virus entry and replication are key determinants of viral virulence, host specificity, and disease pathogenesis and symptoms. Spontaneous mutations in CoV S protein gene due to selection pressure sometimes may lead to a high affinity of RBD for its receptors present on certain host cells; this can cause certain strains of CoV to jump species barrier or spill over to cause outbreaks among humans or another animal species (Chen *et al*., 2020).

Since the initial reports of mysterious pneumonia (COVID-19) in China (Wuhan), there has been significant debate on the origin of SARS-CoV-2, and several theories have been proposed. According to an article published in Nature Medicine, mutations in the RBD of S protein of SARS-CoV-2 due to natural selection might have resulted in higher affinity of RBD for human angiotensin converting enzyme-2 (ACE2) receptors on human cells (Wan *et al*., 2020). ACE2 receptors are expressed in nasal mucosa, esophagus, bronchus, heart, lungs, kidneys, stomach, ileum and bladder making these human organs highly susceptible to SARS-CoV-2 (Zou *et al*., 2019). The acquisition of furin polybasic cleavage sites along with three O-linked glycans at the junction of S1 and S2, the two subunits of SARS-CoV-2 S protein may increase the tissue tropism, host range, and transmissibility of the virus. More cellular proteases could potentially cleave at polybasic sites, thus enhancing virus attachment and its entry into the host cells. More research studies are desired to determine the functional significance and role of polybasic cleavage site and three O-linked glycans in S protein in SARS-CoV-2 transmission.

Though SARS-CoV-2 shares 96.2% similarity at the nucleotide level with CoV RaTG13, which was identified in horseshoe bats, this virus has not formerly been identified in humans; may be because the bat habitations are usually far away from dense human populations. It is possible that due to selection pressure, SARS-CoV-2 S protein RBD, after evolving in horseshoe bats, further evolved in an intermediate animal host before zoonotic transfer to humans. The RBDs of S protein of Malayan pangolin CoVs and SARS-CoV-2 indicate strong similarity. It is likely that SARS-CoV-2 S-protein RBD became optimized through pre-adaptation in pangolins for binding to human ACE2 receptors with high affinity before infecting humans. Biophysical and structural evidences suggest that RBD of SARS-CoV-2 S protein is likely to bind human ACE2 with 10 to 20-times high affinity than SARS-CoV-1. It has been reported that the S protein binding site (SPBS) of ACE2 of human, macaque, and chimpanzee are identical. When amino acid sequence was compared, SPBS of cat ACE2 is only three- amino acid different from that of human ACE2; it is therefore imperative to monitor the presence of SARS-CoV-2 infection in cats because human patients with COVID-19 may potentially transmit this virus to infect cats. However, as of today, there is no evidence to conclude that cats, dogs or zoo animals can transmit this virus to humans.

Another theory is that a progenitor of SARS-CoV-2 jumped into humans adapted into a more virulent strain of SARS-CoV-2, responsible for the current pandemic. The viral quasi-species dynamics due to genetic variation, competition and selection might have played a role in SARS-CoV2 adaptation to infect human cells as hosts. The quasi-species phenomenon has been reported for several RNA viruses including SARS CoV and MERS-CoA. The genomic analysis of 103 viral isolates by Tang and coworkers recently suggests that SARS-CoV-2 has evolved into L and S types; the clinical significance of these two types are uncertain. It is possible that one strain type is more pathogenic than the other. Several genotypes and pathotypes of SARS-CoV-2 are possibly circulating among the human population. Studies involving a large number of viral isolates in future might help to characterize genotypes and potential antigenic variants of this virus.

