REVIEW ARTICLE

INSIGHT OF PANDEMIC COVID-19: DEVELOPMENTS AND CHALLENGES

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ABSTRACT: This review focuses on the characteristics of coronavirus disease-19 (COVID-19) including virus structure, ecoepidemiology and pathophysiology, signs and symptoms in infected people, and data on virus pathogenicity, severity, and survivability in COVID-19 infected patients. The emphasis is on immunological reactions, diagnosis, prophylactic methods, and the zoonotic significance of COVID-19. The authors feel that the review's contents will be valuable to epidemiologists, virologists, public health officials, diagnosticians, laboratory workers, environmentalists, and socioeconomic experts. It has information on the many types of coronavirus variants, the disease situation in Pakistan and the WHO criteria for COVID-19 prevention is given. Moreover, lessons learned from the COVID-19 pandemic are also outlined.

Keywords: COVID-19, Epidemiology, Zoonosis, Characteristics, Preventive Measures.

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INTRODUCTION

COVID-19 a disease caused by a virus named SARS-CoV2 (Severe Acute Respiratory Syndrome Corona Virus-2), was initially diagnosed in December 2019 in Wuhan, Hubei Province of China. COVID-19 was declared a global pandemic by the WHO on 11th th March 2020. Till 26th April 2023, this contagious disease has infected 237 countries around the globe. As of 26th April 2023, a total of **764,474,387** confirmed cases and **6,915,286** deaths due to COVID-19 had been reported around the globe. However, over 676 million people had recovered by April 2023. In addition, by 26th April 2023, a total of **13.3426** billion COVID-19 vaccines dose had been administered in various countries (WHO, 2022). The highest rate of mortality has been reported in countries including the USA, Brazil, India, Mexico, the UK, Russia, France, Iran, Argentina, Colombia, Italy, Indonesia, and the Indian Sub-continent countries. The causative agent of COVID-19 disease belongs to the family Coronaviridae, and members of this family can infect animals, birds, and humans (Abbas *et al.,* 2019; 2021a; Ahmad *et al.,* 2020; Abbas *et al.,* 2021b). Such viruses in the past have caused outbreaks of MERS and SARS-CoV-2 in humans (Sohrabi *et al.,* 2020). The primary mode of transmission of COVID-19 is respiratory droplets from an infected person to a susceptible person (Hayat *et al.,* 2020).

Pakistan is a low and middle-income country, with a population of around 230,393,031 individuals (Worldmeter, 2022). In Pakistan, the first two confirmed cases of COVID-19 were reported on 26 February 2020 and presently new cases are being constantly reported ever since (GOP, 2021). The government of Pakistan strategically handled this emergent situation by implementing measures like the partial lockdown, imposing travel restrictions, suspending intercity city transport, and advocating for adopting practices like social distancing, wearing face masks, hand sanitization, and restrictions on the mass gathering (Abbas *et al.,* 2019, 2021a; Iqbal and Abbas, 2020; Imran *et al.,* 2021) However, despite implementing all above measures, new cases are being reported at quite a high rate; thus posing a significant challenge to control infection (Hayat *et al.*, 2020). COVID-19 has been isolated from different animal species including birds, mammals such as bats, camels, mice, cats, dogs, masked palm civets, and livestock (Hassan *et al.*, 2020; Vetter *et al.*, 2020). Viral RNA are reported in the nasal secretions kittens that were inoculated with SARS-CoV-2 for 8–9 days. (Shi *et al.*, 2020). According to WOAH (2020) cats acquired infection from being cohoused with cats that were experimentally infected and shed virus in respiratory tract secretions longer (7 days) than directly inoculated cats (5 days).COVID-19 produces infection of the upper respiratory and GI (gastrointestinal tract) of mammals and birds (Hu *et al.*, 2021a; 2021b). The incubation period of SRS-CoV-2 varies from 2 to 14 days (Ali and Alharbi, 2020).

SARS-CoV-2 is a positive-sense single-stranded RNA. The viral RNA genome size ranged from 26-32 Kb in the length (Lu *et al.*, 2020). The formation of crown-shaped projections on the virus envelope gave rise to the term coronavirus. SARS-CoV-2 has four structural proteins named spike (S) protein, nucleocapsid (N) protein, envelope (E) protein, and membrane (M) protein. The structural Spike (S) protein is the largest multifunctional transmembrane protein that plays a significant role in the virus attachment and entry into the host cell (Wrapp *et al.*, 2020). The mechanism of COVID-19 entry depends upon the cellular proteases. Transmembrane protease serine 2, cathepsins, and human airway trypsin-like proteases are important enzymes capable of cleaving the spike protein to initiate membrane fusion. SARS-CoV and HCV-NL63 need angiotensin-converting enzyme type 2 as the main receptors while MERS-virus hires dipeptidyl-peptidase 4 as a crucial receptor (Luan *et al.,* 2020). As per the most recent report cat has been known to harbor a virus, indicating the importance of zoonosis (Sila *et al.,* 2022)

Since multiple variants of COVID-19 are constantly evolving and circulating globally i.e., [Alpha (B.1.1.7), Beta (B.1.351), Delta (B.1.617.2); Kappa (B.1.617.1), Epsilon (B.1.427, B.1.429), Gamma (P.1); B.1.1.207, A.23.1, COH.20G/501Y, Iota (B.1.526), Theta (P.3), Lambda (C.37), Mu (B.1.621), Omicron (B.1.1.529), Zeta (P.2)]. The purpose of this review article is to assess the current status of COVID-19 in various countries including Pakistan, considering its modes of transmission, epidemiology, diagnostics, detection capabilities, and challenges to control its spread.

Covid-19 Epidemiology: On December 31, 2019, the World Health Organization (WHO) was notified of a cluster of unidentified pneumonia cases in Wuhan City, Hubei Province, China. In the first cluster of samples, a new coronavirus was discovered and this novel virus then spread from China to twenty other countries by the end of 2019, with incidents reported on all continents except Antarctica (Muneer *et al.*, 2021; Zahid and Perna, 2021).

On January 30, 2020, the WHO Director-General declared the outbreak to be a Public Health Emergency of International Concern (PHEIC), the highest level of concern recognized by the International Health Regulations. The International Committee on Taxonomy of Viruses (ICTV) and the World Health Organization (WHO) named this disease COVID-19 and the virus SARS-CoV-2 on February 12, 2020. COVID-19 was declared a pandemic by the World Health Organization on March 11 (Zahid, 2021). On February 25, 2021, over 112 million cases had been identified worldwide, with over 2.5 million deaths (Bulut and Kato, 2020). The most recent COVID-19 infection is a mixed infection caused by both the old Wuhan strain and new emerging strains. Because the virus's genome is hyper-mutable, multiple variants are evolving. The death rate from COVID-19 infection has decreased over the last month, but the peak rate of infection has recently increased. The reason for the increase in cases is that people are not following standard operating procedures correctly, and many people have not yet been immunized against the infection. Another factor contributing to the rise is a lack of cross-protection. Even those who have been immunized are vulnerable to re-infection (Ji *et al.*, 2020).

Transmission: Early outbreak investigations in Wuhan revealed an initial link to a seafood market selling live animals, where the majority of patients worked or visited and was later closed for disinfection (Hindson and Hepatology, 2020; Li *et al.*, 2020a). Person-to-person interaction eventually took over as the primary mode of transmission (Morawska *et al.*, 2020).

SARS-CoV-2 can be transferred directly from person to person through the respiratory route. According to evidence, transmission occurs primarily through close contact, i.e., within six feet or two meters, such as through inhalation of respiratory droplets (Tindale *et al.*, 2020; Qu *et al.*, 2020). Aside from this, the virus can be transmitted through contaminated surfaces. Nonetheless, adequate and proper ventilation is a strong recommendation for reducing airborne transmission. Some SARS-CoV-2 outbreak reports highlight the possibility of airborne transmission beyond two meters in poorly ventilated indoor environments (Jarvis *et al.*, 2020). The SARS-CoV-2 virus has been found in feces, blood, eye fluids, and sperm, but the role of these sites in the transmission is unknown. SARS-CoV-2 transfer via blood transfusion has not been reported. Furthermore, there is insufficient evidence to support blood transfusions via embroidered skin (Hindson and Hepatology, 2020). The risk of transmission from a person infected with SARS-CoV-2 varies depending on the type, closeness, duration of contact, and use of preventive measures. In indoor settings, it appears to be highest with prolonged contact (Wu *et al.,* 2020a). The majority of secondary infections have been reported among household contacts, in health care settings, but in other indoor settings where people live or work in close quarters, such as cruise ships, homeless shelters, detention camps, college dorms, and food processing areas. In addition, restaurants and playgrounds have been linked to an increased risk of infection transmission (Gao *et al.,* 2020). The transmission of SARS-CoV-2 from asymptomatic patients has been well-documented (Pollock and Lancaster, 2020; Park *et al.,* 2020).