Although the pandemic caused by an accidental leakage of SARC-CoV-2 from a virology laboratory working on bat CoVs cannot be discounted, the accidental release of a laboratory manufactured or bio-engineered virus is least likely owing to SARS-CoV-2 S protein features reported so far, mutation patterns in genomes of

SARS-CoV-2 isolates, and strong similarity of SARS-CoV-2 S protein RBD to that of pangolin CoVs. It is possible that scientists working with bats could have been infected from bat-CoV that might have mutated through preadaptation in another animal or through human-to-human passage to a more virulent strain. However, the circumstances and species from which the virus crossed the species barrier to infect humans remain to be conclusively determined. It would be interesting to see what animal models and parallels in those animal models will be used to study the characteristics of this virus. Rhesus macaques, Syrian golden hamsters and ferrets might be suitable animal models as disease has been produced in these animal species by inoculating them with SARS-CoV-2.

SARS-COV-2 PHYSICOCHEMICAL PROPERTIES, TRANSMISSIBILITY AND REPLICATION

SARS-CoV-2 has a diameter of 60 to 100 nm (occasionally 120 nm) with an RNA genome of about 30 Kb. It can be inactivated at 56 ^{o}C for 30 minutes; the virus is sensitive to ultraviolet rays, most disinfectants including diethyl ether, 75% ethanol, chlorine, peracetic acid and chloroform as well as alcohol-based hand sanitizers and common hand soap. A recent unpublished study found that serial increases in temperature and relative humidity decrease the transmission of this virus. High temperature and humidity have also been reported to reduce the transmission of SARS-CoV-1 and influenza viruses.

The first step in infection cycle of SARS-CoV-2 is the attachment of the RBD of S protein of viral particle to ACE2 receptors on the surface of host cell membrane. After entering the cell through a process called endocytosis, the virus releases its genomic RNA into the cell cytoplasm. Viral RNA-dependent RNA polymerase with the help of host cell's machinery makes copies of its genome and synthesizes its proteins including the packaging proteins. The new packaging proteins assemble around newly formed viral RNA copies to make new virions. The new viral particles exit through the cell membrane (exocytosis) into the extra-cellular environment and are ready to attach to the neighboring host cells and repeat the new cycles of infection process. Viral genomic and multiple sub-genomic messenger RNA species and ribosome frameshifting during translation of the viral genome are hallmarks of CoV replication. SARS-CoV-1 is known to evade the early immune response in at least two ways: first, SARS-CoV-1 produces non-structural protein-1, which degrades host mRNA and restricts host gene expression leading to less interferon response in host cells; second, SARS-CoV-1 also produces interferon antagonist proteins in host cells, which reduces cell's antiviral activity. Research is needed to study whether such mechanisms are also used by SARS-CoV-2 for early immune evasion. The other questions that need to be answered relate to pathways involved in SARS-CoV-2-induced membrane modelling, temporal and functional coordination of various stages of viral replication and transcription complexes in host cells, maintenance of large genome but still undergoing sufficient mutations for adaptation and trans-species jump or preadaptation in humans to become more virulent, and finally but not the least functions of SARS-CoV-2 accessory proteins and their impact on *in-vivo* growth and virulence of the virus; this is important to study, explore, find and develop potential antiviral treatment against SARS-CoV-2.

The transmissibility or contagiousness of an infectious disease (R_0) is measured as average number of individuals who tend to contract the disease from each infected individual. Any disease with a $R_0 \geq 1.0$ is likely to spread over time. In one study conducted in 2014, the median result for R_0 (basic reproduction number) of seasonal flu (influenza) was reported to be between 1.28 to 1.8. In a study published in The Journal of Travel Medicine, the R_0 for COVID-19 was estimated to fall between 1.4 and 6.49 with an average of 3.28 and a median of 2.79. WHO has estimated R_0 of SARS-CoV-2 to be around 2.6 and one infected individual could infect 2 to 3 individuals within three to six days. Thus, SARS-CoV-2 transmissibility or contagiousness is higher than the seasonal flu, explaining its rapid spread. It is important to mention that R_0 may vary due to data size, biological, socio-behavioral and environment factors as well as disease mitigation, infection period, herd immunity and intervention measures. The most alarming aspects of viral transmission are asymptomatic or subclinical infectious cases of SARS-CoV-2. Individuals who are incubating the virus as well as recovered individuals are known to shed the virus. The duration for which an infected person is infectious is uncertain. Most of the studies published so far have used viral genome detection as a viral load parameter. However, it should be noted that detection of viral RNA or its segment does not always correlate with presence of infectious virus in samples from population tested.