The SARS-CoV-2 infection is thought to have been transmitted to humans via an animal host, however, the risk of transmission through animal interaction is uncertain. There is no evidence that animals, particularly domesticated ones, are a major source of contamination in people (Ahmad *et al.,* 2020). SARS-CoV-2 appears to be highly contagious in mink, with outbreaks from mink farms documented in Europe and the United States (Fenollar *et al.,* 2021). Considering the ambiguity surrounding the risk of transmission and the probable sensitivity of some animals to SARS-CoV-2 infection, pets should be kept away from other animals and sick people. There have been no cases of domesticated animals transmitting SARS-CoV-2 infection to humans, except for mink (Tiwari *et al.,* 2020).

Overall, the short-term risk of reinfection within the first few months after the initial infection is reportedly low. Nevertheless, sporadic cases of reinfection have been documented. Reinfection with the multiple variants of Concern (VOC) 202012/01 of the B.1.1.7 lineage has also been documented following infection with a wild-type virus (Jabbari *et al.,* 2020). However, having a positive SARS-CoV-2 viral test after recovery does not necessarily indicate reinfection; sequencing that demonstrates a different strain at the time of presumptive reinfection is necessary to make the distinction (Alinaghi *et al.,* 2020).

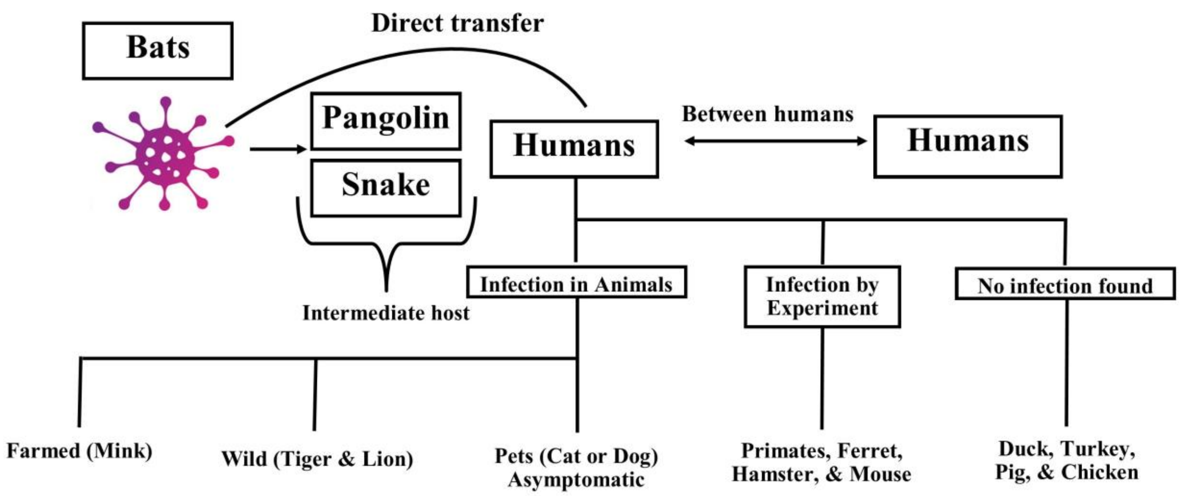

Figure 2: Covid-2 transmission

Immune Interaction With Covid-19: An interaction between the SARS-CoV-2 virus and the individual immune system leads to clinical signs of COVID-19 disease. A well-understood mechanism between the biology of SARS-CoV-2 and the human immune response is crucial for the development of a vaccine against COVID-19 (Kruglikov *et al.,* 2020). These viruses may manipulate the immune system and disrupt the interferon (IFN) pathway and both the humoral and cellular innate immune systems significantly contribute to the elimination of the virus (Ansariniya *et al.,* 2021).

Innate immunity is a conserved evolutionary series of chemical and cellular defense mechanisms essential for the detection and restriction of infectious agents. Macrophages and dendritic cells may stimulate the initial immune response against SARS-CoV-2, resulting in lymphocytosis and cytokine release (Raony *et al.,* 2020; Phan, 2020). Cytokines stimulate interferon-stimulating genes (ISG), a key component of natural antiviral defense that restricts viral entry and replication when the virus enters host cells. SARS-CoV-2 has developed several pathways to escape this essential antiviral response. It has recently been confirmed that angiotensin-converting type 2 enzyme (ACE2) is similar to interferon-stimulating genes (ISGs) inferring that SARS-CoV-2 might be using ACE2 IFN-driven up-regulation to boost infection. However, the exact mechanism is still unknown (Chua *et al.,* 2020; Ren et al., 2021). Recently, interferon-alpha (IFN-α) has been reported to strongly inhibit the replication of SARS-CoV-2 in vitro. Therefore, the Chinese National Health Commission has proposed guidelines for the use of IFN-α against COVID-19 infections. IFN-α combined with antiviral drugs such as ribavirin and lopinavir has beneficial effects in the treatment of patients with SARS CoV-2 infection (Abdolvahab *et al.*, 2021).

The level of pro-inflammatory cytokines such as Interleukins (IL-2, IL-7, IL-10), MIP-1a, granulocyte colony-stimulating factor (G-CSF), tumor necrosis factor (TNF), monocyte chemoattractant protein-1 (MCP-1), and IP-10 increase rapidly in COVID-19 infection (Li *et al.,* 2020b). These pro-inflammatory cytokines damage the neutrophils and myeloid cells in the lungs, resulting in hyper-inflammation and significant immunopathology (Tay *et al.,* 2020). Closely related immunopathology has been reported in SARS-CoV and MERS-CoV diseases. The severity of COVID-19 depends mainly on the activation of cytokine storms, particularly high levels of IL-6. The activation of IL-6 is a highly dynamic process that can govern the pathways of nuclear factor kappa (NF-κB) and stimulation of signal transducer and transcription 3 (STAT3) in epithelial cells and endothelial cells of the respiratory system (Manganotti *et al.,* 2021).

These findings suggest that acute respiratory disease syndrome (ARDS) induced by cytokine storms is the leading cause of death in COVID-19. It is worth noting that intravascular coagulation poses a risk to more than one organ, predominantly induced by inflammatory cytokines, IL-6. The COVID-19 patient showed several organ failures, as well as abnormalities in the lungs, brain, heart, kidney, and liver due to thrombin accumulation, demonstrated by a decrease in platelet count and an increase in D-dimer associated with poor prognosis. Similar observations have been shown in most SARS-CoV infected patients with renal failure. Another reason for multiple organ failure is that SARS-CoV-2 infection in endothelial cells may lead to cell damage, resulting in vascular leakage (Coomes and Haghbayan, 2020; Garg *et al.,* 2021; Singh *et al.,* 2020).

Cells that play an important role in establishing innate antiviral immunity to SARS-CoV-2 infections are natural killer (NK) cells. They initiate the process of internal and external apoptosis in different ways by releasing two important proteins, such as granzymes and perforin. These cells present FAS ligand and Tumor necrosis factor to virus-infected cells and secrete cytokines, such as GM-CSF, IL-6, IL-7, IFN-γ, and TNF-α. It has been stated that the number of NK cells can decrease during infection with SARS-CoV-2. (Udomsinprasert *et al.,* 2020; Alrubayyi, 2020).

It has been noted that the complement system is activated by past viruses such as SARS-CoV and MERS-CoV. Recent reports of complement activation were described in COVID-19 patients (Maucourant *et al.,* 2020). Epithelial and endothelial lung cells are key sites of infection with SARS-CoV-2 and complement activation. The SARS-CoV-2 initiates an alternative complement pathway by spike proteins (Gattinoni *et al.,* 2020). Serum concentrations of C5a and C5b-9 vary with a mild, moderate, and severe form of the disease, which may be considered to be sensitive indicators of the severity and activity of COVID-19 (Java *et al.,* 2020; Quinti *et al.,* 2020).