The major route of SARS-CoV-2 transmission is by inhalation of respiratory or saliva droplets discharged by infected individuals via coughing, sneezing, and talking. The virus remains viable in droplets from infected patients for at least three h. SARS-CoV-2 infection also occurs when an individual touch his or her eyes, nose and mouth after contacting contaminated surface(s) or hand shaking with infected persons or asymptomatic infectious cases. In a study, SARS-CoV-2 was found to survive on copper and cardboard for 4 and 24 h, respectively and on plastic and stainless-steel surfaces up to 72 h; Individuals recovered from COVID-19 have been reported to shed virus from a few days to about three weeks. Respiratory droplets generally do not travel more than six feet because they are heavier in size. However, depending upon the force behind the sneeze and cough, the virus might travel even longer distances. The bioaerosols containing infectious virus could travel up to 13 feet distance as reported in one of recent studies. Bioaerosols are more likely to occur in hospital settings or laboratories, thus requiring standard aerosol precautions in those facilities. Guidelines should be modified to include face masks or coverings for all individuals in work and public places, and social distancing be re-defined as per new research findings. SARS-CoV-2 has also been detected in blood and stool; less significant but fecal-oral transmission of virus is a possibility. Until now, there is no evidence of vertical (intra-uterine infection) virus transmission in pregnant women.

Initial studies have suggested that asymptomatic or subclinical infectious cases occur mostly in young individuals. The exact frequency of asymptomatic infections and their contribution in virus transmission remains to be determined and quantified. Large-scale serological screening may provide a better understanding on the seroprevalence of asymptomatic infections, population exposure and herd immunity. Several factors including geographic location, population density, age distribution, immuno-competency and presence or absence of underlying health conditions might affect the percentage and extent of virus shedding as well as host susceptibility.

It is therefore important to follow guidelines such as social distancing, frequent hand washing, no-hand shaking, no-face touching, no-eye rubbing and using face masks when going outside. Social distancing means staying at least 6 feet (perhaps more) away from others and avoiding mass gatherings and crowded places. All these measures assist in flattening the curve, reducing the risk of human to human infections, and indirectly decelerating the pandemic so the health system can better cope with sick patients.

COVID-19 PATHOGENESIS

The incubation period from contact of virus to onset of symptoms is between two and six days with median incubation time being 5.1 days (Lauer *et al*., 2020). The SARS-CoV-2 infection, which usually starts as upper respiratory illness can easily develop into serious lower respiratory tract disease; viral pneumonia leading to fatal respiratory failure in susceptible persons 10-14 days post-infection.

According to a study involving 138 patients, the common clinical features of COVID-19 were fever (99% of cases), dry cough (59% of cases), fatigue and myalgia (70% and 35% of cases, respectively), sputum production (27% of cases), anorexia (40% of cases) and 31% of cases showed dyspnea (Sharma *et al*., 2020). In another study, headache (8% of cases), hemoptysis (5% of cases) and diarrhea (3% of cases) were also present. Nervous signs, itchy eyes, loss of smell (anosmia) and loss of taste (ageusia), nausea, rhinorrhea and sore throat have also been reported as signs of SARS-CoV-2 infection in some cases. In another report, roughly 50 % of COVID-19 patients present with main digestive symptoms as their primary complaint in addition to respiratory signs; moreover, disease course is more prolonged and severe in patients with gastro-intestinal signs. According to another report A dysregulated excessive "cytokine storm" causing fatal pulmonary inflammation during SARS-CoV-2 infection is most likely the principal cause of case fatality. Rapid viral replication and cellular damage, virus-induced ACE2 downregulation and shedding, and antibody dependent enhancement (ADE) are likely responsible for the intense inflammation caused by SARS-CoV-2. The virus may also affect heart directly by causing uncontrolled inflammation. It is not known whether SARS-CoV-2 causes ciliostasis in the upper respiratory tract of humans. However, CoV of avian infectious bronchitis is reported to cause ciliostasis in tracheal organ cultures of chicken origin. It is possible some circulating strains of SARS-CoV-2 have more affinity for certain organ system; thus, presence of pneumo-tropic, entero-tropic and neuro-tropic strains of SARS-CoV-2 is a possibility but this needs to be verified through research.

According to a report from Chinese Center for Disease Control and Prevention, infection of humans with SARS-CoV-2 could result in five outcomes: asymptomatic carriers (1.2%), mild to moderate cases (80.9% with mild or no pneumonia), severe clinical cases (13.8%), critical cases (4.7%) and death (2.3%) in all the reported human cases (Read *et al*., 2020; Huang *et al*., 2019, 2020; Chen *et al*., 2020). Severe cases involve patients with shortness of breath and high respiratory rate with blood oxygen saturation at or less than 93%. Acute respiratory distress syndrome (ARDS) is a main complication in serious patients, which usually follows dyspnea (Chen *et al*., 2020). The alveolar damage prevents sufficient oxygen from getting to the lungs and into the circulation, causing mortality due to acute lung injury and ARDS. Patients who suffer respiratory failure, septic shock, and/or multiple organ impaired function or failure are in critical health condition. According to one report, 12.3 % of the severe to critical cases require mechanical ventilation. Lymphopenia is another common feature of critical COVID-19 patients;

it is not clearly known whether lymphopenia results only from direct immunosuppressive effects of SARS-CoV-2 or because of the cytokine storm. Pathological findings from severe patients show pulmonary bilateral diffuse alveolar damage.

The percentage of asymptomatic carriers is believed to be much higher than 1.2%. A recent study from China reported 78% of new infections to be asymptomatic infections; however, sample size was very small and it is likely patients tested also included pre-symptomatic cases. According to the US Center for Disease Control and Prevention (CDC), the percentage of asymptomatic spreaders of SARS-CoV-2 might range from 25 to 50%.

COVID-19 affects all human age groups. However, the disease has a higher case fatality rate in immune-compromised patients and/or elderly people especially those with co-morbidities such as asthma, cardiovascular disease, severe obesity, liver disease, diabetes mellitus, hypertension, chronic kidney and/or respiratory disease and cancer.

The case fatality rate (CFR) varies with location. In Italy, CFR was 7.2 percent in mid-March of 2020 with median age of infected people being 64 years. In contrast, the approximate CFR in South Korea in mid-March was only 0.9 with median age in 40s of patients. Traditionally, respiratory infections are more common either in elderly people or very young children. In Italy and China, higher CFRs were reported in males compared to females.

In the USA, the mortality rate in African Americans is disproportionately higher. In some patients recovered from COVID-19, relapses or re-infection with SARS-CoV-2 have been reported; this could be due to poor protective immune responses in recovered patients, incomplete clearance of virus from the body of infected host, asymptomatic carriers, initial infection with antigenically variant strain, further transformation of the virus into more virulent strain, and possible ability of certain virus strain to cause infection through antibody mediated enhancement of infection; moreover, false negatives in initial testing and false positives in the recovered patients have been reported due to poor sensitivity and variable quality among batches of invalidated RT-PCR kits.

The lack of widespread and systematic testing is the main source of discrepancies in mortality and morbidity rates in various countries. The other plausible explanation for differences in morbidity and mortality rates due to COVID-19 could involve under-reporting of COVID-19 cases, under-testing, variation in herd non-specific immunity, geo-genetics, climate/temperature differences, variation in circulating strain virulence, higher percentage of younger population, less economic capacity for people to travel nationally or internationally and contract or transmit SARS-CoV-2, and timely implementation of travel restrictions and early social distancing guidelines by some government(s).