The binding of the spike protein of SARS-CoV-2 with different host cellular receptors such as angiotensin-converting enzyme-2, neuropilin-1, CD147, and TMPRSS2 is a prerequisite for entrance into the host cells. Spike protein harbors two subunits S1 and S2. S1 contains RBD (receptor binding domain) and S2 mediates membrane fusion for viral entry. The main focus of innovative immunization techniques is the production of immunity prohibiting SARS-CoV-2 from entering the cells by blocking S1-ACE2-RBD or S2-mediated membrane fusion. Antibody-dependent enhancement (ADE) mechanism is a major obstacle to the development of a COVID-19 vaccine (Tetro, 2020; Earle *et al.,* 2021; Gilbert, 2020). The antibody-antigen immune complex accumulates in different respiratory tissues and activates complementary pathways. The cytokines produced as a result cause hyper-inflammation and blockage of the airways. A similar type of observation has been reported in COVID-19 clinical cases. Hyper-activation of the complement system has been reported in COVID-19 patients contributing to lung injury (Mazzoni *et al.,* 2020).

The humoral immune response plays an important role in the resistance to SARC-CoV-2 infection, which is usually driven by increased serum antibody response in human B-cells (Quinti *et al.,* 2020). IgM and IgG are produced by SARS-CoV-2 spike and nucleocapsid proteins as primary and secondary immune responses respectively. IgM reached a peak within 4 weeks and was no longer observable after 3 months of illness. However, IgG peak was observed around the 14^{th} day and detected up to 36 months after post symptoms onset (Traggiai *et al.,* 2004). A recent study confirmed the presence of IgM and IgG in COVID-19 patients after 3 weeks of the onset of symptoms, implying the stimulation of the humoral immune response to the viral infection (Andreano *et al.,* 2021)

Poljak *et al.* (2021) reported an early antibody response to Nucleoprotein in patients infected with SARS-CoV-2. IgM and IgA have been measured in the plasma samples of the patients. IgA was detected due to the production of pro-inflammatory cytokines. At this stage, the full characteristics of the SARS-CoV-2 humoral immune response and systematic population studies are lacking to target it for vaccine production (Iba et al., 2020; Lorenzo-Redondo et al., 2020). Most of the people who recovered from infection with SARS-CoV-2 developed T-cell and B-cell memory. COVID-19-infected patients have memory B cells (MBCs) in their blood for up to six months (Quast and Tarlinton, 2021; Jarjour *et al.,* 2021).

SARS-CoV-2 infections may cause serious complications during pregnancy and early childhood. Innate immunity and maternal antibodies have a significant role in the protection of infants from infection (Moore *et al.,* 2020). A recent study found that SARS-CoV-2 maternal antibodies (IgM and IgG) were transferred to the fetus by the placenta in 88% of pregnant women infected with COVID-19. This maternal antibody transmission depends primarily on the type and amount of antibodies during pregnancy (Zeng *et al.,* 2020).

Numerous SARS CoV-2 variants, such as B.1.1.7, B.1.351, and PI, were identified in the United Kingdom, South Africa, and Brazil at the end of 2020. The target mutation site is the SARS-CoV-2 spike protein, in particular, the receptor-binding domain (Duong, 2021). SARS-CoV-2 reinfection was documented in Brazil due to the SARS-CoV-2 variant comprising the E484K mutation. South African and Brazil-based SARS-CoV-2 variant i.e., B.1.351 and P.1 has multiple mutations in the S protein such as N501Y, E484K, and K417N (Choudhary *et al.,* 2021; Tegally *et al.,* 2020).

SARS-CoV-2 is capable of mutating and evading the immune response, impacting the effectiveness of current vaccines and antibody therapies. The receptor-binding motif is a highly variable spike protein region responsible for the entry of SARS-CoV-2 into the host cell via ACE-2 receptors. Genetic variation in this region may be important for the mitigation of SARS-CoV-2 variants through an immune response. However, these mutations will keep on increasing with a risk of re-infection (Starr *et al.,* 2021). D614G SARS-CoV-2 variants comprise glycine amino acid at position 614 instead of aspartic acid. Various vaccines including BNT162b2, Moderna mRNA-1273, Oxford-Astra Zeneca ChAdOx1, and CoV-19 have recently been given licenses. The safety coverage of these vaccines varies from 60-90%. These vaccines were developed against the type-1 Wuhan genotype identified in 2019. Questions were raised as to whether the vaccine would be effective against newer versions of SARS Covey 2, such as B.1.1.7, B.1.351, and P.1 that originated in the United Kingdom, South Africa, and Brazil and have now spread to more than fifty countries (Nawaz *et al.,* 2021). A recent study was conducted to determine the immunogenicity of the BNT162b2 vaccine manufactured by Pfizer-BioNTech against pseudoviruses expressing the wild-type Spike protein or the 8 mutations found in the B.1.1.7 Spike protein. Neutralizing antibody response was measured by administrating a single shot of the vaccine. The BNT162b2 vaccine produced a variety of neutralizing antibody titers such as <3449 against pseudoviruses, which declined by 3.85 times against the UK-based B.1.1.7 variant. This decrease was also observed in those patients who recovered from COVID-19. Monoclonal antibody neutralization was decreased when targeting the NTD and RBM of spike protein in the B.1.1.7 SARS-CoV-2 variant (Lurie *et al.,* 2020; Jones and Roy, 2021; Jones *et al.,* 2020).

Mechanism of genetic variation in coronaviruses: The average substitution rate for coronaviruses is estimated to be 10^{-4} substitutions per site each year. However, the mutation rate of SARS CoV-2 is controlled due to the presence of exonuclease, a non-structural protein 14 (nsp14) in the RdRp complex (Chen *et al.,* 2020; Debnath *et al.,* 2020; Feng *et al.,* 2020; Portelli *et al.,* 2020). Despite this, coronaviruses have a large RNA genome which makes them prone to recombination and point mutations. Genetic variations may occur through antigenic drifts also. The process of genetic variations and recombination in SARS-CoV-2 is still unclear (Chakravarty, 2020). The common recombination breakpoints for SARS coronaviruses are in the accessory-protein encoding orf8 gene and a gene encoding receptor-binding domain (RBD) in Spike proteins. It is still unclear whether this recombination occurs in an intermediate host between bats and humans, and if it is so, in which animal (Kaser, 2020).

Covid-19 Diagnostics

Serological testing of COVID-19: Serological testing is a powerful tool in epidemiologic studies to understand viral circulation and evaluate the effectiveness of virus control measures, as in the case of SARS-CoV-2, the pathogenic agent of COVID-19. Determination of antiviral antibody titers can provide information on viral exposure, and changes in IgG levels also indicate a reduction in viral circulation. Serologic assays detect anti-SARS-CoV-2 antibodies, measurable approximately two or more weeks after the onset of illness (Guo et al., 2021). Commercially available tests required Food and Drug Authority (FDA) Emergency Use Authorization (EUA) and should have a sensitivity and specificity of more than 99.5% to be qualified as a diagnostic test. These assays target two antigens i.e., structural nucleoprotein (NP) and spike (S) protein. Commercially available antibody testing varies in the detection techniques, the type of specimen required (serum, plasma, venous whole blood; Wang *et al*., 2021), characterization of antibodies and their targets i.e., NP, RBD, or S1 subunit of the trimeric spike protein. Lateral flow immunoassay (LFA), Enzyme-Linked Immunosorbent Assay (ELISA), Plaque reduction neutralization test (PRNT) and chemiluminescent immunoassay (CLIA) designs differ in performance and accuracy of the test results and fluctuate considerably in mild, severe, and asymptomatic patients of SARS-CoV-2 (Ghaffari *et al*., 2020; GeurtsvanKessel *et al.,* 2020).

Molecular detection of COVID-19: Nucleic acid testing (NAT) is the most widely accepted epidemiological tool for the early detection of infectious pathogens since it directly targets nucleic material. There are three categories of testing for COVID-19. The first one is to see if there is the actual genetic material of the COVID-19 virus, and this is called the NAAT test, N-A-A-T. It's PCR testing where you would have a nasopharyngeal swab or a pharyngeal swab. And then they look for the genetic material of the virus itself. Before detection through RT-PCR, no method is available to assess Covid-19 infection during incubation and after the onset of symptoms (Tahamtan, and Ardebili, 2020; Niu et al., 2020). The second type of testing is when they try to identify one of the outer proteins of the viral envelope (Bianchi *et al.,* 2020), and this is called antigen testing. So, it tries to detect the outer protein of the virus. And the third type is to find out in the human body if they have developed antibodies. So it looks for antibodies that are specific to the outer part of the virus itself. So it shows whether an individual has mounted an immune response or developed immunity to that specific virus or COVID (Afzal, 2020).

Soon after the first SARS-CoV-2 sequence was released on 10th Jan 2020 multiple manufacturing companies and research groups developed the primer/probes for real-time RT-PCR-based diagnostics (WHO COVID-19, FDA). To increase the sensitivity and reliability of the molecular testing duplex and multiplex real-time RT-PCR techniques are also approved by FDA. Multiplex kits use different fluorophores to target various viral genes (N, S, E, ORF1ab, RdRP) and a human gene as an internal control to rule out sampling bias (Tang *et al*., 2020). COVID-19/SARS-CoV-2 S1 serology ELISA kit was developed and validated COVID-19 with negative samples (Bond *et al.,* 2020; Rashid *et al.,* 2020). The specificity of the ELISA kit was 97.5% (Afzal 2020; Qiu *et al.* 2020).

Other alternative and evolving techniques include Loop-mediated isothermal LAMP assay (Mori *et al*., 2020), and Cluster regularly interspaced short palindromic repeats (CRISPR) (Pan *et al*., 2020), molecularly imprinted polymer (MIP)-based detection and microarray. LAMP and CRISPR assay approved by FDA promises 100% sensitivity, one hour turn-around time, and approx. 4-6 copies/µl LoD (Zhang *et al*., 2020).

Clinical diagnosis of SARS-CoV-2 is dependent on the timely collection of specimens from suspected cases. The Upper and lower respiratory tract remains the primary focus for detecting the virus depending on the ease of collection and severity of the symptom. A mean incubation period of 5.2 days for SARS-Cov-2 has been reported to cause the onset of symptoms and a mean of 12.5 days for hospitalization from the day of infection (Baum *et al* 2020). The viral load of each type of specimen is variable. Bronchial alveolar lavage specimens have 93% positivity rates as compared to sputum (69%), nasal swabs (62%), and pharyngeal swabs (32%). In a similar study, early detection of SARS-CoV-2 was suggested from deep-throat saliva specimens (Binnicker 2020) with a positivity rate of 92%. Irrespective of the type of specimen the first 5 days after the onset of symptoms is critical for molecular testing sensitivity. A recent study suggested retesting initially negative patients on clinical suspicion within 7 days increased positivity by 3.5% (Hong *et al*., 2020; Lin *et al*., 2020).

Infection control strategies: Many studies and clinical trials have been conducted to demonstrate the effect of different antiviral drugs against infection. Unfortunately, no antiviral drug has been approved by FDA for the treatment of COVID-19 infection (Aiewsakun *et al.,* 2020). So, the current strategy being employed worldwide is preventive measures to prevent the spread of this infection. To limit the spread of this infection early screening of individuals, diagnosis and treatment are necessary.

Various strategies have been suggested by WHO to be undertaken by the population to reduce its transmission. One of the most important strategies is to wash hands properly and use portable hand sanitizers, to minimize contact and possible interaction with contaminated surfaces and objects (Korber *et al.,* 2020). Overcrowded places, close contact with people, and respiratory hygiene should also be practiced reducing the transmission risk in the community. Different health organizations including the WHO have developed multiple posters, videos, and brochures to make people aware of different issues related to COVID-19. An exponential increase in the number of cases has been observed in countries where these preventive strategies have not been implemented strictly. Different measures for social distancing and quarantine of the suspects have been developed in many countries to prevent the spread of this virus (Ozono *et al.,* 2020).

WHO has recommended the use of face masks for people with respiratory symptoms (Bhattacharya *et al.,* 2020). Social distancing can be practiced by promoting work from home, closure work and educational places, prohibiting mass gatherings and meetings, etc., (Dumonteil *et al.,* 2021). A mathematical model study conducted in Italy demonstrated that this global pandemic cannot be controlled without strict quarantine rules (Rambaut *et al.,* 2020). The recommended time for the quarantine of a person who has contracted a positive patient or contaminated environment is 14 days (Guzik *et al.,* 2020). Disinfection of high-touch areas i.e., tables, door handles, etc., with household bleach should be practiced daily for preventing the spread of the virus. 70% ethanol can also be used as an alternative to disinfection. Bathrooms and toilets should be cleaned and disinfected with 0.5% sodium hypochlorite solution (Liu *et al.,* 2021).

The development of new test strategies and increasing the laboratory capacity is also effective way to reduce the spread of the disease throughout society. Different rapid test kits**,** self-collected specimen testing, and serological testing should be developed with good sensitivity and specificity (Mahase, 2021). All the preventive measures and quarantine rules should be published by all the public and private health agencies for effective enforcement (Wu *et al.,* 2020b).

The Centers for Disease Control has issued safety advisories that may help prevent infection in the public. Specifically, guidelines are to avoid close contact with infected people, stay at home if someone has symptoms, disinfect household items frequently, and wash hands frequently. Initially, masks were only recommended for people with symptoms of COVID-19, medical staff, and people in close contact with sick persons, but now it is noted that wearing a mask in public places can effectively minimize the chance of transmission of COVID-19. Also, World Health Organization has issued advice to wear face masks, eat properly cooked food, wash hands & covering your face when you sneeze or cough (Güner et al., 2020). SARS-CoV-2 causes disease in people of all ages. However, mortality is higher in older and immune-compromised people. In 2020, Daoust's found that older adults are more vulnerable and disproportionately affected by COVID-19. Elderly people are advised to stay home to minimize transmission of infection. Furthermore, it has been reported that government efforts to overcome the pandemic and reduce COVID19-related mortality should be directed efficiently and strategically to save the elderly (Güner et al., 2020).

Airport advisories are strictly following and implementing specific travel measures to prevent the spread of novel coronaviruses between borders. For this screening, stations have been established in many airports across the world to identify symptomatic travelers (Elachola *et al.,* 2020). Many airlines have made it compulsory to go for COVID-19 PCR testing before they departed from the country. Some countries i.e., Japan have established quarantine control checks for all the entry points of the country and all airports (Kim *et al.,* 2021). Almost all airline companies have published specific travel guidelines specifically for the prevention of COVID-19 spread from and to different countries especially China. Lockdown operations and movement control has been strictly enforced in many countries and a significant decrease in incidence has been observed (De Miguel *et al.,* 2021). The best way to avoid exposure with carriers is to stay as much at home at possible, restrict the movement and avoid the physical contact with other people.

A very novel approach to prevent infection and confer immunity that has recently been identified in patients is T-cell mediated protection (Bachanova *et al.,* 2020). High level of specifically sensitized T-cells (CD8+) has been identified in patients. Many animal models supported this finding where T-cells have been proved to be protective especially in patients with weak humoral immune response (Ishay *et al.,* 2022). So sensitization of T-cells with a pre-exposure of antigen can be a novel approach to confer protection in individuals and ultimately can be helpful in prevention of infection.

Management and Treatment

Nutritive management: Like other coronaviruses, COVID-19 is sensitive to heat and killed at cooking temperature therefore avoid raw cooked foods. Minerals, vitamins, and antioxidants-rich food help in repairing the cells damaged by the virus. Consuming probiotics like yogurt, honey, fruits, fresh and/or cooked veggies, and herbs improves the innate and adaptive immune response. Stay well hydrated, it will increase the mucus coating on the mucus membrane, airways, and lungs. Vitamin c (tomatoes, citrus fruits, papaya, strawberries, watermelons) rich food is also advised as it boots up the immune system. Green tea contains *L-theanine* which is reported to increase the proportion of T regulatory lymphocytes to cytotoxic lymphocytes and is helpful to increase the immune response (Edara *et al.,* 2021). Plentiful regular use of ginger cinnamon, onion, and garlic in food or in the form of extract may also be helpful, however, it is just a suggestion and sufficient research data is needed to prove the beneficial effect of these herbs against coronavirus infection.