IMMUNITY TO SARS-COV-2

Antibodies in SARS-CoV-2 infected patients are produced, and these could be neutralizing and protective. However, this needs to be established decisively. The evidence for protective effect of neutralizing antibodies against this virus comes from the fact that convalescent plasma (CP) of recovered patients confers some degree of protection in recipients, who suffer from the disease. Antibodies to RBD of S protein and nucleocapsid protein have been determined by enzyme-linked immunosorbent assay (ELISA) two weeks after the onset of disease symptoms. In the USA, a serological test, which qualitatively detects IgM and IgG antibodies in serum of patients, infected with SARS-CoV-2, has recently been approved by Food and Drug Administration (FDA). Once soundly established that presence of IgM and/or IgG in serum offers protective immunity, serological screening will play a significant role in understanding the herd immunity and differentiating individuals, who are at higher risk from those with lower risk of infection. The baseline antibody titers required to confer protection in a susceptible patient also needs to be established. According to herd immunity formula (R_0-1/R_0), 50 to 70% human population in a community have to have protective immunity provided effective and safe vaccines are available against COVID-19.

VACCINES FOR COVID-19

At present, there are no approved vaccines available for COVID-19; however, there are many dozens of vaccine candidates globally. Most of them are presently at investigating or preclinical trail stages. There are at least five advanced vaccine candidates that have moved into Phase I Clinical Trials. These include novel lipid nanoparticle (LNP)-encapsulated mRNA-based vaccine that encodes for a full-length prefusion stabilized S protein of SARS-CoV-2, recombinant novel coronavirus vaccine (adenovirus type-5 vector expressing S protein), DNA plasmid encoding S protein carried by electroporation, Covid-19 minigenes engineered consisted of multiple viral genes, using an efficient lentiviral vector system (NHP/TYF) to express viral proteins and immune modulatory genes to modify dendritic cells and to activate T cells, and pathogen-specific artificial antigen presenting cells modified with lentivirus vector expressing synthetic minigene based on domains of selected viral proteins.

Some laboratories are also working to develop traditional live attenuated and/ or inactivated viral vaccines. The complete vaccine development process and availability on global scale might take 12 to 18 months or even longer.

In previous studies, memory B cells and neutralizing antibody response against SARS-CoV-1 were found to be short-lived. The fundamental questions requiring definite answers through further research include seroconversion rates in human population for SARS-CoV-2, type and duration of immunity protective against SARS-CoV-2, kind of vaccines required to produce such an immunity, comparison of natural and vaccine -produced immune responses to this virus, and immuno-pathology in older people due to this virus. Recently, some Chinese scientists have identified 33 mutations in SARS-CoV-2 genome, out of 19 are new mutations; this finding suggests antigenically different strains or various pathotypes of SARS-CoV-2 might be circulating in human population. This has important implications for vaccine development.

ANTIVIRALS

At present, there are no specific antiviral drugs available to treat SARS-CoV-2 infection in humans. Hundreds of drugs and chemicals are being evaluated and explored as potential antiviral treatment for COVID-19. Several drugs, including remdesivir, hydroxychloroquine and chloroquine are in Phase I Clinical Trials (Dong *et al*., 2020; Elfiky, 2020; Gordon *et al*., 2020). Remdesivir is a nucleotide analogue, which has been used for treatment of a distantly related CoV responsible for causing infectious with a highly fatal disease (feline infectious peritonitis) in cats. The *in vitro* study has shown that antiviral effects of chloroquine or hydro-chloroquine are believed to be because these drugs can make the host cell pH slightly basic, which interferes with the replication of SARs-CoV-2 in host cells. Ivermectin, an antiparasitic drug being used for a long time for prevention of heartworm disease in dogs, has shown to inhibit SARS-CoV-2 replication in *Ver*o Cells as detected by about 5000-fold reduction in SARS-CoV-2 specific viral RNA with a single treatment of ivermectin. Ivermectin therefore warrants further investigation. Recently favipiravir, a drug which selectively inhibits RNA (ribonucleic acid)-dependent RNA polymerase necessary for influenza virus replication and being tested in Japan and China for its antiviral effects against SARS-CoV-2, has been approved for the Phase I Clinical trials in USA. The injectable form of famotidine, an over the counter heartburn drug, is being tested to treat patients infected with SARS-CoV-2. The other approaches being explored as treatment options for COVID-19 involves antibody-based, RNA-based and cell therapy-based products.