Herbal medicine: The Chinese government, which is the first country to be severely affected by the COVID-19 epidemic, attributes its rapid turnaround in managing COVID-19 to the integration of herbal medicine with conventional medicine. Clinical practice guidelines or memoranda on the use of herbal medicine for COVID-19 have also been issued in several countries such as South Korea, Japan, Malaysia and India. China and India use herbal medicine alongside modern medicine to boost patients' immunity as well as reduce the severity and prevention of COVID-19 (Ni *et al.* 2020, Shankar *et al.* 2020). In Ethiopia, the number of COVID-19 cases is increasing rapidly. Health facilities are more fragile and have poor infrastructure. They use plant material to treat these comorbid conditions in addition to supportive care. Elderly people living in rural areas have more knowledge about herbal plants to treat COVID-19 (Xu and Zhang 2020; Xu et al., 2020).

Since many herbal medicines show antiviral activity, the use of herbal medicine for therapeutic purposes should not be underestimated. Currently, well-known herbal medicines with antiviral activities are used as adjunctive treatment to suppress SARS-CoV-2, as conventional treatment is still not very successful. Previous studies have revealed that echinacea supplementation may be associated with decreased levels of the pro-inflammatory cytokines TNF, IL-6, IL-8 and increased anti-inflammatory cytokines IL-10. Interestingly, curcumin was found in in-silico studies to prevent SARS-CoV-2 from entering cells and viral replication, while a recent experimental finding showed that bromelain can also inhibit viral entry into cells. Further, potential specific antiviral agents such as the decoy mini protein CTC-445.2d, protease inhibitors, mainly against the core protein, nucleoside analogues such as molnupiravir, and compounds blocking proteins of the transcription replication complex such as plitidepsin and zotatifin are also being investigated. against COVID-19 (Kritis *et al.*, 2020, Luo *et al.*, 2020, Aucoin *et al.*, 2021; Zhou *et al.*, 2020). China to protect respiratory function a kind of prevention value is chosen because of the nature of viral condition by using *Yupingfeng San* (Xu and Zhang 2020).

It is an ancient Chinese herbal medicine that contains three types of plants: *Astragalus* can (improves lung function), *Fangfeng* (to relieve pathogen) and *Atractylodes* (improves spleen and digestion and absorption functions of our body). Studies have shown that *Yupingfeng* San could optimally regulate the body's immune functions. To clear lung heat, expel, relieve cough, regulate patient's lungs, and restore normal breathing function. Clinically in China, patients who had a high fever used *Yinqiao san* and patients who had a severe cough used Sangju yin. A study conducted on various herbal supplements has shown that ginger, lemon/orange, vitamin C, honey, black seed, costus have a significant effect on the treatment of COVID-19 (Liu *et al.*, 2015).

Chinese doctors have also used *4-methylumbelliferone* (4-MU) and its derivatives, which are commonly present in various traditional Chinese medicinal herbs, to treat pneumonia associated with COVID-19. If early in the course of treatment for COVID-19 the infection is not easily controlled, worsens with respiratory failure, multi-organ failure and death, then serious infection should be considered. These infectious patients have the following main symptoms: high fever, dry cough, difficulty breathing, sweating, chest tightness, fatigue, nausea, bloating, red or dark red tongue, yellow coating, and oiliness. Some of the preventive and supportive treatments for COVID-19 through immune-modulating *G. glabra, Thymus vulgaris, Allium sativum, Althea officinalis* and *ginseng* may be effective (Xu and Zhang 2020).

Vernonia amygdalina is mostly found in Asia and the countries of high tropical Africa. It belongs to the family (*Asteraceae*); an angiosperm species classified as the genus *Vernonia*, which contains about 1,000 species. It has demonstrated immune effects as an adjunct to vaccines. This plant was usually used to relieve headache, fever, diarrhea, and cough. Aqueous extracts of *G. amygdalina* have shown positive effects in inducing the human immune response by increasing the level of CD4+ and white blood cells. It also has antimicrobial, antidiabetic, antiallergic, antimalarial, antibacterial, anticancer, antifungal, antileukemic, analgesic, antipyretic, anthelmintic, hypolipidemic, hepatoprotective and antioxidant properties. Despite the described strong activity of *G. amygdalina*, which maintains immune and inflammatory reactions, the toxicity of these plants is found. Although no mortality was reported in an acute toxicity study conducted on animals (Grubben and Denton 2004, Momoh *et al.* 2012).

The main clinical symptom of COVID-19 is fever, and *Azadirachta indica* (neem) plants have valuable results in reducing it. Neem leaves are traditionally boiled and consumed in the treatment of fever associated with COVID-19, with anti-inflammatory effects reported in animal studies. Its leaves have positive immunoregulatory effects to enhance the immune response in animal models (Paterson, *et al.*, 2020, Borkotoky and Banerjee 2021).

Based on our review, *N. Sativa* (Black Cumin) seed was one of the herbal products with the most published positive evidence. Ethanol extracts of *N. sativa* seeds have shown antiviral properties by reducing viral load, alpha-fetoprotein and improving liver function parameters in hepatitis *C patients*. In an animal study, its seed oil exerts antiviral and immunomodulatory effects against cytomegalovirus and reduces viral load to an unpredictable value. It can boost the immune response by increasing CD3 and CD4 counts, as well as increasing the release of interferon-gamma (IFN-γ) from natural killer T-cells and macrophages (Salem *et al.*, 2011, Abdel-Moneim *et al.*, 2013, Salem *et al.*, 2017).

Peppermint (*M. Piperita)* is the oldest herbal remedy for various disease states in the world. Dry peppermint has been produced since 1000 BC and its importance has been described in ancient Egypt, Greece and traditional Chinese medicine. Peppermint essential oil has significant antibacterial and antifungal activity against gram-negative and gram-positive bacteria, yeasts and fungi, mainly due to the presence of abundant phytochemicals menthol and menthone (Nayak *et al.*, 2020).

Garlic (*Allium sativum*) and onion (*Allium cepa*) are commonly used in Ethiopia as a home remedy for various medical conditions. The onion, which has long been used in traditional medicine to treat various conditions and infections, was obtained to destroy the bird flu virus (H9N2). However, the method of preparation plays a crucial role, as boiled or fried onions are relatively ineffective. The researcher’s said onion is a good candidate for treating patients with COVID-19 due to its anti-inflammatory, anti-thrombotic and antiviral effects. it is mostly used for its immunomodulatory, antimicrobial, antioxidant, anti-inflammatory, anticarcinogenic, antihypertensive, antithrombotic, antidiabetic, antimutagenic and prebiotic activities. The active metabolites of garlic can be divided into two, such as sulfur-containing compounds and non-sulfur-containing compounds. allicin and alliin are the major sulfur-containing compounds, while the major non-sulfur active compounds include flavonoids and saponins. The ability of garlic to inhibit SARS-CoV-2 was perceived in silico by the formation of hydrogen bonds between amino acids with the binding site of the main structural protease of SARS-CoV-2 and its bioactive parts, which protease is responsible for the production of the virus (Ahmadi *et al.,* 2018; Dorsch and Ring 2021).

It also explains the combination of herbal therapy with FDA-approved drugs in recovered patients in China (Callaway and Ledford, 2021). It can also open research gateways to look for herbal and other plant-based medicine to manage the symptoms associated with COVID-19.

Use of plasma or serum Transfusion: The use of convalescent plasma might be helpful to combat the recent devastating situation. Plasma transfusions have also been somewhat successful in the past in fighting against the H1N1, SARS MERS epidemics, and other viral outbreaks (Siddique *et al.,* 2021).

Medicines/chemical therapy Currently, there is no proper remedy available for COVID-19. Yet, symptomatic, and oxygen therapy is being used to treat serious infections (Chhikara *et al.,* 2020). Mechanical ventilation is needed in cases of respiratory dysfunction and hemodynamic support is required for managing septic shock. The use of inappropriate antibiotics and corticosteroids is not recommended for the treatment of acute respiratory distress syndrome and/or viral pneumonia (CPAM; Khan *et al.,* 2020; 2021). Although the scientific basis is least, Lopinavir 400 mg; Ritonavir 100 mg (2 tablets by mouth BID) or Chloroquine (500 mg by mouth BID) and/or Hydroxychloroquine (400 mg by mouth OD) treatment is claimed to reduce viral load in patients of COVID-19 (Gautret *et al.,* 2020; Sahin *et al.,* 2020). Aspirin is an anti-inflammatory drug that also has anti-platelet aggregation and anti-lung injury effects. It inhibits the replication of the virus, therefore may have a promising effect to reduce the critical effect of COVID-19, especially in diabetic and cardiovascular patients. However, sound scientific reports about the use of these medicines are not present in viral infections. Similar effects of Sofosbuvir in combination with Ribavirin (Muik *et al.*, 2021), Arbidol, Remdesivir, and Aavipiravir against COVID-19 have also been reported to be promising (Buckland *et al.*, 2020; Wang *et al.*, 2021). Alpha-interferon @ 5 million units by aerosol inhalation BID and Lopinavir/ Ritonavir has also been suggested by research studies (Muik *et al.*, 2021). Wibmer *et al.* (2021) suggested that Remdesivir (GS5734) may be a promising and effective drug to treat COVID-19 infection. Remdesivir is an inhibitor of RNA polymerase against multiple RNA viruses including Ebola and has shown therapeutic and prophylaxis effects for HCoVs disease (Faria *et al.,* 2021). Wang *et al.* (2021) reported the effect of Remdesivir in a rhesus macaque model of MERS-CoV infection. However, the uses of these drugs are not advised as first-line treatments due to potential side effects (Guo *et al.,* 2020). Moreover, there is great dispute and controversy about the use and misuse of these anti-inflammatory drugs; therefore, standard research is needed to generate sufficient data to decide on the facts (Liu *et al.,* 2021).

Effects of sars-cov-2 variants on vaccines: Mutations in the Spike protein of SARS CoV-2 are a major concern for effective vaccine development. The first and most notable D614 to G614 mutation in spike protein, though did not increase disease severity, but resulted in an increased transmission rate (Caldwella *et al.*, 2021). As almost all of the COVID-19 vaccines are designed against the receptor-binding protein (RBD) of the spike protein of the original Wuhan-Hu-1 isolate, this part of the virus is under constant pressure to mutate. (Conti *et al.,* 2021).

The two most important variants, B117 and B1351, were identified in the UK and South Africa. In a study, it was observed that B117 and B1351 lineages differ in the interaction of RBD and ACE2. The B1351 lineage formed more flexible and new hydrogen bond networks between the RDB and the ACE2 resulting in greater flexibility and higher stability in the RDB loop segment with K484 residue. This may result in increased infectivity of the lineage (Moore, 2021).

Variants

B.1.1.7. Since its identification, multiple studies have reported little to no antibody-mediated protection of the variants of lineage B117 (Table 1). The location of N501Y has made it unlikely to stop the antibodies from neutralizing the virus. The BNT162b2 vaccine of Pfizer-BioNTech and mRNA-1273 vaccine of Moderna show neutralizing effects on N501Y. The US-based Novavax has released its interim results from a phase-III trial carried out in the UK. It included 15000 volunteers; out of which 62 cases of symptomatic COVID-19 were recorded (Lacobucci, 2021). The results show that the NVX-CoV2373 vaccine is 95.6% effective against the original variant of SARS-CoV-2 and provides 85.6% efficacy against the newer variants B.1.1.7. studied the neutralization activity of antibodies generated by mRNA-1273 against SARS CoV-2 variants. The group utilized the live-virus Focus Reduction Neutralization Test (FRNT) assay and compared the convalescent sera from infected and vaccinated people. Their results confirm that the vaccine- and infection-induced antibodies are effective against the UK variant. Some studies were conducted on one or two mutations found in the B117 variant, and the results showed no change in BNT162b2-immune sera neutralization on mutation-induced pseudoviruses and original strains. However, another study was conducted in which a full set of B117 spike protein mutations (H69/V70, Y144, N501Y, A570D, D614G, P681H, T716I, S982A, and D1118H) were introduced in pseudoviruses and their escape on neutralizing vaccine-induced antibodies was studied through 50% neutralization assay. No difference in neutralizing activity of the B117 spike protein pseudovirus and Wuhan strain pseudovirus was observed, proving that the vaccine is efficient against its seed strain and B117 variant. Another study reported resistance against monoclonal antibodies due to N-terminal domain mutations, and low resistance of RBD mutations to Moderna and Pfizer-BioNTech vaccine sera. (Stokel-Walker, 2021).

B.1.351. As discussed above, the B.1.1.7 variant is susceptible to most vaccines currently in use worldwide. However, the South African variant B.1.351 (Table 1) poses threat to vaccine research. This variant has N501Y mutation in RBD along with an important E484K, which is termed an "escape mutation" as the presence of E484K increases resistance to neutralizing antibodies. This lineage has nine spike protein mutations; including the D614G, a cluster of mutations including deletion 242-244 and substitution R246I, L18F, D80A, D215G in the N-terminal domain (NTD), and three RBD mutations K417N, E484K, and N501Y as well as one mutation A701V near the furin cleavage site (Williams *et al.*, 2021) The RBD mutations had 10.3-12.4 fold increased resistance against the BNT162b2 and mRNA-1273 vaccine sera as compared to the 9.4 fold increase of resistance against the convalescent sera. The NTD mutations are found to provide resistance to viruses against the Nabs. Laboratory results indicate that Novavax is 60% protective against B1351 which is considerably reduced activity from their originally reported 95.6 % efficacy against seed strain. Other studies have also reported a decreased susceptibility to convalescent plasma-induced antibodies, and BNT162b2 and mRNA-1273 vaccine-induced antibodies support a rationale for the development of modified vaccines against E484K (Yadav *et al.*, 2021).

P.1. The lineage P.1 was detected in the city of Manaus in Brazil in January of this year and spread to other parts of the world including Japan, the US, Spain, Germany, France, Italy, and the Netherlands (Table 1). This lineage has 17 mutations with three in the RBD of S protein; the two K417T and N501Y interacting with ACE2, and a third E484K present in the loop outside the ACE2 interface (Faria *et al.*, 2021). The vaccines give reduced neutralization against this lineage as well. The convalescent plasma and sera of vaccines LY-CoV555 (Bamlanivimab), REGN10987 (Imdevimab), and REGN10933 (Casirivimab) had shown a reduction in neutralization against P.1, but it was not as substantial as the reduction against B1351 lineage. This enhanced capability to escape the neutralization effect can be due to the E484K mutation present in the loop of RDB (Wang *et al.*, 2021). However, another study reported a 6-fold reduced neutralization against P1 than B1351 in vaccinated sera and convalescent plasma. Apart from reduced neutralization, the P1 lineage caused reinfection in patients in Brazil (Saadat, 2020).

B.1.617.2. Designated as delta variation in WHO naming (WHO, 2021), was isolated from India in September of 2020 and it has spread to more than 66 countries. It has 12 mutations in its Spike protein with the defining SNP of T478K and this lineage lacks the mutations at 501 and 484 at ACE2 RBD (Williams *et al.*, 2021). The BNT162b2 mRNA vaccine has retained neutralizing capability against B.1.1.7, P.1, B.1.351, and B.1.617.2 (Table 1) but with reduced ability. A study conducted in the UK compared the neutralizing antibody titers generated by the BNT162b2 vaccine against the wild type and its variants. The B.1.6.1.7.2 had produced a 5.8-fold reduced activity as compared to the neutralizing activity of the vaccine against the wild type. The P.1 and B.1.1.7.1 had 4.9 and 2.6 folds reductions as compared to the wild type, respectively (Campbell *et al.*, 2021).

Up till now all the studies on neutralizing the effects of antibodies on variants either use single mutations or a set of mutations to study the effect. However, it is still to be identified how the variants will work in real-life environments when the variants face T-cell responses too. Whether the SARS CoV-2 variants are resistant enough to elicit reduced immunity in humans, remains to be confirmed through the vaccination drives data. All the studies point toward the reduced capability of the current vaccine against the variants of SARS Cov-2, this directs us toward effective mass vaccination drives which will give less time for the virus to mutate and accumulate variations in its genome. The yearly boosters are applicable for use in the future and with the enhanced sequencing abilities we have seen during the COVID-1919 pandemic, the researchers will be able to track the variations occurring each year to manufacture boosters for the particular variations.