The epidemiological studies suggest that countries where the majority of human population receives BCG (TB) vaccine and anti-malarial treatment with chloroquine and hydro-chloroquine have experienced low mortality and morbidity due to COVID-19.

Nitric oxide is an essential signaling molecule between cells which is reported to have an antimicrobial activity for several viruses, protozoa and bacteria (Coleman, 2001). During the recent pandemic multiple drugs were used in an effort to lessen the effect of COVID-19 infection, however, these are not actual therapeutic treatment for COVID-19. Effect of use of combination of Doxycycline and Ivermectine are reported and some clinical trials have shown effect of dexamethason in serious patients of COVID-19. Recently, Government of the Pakistan has approved the use of Tocilizumab (Actemra) to treat COVID-19 patients. The Corona Experts Advisory Group has issued SOPs (standard operating procedures) for the use of Actemra injection to treat COVID-19 patients. However, Actemra injection initially will be used on trial basis for critically ill patients admitted in ICUs at some hospitals.

PLASMA OR SERUM TRANSFUSION

The convalescent plasma (CP) treatment involves infusion of antibody-rich plasma to patients (from people who have recovered from an infection with SARS-CoV-2). The optimal dose, time point, and the clinical benefits of CP therapy require additional exploration in larger well-controlled trials. Plasma transfusions were also been successfully used to fight against the SARS MERS and H1N1 epidemics and other viral outbreaks in the past. Recently, the FDA has issued guidance for study of investigational convalescent plasma donated from who have recovered/improved from COVID-19. Efforts are also underway to purify the SARS-CoV-2 specific immunoglobulins from recovered human patients or animal models post-infection.

NUTRITIONAL STRATEGIES AND HERBAL MEDICINES

Nutrition plays an important role to boost the immunity against various types of pathogens (Calder, 2020; Ferrara *et al*., 2020). Protein has been reported to be helpful in boosting immunity in COVID 19 infections. Imbalanced and protein deficient diet may lead to lower phagocytic activity which leads to higher risk of viral and bacterial infections. Milk, yogurt, eggs, chicken meat and fish meat are more favored due to their safe use for cardiovascular health. Chicken meat and eggs cooked in broth may help to improve the symptoms of flu and cold. The immune system got boosted by intake of minerals (calcium, phosphorus, iron, zinc, copper, chromium) and vitamin especially Vitamin D, B1, B6 and B12 (Chakhtoura *et al*., 2020; Name *et al*., 2020). Eggs and white meat are enriched with these nutrients.

Corona viruses including novel COVID-19 are sensitive to heat and got killed during normal cooking process. Hence, there is no danger of contamination in cooked food. Drinking of a plenty of water is needed to increase the mucus membrane activity of lungs and airways to fight against the pathogens. Vitamin C may helpful to boost immunity against COVID-19 as it enhances the neutrophils, T cell concentration, chemicals released by T cells and cytokines (de Melo and Homem-de-Mello, 2020; Feyaerts and Luyten, 2020). Vitamin C rich foods and fruits items (tomatoes, lemon, citrus fruits, papaya, strawberries, watermelon, cherry etc.) are reported to boost immunity against COVID 19 and most of the other pathogens. The junk and fast foods e.g. sodas, cold drinks, alcohols and drugs may have negative effect on vital organs like heart and liver thus can increase the danger of COVID-19. Regular use of neutraceuticals honey, cinnamon, ginger onion and garlic in food or in the form of extract may be helpful (Ahmad *et al*., 2021).