Challenges of covid-19 pandemic in pakistan The pandemic Coronavirus (COVID-19) which emerged from China Wuhan in December 2019 posed a devastating challenge to the global economic, social, and healthcare systems. The disease has spread promptly in higher proportions around the globe and we cannot estimate when this will be overcome (Mamun & Griffiths, 2020). As of 20th September 2022, 609,848,852 confirmed cases of COVID-19, including 6,507,002 deaths, have been reported to WHO. This pandemic has altered the dynamics of the world adversely affecting various sectors like economics, governance, health, markets, education, tourism, and national as well as international affairs.

Table 1: WHO proposed variant of concern.

Labeled by WHO	Region	Date	Lineage
Alpha	United Kingdom (UK)	09-2020	B.1.1.7
Beta	South Africa	05-2020	B.1.351
Gamma	Brazil	11-2020	P.1
Delta	India	10-2020	B.1.617.2

Pakistan being a developing country has limited resources and very weak health and economic infrastructure. To encounter the pandemic situation, Pakistan has strengthened its preparedness against the virus by formulating and implicating many new policies to combat national emergencies, i.e. ensuring thermal screening at entry points and contact and surveillance tracing by data collection and monitoring at surveillance units (Mughal, 2020). Moreover, diagnostic and testing capacities have also been enhanced by importing the technological detection assay using commercially available Polymerase Chain Reaction (PCR) kits for SARS-COV-2. To facilitate the suspected cases of COVID-19 resources have also been mobilized for setting up quarantine facilities at hospitals and isolation centers as per recommendations of WHO (Noreen *et al.,* 2020). However, Pakistan, with a unique challenge of highly porous borders, is sandwiched between two epicenters of Corona - China and Iran. The death toll associated with COVID-19 has drastically increased in Pakistan (Anser *et al.,* 2020). Due to this reason, there is a dire need of developing robust strategies, and reinforce public health facilities, laboratory networks for diagnostics, infrastructures, trained human capacities, and hostile disease surveillance systems (Noreen *et al.,* 2020).

COVID -19 PANDEMIC: Challenges to the Health Care System of Pakistan: The SARS-Cov2 pandemic has posed big challenges to the healthcare system globally. In most of the affected countries, one of the major concerns outsets of this pandemic was access and provision to PPE (personal protective equipment). Even despite having strong healthcare systems, the developed world is also facing a lot of issues as their systems were put under strain. The worldwide healthcare systems can work at maximum capacity for months, yet the need for medical staff, wards, and ventilators cannot be accomplished within a short time (Peckham, 2020). The alarming increase in COVID-19 cases resulted in a shortage of medical equipment such as PPE, masks, and ventilators for critical patients (Wood, 2020). This worldwide situation is also the same for Pakistan and our healthcare system is also overburdened there is a huge scarcity of medical facilities including PPEs, diagnostic/testing kits, and other necessary equipment.

Testing Facilities In Pakistan: Pakistan is a poor country with limited resources and weak infrastructure where a majority of the population is living based on their daily wedges. A PCR-based diagnostic test which has been declared a gold standard test worldwide is not affordable for a majority of people in Pakistan. Besides this, although the testing facilities have been improved significantly still facilities available to date are insufficient as compared to the large population. The first diagnostic testing facility was established at NIH (National Institute of Health) in Islamabad followed by Sindh and Punjab. Currently, there are a total of around 35 testing facilities across different cities in Pakistan (Islamabad-01, Punjab-06, Balochistan-10, KPK-07, Sindh-04, AJK-03, and Gilgit Baltistan-04). However, at the beginning of the pandemic testing started from 1000 cases/tests per day but now it has reached up to 11,000 tests/day and a total of 520 017 tests have been conducted yet (Habib and Abbas, 2021).

Quarantine Management: The quarantine centers are set up to facilitate the isolation, monitoring, and treatment of individuals who are infected or suspected to be infected with COVID-19. Such quarantine centers have been distributed in several cities in all four provinces of Pakistan. A total of 23,557 numbers of quarantine facilities have been set up across 139 districts to control the expected outcomes of COVID-19 (Rana *et al.,* 2020).

Lack of Awareness and Education: Despite several policies and efforts set by NCOC to reduce the transmission of Coronavirus in Pakistan, some key shortcomings resulted in the worsening of the pandemic situation. One of the major factors behind the current situation of COVID-19 is the lack of awareness and education particularly in rural and backward areas (Kamran & Ali, 2021). The government of Pakistan has taken many initiatives regarding making new policies and issuing directives on risk alleviation strategies to prevent the spread of social distancing by maintaining at least six feet distance between individuals, regular hand washing and sanitization, wearing masks, and avoiding public gatherings, regular hand washing, social distancing, and maintaining at least a distance of two meters or six feet (Noreen *et al.,* 2020). The majority of people do not abide by these regulations of Government which results in a massive increase in COVID-19 cases and conditions being complicated with each passing day (Javed *et al.,* 2020).

Covid-19 Vaccine Challenges:

Development of effective vaccines : The development and distribution of effective and safe vaccines against SARS-CoV-2 have been a major and global concern. Since COVID-19 was declared a pandemic, several clinical trials of vaccines have been authorized for emergency use in different countries. However, despite this scientific breakthrough in vaccination trials, the journey from discovering and developing effective vaccines to inducing global herd immunity in a big population against the COVID-19 virus still faces a lot of challenges that are not possible to address without a collaborative global response. These challenges mainly include maintaining and developing R&D incentives, extensive clinical trials, authorizations, post-market surveillance, manufacturing, and supply. Moreover, the development of many first-generation vaccines is a very costly process due to the use of ultra-cold chain technology which is one of the major hurdles in various global vaccination campaigns, particularly in middle- and low-income countries like Pakistan. So, besides the developmental aspects, some other challenges regarding ethical and financial concerns also need to be addressed (Forman *et al.,* 2021).

Vaccine development status: The vaccine development includes six basic steps that are Preclinical, Clinical trials-Phase 1, Clinical trials-Phase 2, Clinical trials-Phase 3, approval and manufacturing followed by post-marketing surveillance (Sharma, 2020). Some of these steps were combined to bridge the gap between the rapid manufacturing of unlicensed Covid-19 vaccines and fulfilling the need for efficient and safe vaccination facilities. The first FDA approval was issued for the Pfizer vaccine by BioNTech also known as BNT162B2. Currently, eight different types of vaccines have been approved by WHO for administration for COVID-19 prevention and treatment. These vaccines are designed based on different strategies i.e. mRNA based vaccines designed using selected sequences from the spike protein gene (Moderna and BioNTech Pfizer/BNT162b2; Jackson *et al.,* 2020), inactivated/killed virus vaccines (BIBP CorV/Sinopharm and Corona Vac/Sinovac Biotech; Xia *et al.,* 2021; Yang *et al.,* 2020) and non-replicating vector-based vaccines (AstraZeneca, Cansino Biologicas, Janssen/Johnson & Johnson). Besides these approved vaccines, there are some other vaccines like Sputnik V by Gamaleya Research institute and Covaxin and Novavax (NVXCoV237) by Bharat Biotech, which are being widely used but merely have national approval (WHO, 2021).

Since the outbreak of the pandemic in February 2020, UNICEF has worked closely with the Ministry of National Health Services, Regulations, and Coordination, Pakistan (MoNHSRC) to support its response to the COVID-19 emergency. UNICEF has supported the government's efforts to reduce the transmission of the virus by promoting adherence to public safety health measures and is supporting the vaccination campaign against COVID-19 in line with the National Deployment and Vaccination Plan (NDVP; Chandir, *et al.*, 2020; Pearson, *et al.*, 2021). Despite limitations in global vaccine availability, the Pakistani government has procured nearly 30 million doses of China-licensed COVID-19 vaccines through bilateral agreements. The country recently began bottling PakVac with the help of China's Cansino Bio after rigorous quality control checks, a single-dose COVID-19 vaccine, to deliver 3 million doses per month. According to government sources, the vaccine has passed rigorous internal quality assurance testing, considering it an important step to help the vaccine supply line. The first batch of bulk CanSino vaccine is being processed at the National Institute of Health's plant, in Pakistan. Accordingly, the government started co-production of a single-dose Ad5-nCoV vaccine and repackaged the vaccine as PakVac for the masses. Such a procurement practice represents another way of obtaining vaccine supplies. It could allow the government to provide the vaccine at a lower price than the global price of the vaccine. In addition, locally produced vaccines could help the country achieve its Sustainable Development Goals and in the long run, it could export low-cost vaccines to other underdeveloped countries (Siddiqui, *et al.*, 2021).