Chinese physicians reported the effect of use of 4-Methylumbelliferone (4-MU) and its derivatives (found in traditional Chinese medicinal herbs) to manage the lung inflammation associated with COVID-19. These herbal medicines may be used with combination of FDA approved drugs in China. However, detailed documented evidence still needs to be provided through research.

LABORATORY TESTING

For the safety reasons, samples for virus isolation from suspected or confirmed COVID-19 patients should not be submitted to or processed in traditional laboratories. The laboratory testing using RT-PCR, however, can be performed in Biosafety Level 2 (BSL-2) laboratories as recommended by WHO and CDC. The viral culture must only be performed in BSL-3 laboratories. Virus isolation has been performed in human and animal cell cultures such as Vero CCL-81, Vero 6, HUH 7.0 and 293T. More cell lines need be studied for their susceptibility to SARS-CoV-2. Genomes of many isolates of SARS-CoV-2 have already been sequenced.

SARS-CoV-2 RNA is detected by either traditional or real-time reverse-transcription polymerase chain reaction (RT-PCR). The traditional RT-PCR is a qualitative (yes or no) test whereas real-time RT-PCR is a quantitative or semi-quantitative assay that measures the viral RNA load. However, its efficacy may not be 100 per cent. The RT-PCR positive patients for SARS-CoV-2 are confirmed to have virus infection. However, both false-positive and false-negative test results are always misleading. False negative results have serious implications for disease control efforts owing to highly contagious nature of this virus.

Serology testing for SARS-CoV-2 once developed, adequately evaluated, and generally available, will help to better quantify the number of cases of COVID-19, including those that may be asymptomatic or have recovered. At present, various formats of IgM and IgG based rapid diagnostic test, different formats of ELISA, and neutralization assays are being used in various diagnostic and research laboratory settings. Rapid diagnostic testing is a qualitative (yes or no) test, used for screening the presence or absence of antibodies against the virus in patients' serum. Typically, when a person is infected, the IgM response appears earlier and IgG response later. On the other hand, ELISA and neutralization assay do provide quantifiable amounts of antibodies against SARs-CoV-2 in the patient serum. Neutralization assay specifically provides the titers of antibodies that can inhibit virus growth in cell cultures, and perhaps those antibodies can protect the patient against re-infection in future.

Dr. Bruce Rathgeber, at the Atlantic Poultry Research Centre on the Dalhousie Agricultural Campus reported that the immune system of the chicken will be exploited to produce antibodies against the COVID-19 virus and that antibodies produce in egg of chicken will lead to a good diagnostic test against this disease.

PET ANIMAL INFECTION

Although SARS-CoV-2 is believed to have originated in bats and possibly passed through at least one intermediate host before infecting the humans, a few reports of domestic animals being infected in this pandemic have surfaced. A report from the Australian Animal Health Laboratory suggests that ferrets are susceptible to infection with SARS-CoV-2 and the virus does replicate within this species under experimental conditions. Currently there is no confirmation that domestic animals, including pets such as cats and dogs, could be infected with SARS-CoV-2 under field conditions.

The Chinese Animal Health Laboratory conducted testing in more than 4800 samples of animals such as pigs, poultry, dogs and cats; all were all negative. More research studies involving a larger number of cats free from other covert disease conditions need to be conducted to arrive at certain conclusions. Zhang and co-researchers from China have reported that SARS-CoV-2 also infected the cat population in Wuhan during the COVID-19 outbreak. They tested cat sera collected before and after the

COVID-19 outbreak and found 14.7 % sera were positive RBD of SARS-CoV-2 ELISA. Among the positive cat sera, 11/15 serum samples had various titers of SARS-CoV-2 neutralizing antibodies.

IDEXX Reference Laboratory in USA tested samples of more than 3500 dogs and cats for presence of SARS-CoV-2 viral RNA as a part of validation process for a real-time RT-PCR; nearly all of those samples tested negative. IDEXX has recently launched a test under the commercial name "SARS-CoV-2 (COVID-19) Real PCR Test" to test samples from pets especially cats and ferrets. Antec Laboratory in USA has also been performing surveillance testing on respiratory and gastrointestinal PCR samples of dogs and cats to detect emergence of transmission of SARS-CoV-2 from humans to companion animals.

On April 05, 2020, a four-year old Malayan tigress at the Bronx Zoo in New York tested positive for SARS-CoV-2 after she developed dry cough. Five other tigers and lions showed mild signs of respiratory illness. A zoo employee, who was an asymptomatic carrier of SARS-CoV-2 was the caretaker of these animals, and probably he transmitted the virus to those zoo animals. As per the latest information, all the infected large cats had recovered well from the illness. A few days ago, two cases of domestic cat tested positive for SARS-CoV-2 in the USA; one cat contracted the infection from the owner who tested positive for COVID-19; the other cat presumably became infected after coming in contact with a covertly infected human.

ONE-WORLD ONE-HEALTH CONCEPT

Over the last two decades or so, a noteworthy upsurge in newly emerging and certain re-emerging pathogens was observed. About 60% of infectious diseases in humans are zoonotic and about 70% of emerging infectious diseases in humans are of zoonotic origin. Nearly all the infectious disease pandemics have been caused by organism(s) of animal origin(s). Thus, a better knowledge of causes and consequences of certain human activities such as urbanization, rapid growth and expansion of new geographic areas, lifestyle change, eating habits, increased human national and international travel and increased movements of animals and animal products across the globe for international trade resulting in rapid spread of infectious diseases, climate change, intensive farming systems, deforestation and land use change, and other behaviors affecting the animal health and ecosystems is imperative for a thorough understanding of disease dynamics and to drive public policies. Humans should learn to respect the natural world and live with animals and ecosystem on earth in cooperation and collaboration. Global health security cannot be now understood without integrating human, animal, plant and ecosystem health, and biodiversity. The success of OH approach depends upon crossing interdisciplinary barriers with stronger communication, coordination and collaboration among veterinary and human medical professionals and researchers as well as other relevant partners at all the important levels.

The World Organization for Animal Health (OIE), WHO, Food and Agriculture Organization of the United nations (FAO), United States Department of Agriculture, CDC and several other global and local organizations and institutes have been advocating, promoting and working on several issues under One Health approach. We suggest that veterinarians, medical doctors and human and animal health specialists should be involved in inter-disciplinary collaboration to fight against not only COVID-19 pandemic but also, newly emerging pathogens which threaten human and animal health and food safety and security constantly. Veterinarians, veterinary microbiologists and epidemiologists as well as animal biologists might help to predict the emergence and potential source(s) of future outbreaks of infectious diseases. Surveillance of wild and domestic animal population is the key to prevent SARS-CoV-2 from establishing itself in another animal species, especially companion and food animals. Remember, SARS-CoV-2 may not be the last coronavirus to jump species barrier and infect humans and other animal species.

CONCLUSIONS

This review has been written on emerged pandemic known as COVID-19. This new infection has been observed to have a very high infection rate and resulted as an international health emergency. The emerged infection has severely affected the human health and changed the norms of society and resulted in a worldwide economic crisis. The disease has spread promptly over the world and severely affected the human population being lack of its herd immunity. Although the coronaviruses has been known since long but this mutated form has resulted in devastated losses. Though the infection in varying animal species has been reported but since now there is no evidence of transmission of this virus from animal to human has been reported. In the absence of effective vaccine various supportive therapies have been reported to help/cope the infection. Minimizing the transmission from infected individuals has known to be the only preventive measures. Scientists had made great efforts to produce the effective vaccine. Some of these have gone through trials and are available to provide immunity. However, it is need of the time to meet the demand thus priorities are being set for vaccinating the population. Moreover, these are also some limitations to store, transport and delivery of the vaccine particularly in developing and underdeveloped countries.

Statement of conflict of interest

The authors have declared no conflict of interest.

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