Conducting clinical trials of COVID-19 vaccines in Pakistan can also be a source of their supply. In August 2020, the Drug Regulatory Authority of Pakistan approved a clinical trial of Ad5-nCoV vaccines developed by the Chinese company CanSino Biologics and the Beijing Institute of Biotechnology. More than 17,500 people from different walks of life and rural areas volunteered to participate in the trials held at five different locations in Islamabad, Lahore, and Karachi. Recently, however, Pakistan's National Bioethics Committee has refused to approve at least five double-blind, placebo-controlled phase III clinical trials of COVID-19 vaccines on ethical grounds, citing that the use of placebos cannot be permitted. during these studies at a time when various types of conventional vaccines against COVID-19 are available in the country, although there has not been accepted by the investigators and manufacturers. If this issue is resolved, it could lead to hundreds of thousands of individuals being vaccinated in Pakistan (Haire, 2021, Siddiqui, *et al.*, 2021).

Vaccine acceptance and resistance: Although there are strong shreds of evidence regarding the decrease of new infections and prevention of fatal diseases through vaccination, still some gray areas have raised doubts about COVID vaccine administration. This ambivalence and uncertainty toward the COVID-19 vaccination are prevailing as fast as the pandemic itself (Dror *et al.,* 2020). One of the key concerns is the development of vaccines against coronavirus at unprecedented speeds (Lurie *et al.,* 2020). The vaccines against COVID have recently been introduced across the globe and their design and development proceed in a much faster way as compared to the traditional vaccine development timescale which takes around 15 or more years (Krammer, 2020). Consequently, the acceptance and willingness of people toward SARS-CoV-2 vaccines represented a public health challenge worldwide. The major reasons behind this vaccination hesitancy and resistance are due to many issues, particularly the efficacy and safety, misleading information, accelerated timescale of vaccine development, mistrust or disbelief in medical and scientific communities, lack of awareness and education, and scientific themes politicization. Moreover, the limitation of available data regarding the efficiency and safety of COVID vaccines is also a major concern (Loomba *et al.,* 2021).

The emergence of novel SARS-CoV-2 variants The SARS-CoV-2 coronaviruses belong to the RNA viruses which exhibit a high rate of mutation as compared to DNA viruses. 69 The genome of corona viruses including the SRAS-Cov-2 use an RNA polymerase for their replication which contains proofreading activity thus resulting in higher accuracy in the transcription of these viruses (Boivin *et al.,* 2010). The rapid transmission and the global spread of SARS-CoV-2 resulted in the emergence of many favorable mutations to the virus most of which show no effects on the pathogenesis of the virus. Most of the COVID-19 vaccines developed recently are based on the spike protein which is the major protein that helps the virus in attachment and entrance into the host cell (Le *et al.,* 2010). According to a report at the beginning of 2021, a total of 9654 mutations in the S protein which correspond to 400 different mutation sites have been detected. Among the detected mutations, 44 affect the RBD (receptor binding domain of virus) which ultimately contributes to the immunogenicity of the virus (Bos *et al.,* 2020; Walsh *et al.,* 2020). Hence, a single deletion or substitution of amino acid in the receptor binding domain fails to evade the immune response to a large extent. Among the several variants of SARS-CoV-2, four including B.1.1.7, B.1.617.2, B.1.351, and P.1 have been categorized as Variants of concern. However, with this rapid emergence of new variants, the predictions about the further trajectory of OVID-19/SARS-CoV-2 mutations are much more challenging ultimately resulting in the limited effectiveness of vaccines against it (Makhoul *et al.,* 2020; Malik *et al.,* 2020; Dhama *et al.,* 2020).

Conclusion This review provides substantial information regarding transmission, treatment and vaccination for COVID-19 (Figure 2). The following conclusions can be derived from this review article:

1. The problem of COVID-19 continues to spread around the globe due to the emergence of multiple variants of this SARS-CoV-2 virus.
2. In developing countries like Pakistan vaccination against COV-19 continues using various types of vaccines from the manufacturers and quite a significant number of the population has been vaccinated by receiving, single, double, or booster doses.
3. The virus has killed over 30,607 people in Pakistan to date (20th September 2022)
4. The vaccination percentage in developing countries needs to be increased and vaccination evaluation in terms of seroconversion needs to be monitored regularly, especially because of the emergence of virus variants.
5. COVID-19 has infected a huge number of the human population causing overall economic depression, and a world increase in prices like jobless and difficulty living, especially in the poor and developing nations.
6. COVID-19 infection rate is again accelerating at a high rate in Pakistan in the recent time and again causing economical depression. Again emerging as a panicky situation for the public and of serious concern for medical authorities in Pakistan and WHO.
7. As per the most recent report cat has been known to harbor a virus, indicating the importance of zoonosis.
8. People must be observed for strict preventive measures like wearing masks, and gloves, avoiding congregations, and visiting the patients in the hospitals. Because of the emergent situation vaccination potential in older people needs to be monitored for immunizing.

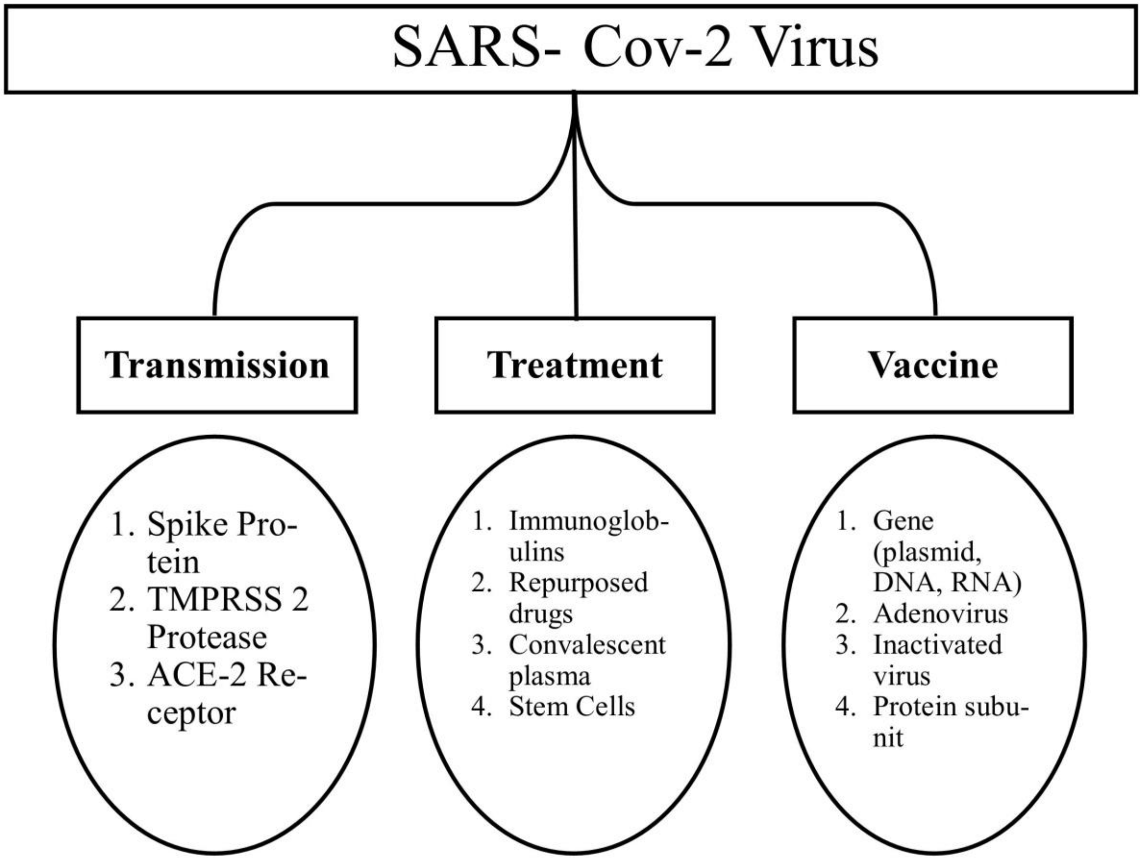

Figure 2: Conclusion regarding the Covid-19 transmission, treatment and vaccine

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