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Review Article

COVID-19: Review on Efforts for Containment, Diagnostics, and Treatment in the Indian Context During the SARS-CoV-2 Global Pandemic

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Abstract

The sudden outbreak in Wuhan City of China in December 2019 by a previously unknown virus, has eventually spread like a pandemic to almost all the countries in the world. It left the healthcare system divested of every single resource, be it the supportive therapeutic regimens, diagnostic kits or personal protective equipment, and even the mask and hand sanitizers. This new virus was later on identified as the novel coronavirus or SARS-CoV-2 and was declared as a public health emergency on January 30, 2020, and as a pandemic on March 11, 2020, by the World Health Organization (WHO).

The ongoing COVID-19 pandemic has been spreading at alarming rates worldwide, calling for better diagnostic and therapeutic strategies. Although our understanding of SARS-CoV-2 has grown tremendously in the past couple of months however a systemic approach to diagnostics, treatment, and epidemiological controls are still lacking, at least in the Indian context. Here we review the efforts on containment, various treatment regimens, diagnostic techniques, and commercial kits available in India and around the world. We also discuss the current disease management protocols and possible therapeutic approaches including vaccines.

Keywords: SARS-CoV-2; COVID-19; Pandemic; RT-PCR; Rapid Cards; Vaccines

Introduction

The novel strain of coronavirus that caused a highly transmissible lower respiratory disease, was first reported in the Chinese province of Wuhan in December 2019 [1,2]. By the end of January 2020, the infection had spread to all the provinces in China and more than 18 countries resulting in the declaration of a Public Health Emergency of International Concern (PHEIC) by the World Health Organization (WHO). The virus was officially named the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by

the WHO in February 2020 and the infection was designated as COVID-19, an acronym for coronavirus disease-2019.

SARS-CoV2 is a single-stranded, positive-sense RNA virus from the *Coronaviridae* family bearing genetic similarity to the SARS-CoV and MERS-CoV viruses [2,3]. These coronaviruses are considered to be zoonotic pathogens which are transmitted from animals to humans and cause severe respiratory illness with symptoms including fever, cough, and shortness of breath. The SARS and MERS are more severe (mortality rate of 15% and 35% respectively) when compared to COVID 19 which has shown an overall global mortality rate is ~2% [4]. Coronavirus infections can easily spread through person-to-person contact via respiratory droplets and even infected body secretions such as blood and stools [3]. However, the duration and frequency of viral shedding in stool and potentially in urine is still under investigation. The severity of these diseases tends to be higher in aged patients and patients with underlying health conditions such as diabetes, cardiovascular disease, chronic respiratory disorder, chronic renal disorder, or cancer.

COVID19 symptoms may take up to 14 days to develop, after exposure to the virus. Asymptomatic positive cases have also been reported [5]. The pre-symptomatic and asymptomatic cases pose as viral carriers and could be accounted for the extensive spread of the disease worldwide.

The majority of the COVID19 cases how mild to moderate symptoms like fever and dry cough, and tend to recover without requiring special treatment. Other symptoms such as headache, diarrhea, sore throat, muscle aches, loss of taste, and smell, as well as conjunctivitis, have also been reported [6]. However, nearly 20% of the patients show severe symptoms such as dyspnoea (shortness of breath) and/or hypoxia and therefore require hospitalization. Critical conditions with manifestations like pneumonia, acute respiratory distress syndrome (ARDS), respiratory failure, septic shock, or multiple organ dysfunction is known to occur in less than 5% of the cases, especially in the elderly and high-risk patients [7,8]. The mortality rate among the vulnerable population is as high as 15% [9].

As of November, 2020, more than 63 million cases have been reported globally. The total number of COVID19 related fatalities is currently 1,465,181worldwide. Wuhan, the epicenter of COVID19, has seen >92,300 cases. According to statistics, Italy and Spain were the first countries to be hit by the pandemic with a very high number of cases and a maximum number of fatalities. Shortly, the USA emerged as a major hotspot, seeing an exponential rise in the case of numbers over the last few months. Currently, the US has more than 13 million cases, and more than 273,072reported deaths.

India has seen a slow rise in numbers in the initial phase due to the imposition of nation-wide restrictions. However, post-lockdown there has been a sudden spike in cases with numbers increasing exponentially. Presently, India ranks second in the highest number of COVID cases with close to 9.4 million cases. State-wise data shows the highest number of patients in the states of Maharashtra and Karnataka, followed by Andhra Pradesh and Tamil Nadu. (https://www.covid19india.org/).

In comparison to SARS and MERS, the global number of COVID-19 cases has substantially risen indicating a longer time to lower the disease cases by half. Government agencies of different countries around the world are working in coalition with health organizations to establish preventive and control measures, and turn down the rapid spread of the virus. Research institutions and pharmaceutical companies are actively studying viral mechanisms and also exploring various diagnostic and therapeutic strategies. The COVID19 scenario is rapidly evolving and many uncertainties have been raised about virus mutations, virus-host interactions, and host immunity.

The SARS-CoV-2

Coronaviruses (CoVs) are positive-sense, single-stranded RNA viruses with a genome of 26–32 kilobases and four structural proteins, namely, spike glycoprotein (S), envelope protein, an envelope protein (E), membrane glycoprotein (M), and the nucleocapsid (N) (Figure 1). The S, E, and M proteins comprise the viral envelope whereas the N protein holds the RNA genome within the virion. The distinct spike proteins which occur as homotrimers on the envelope resemble a solar corona under an electron microscopic therefore a suitable name was given.

The *Coronaviridae* family is further subdivided into four genera: alpha CoV, beta CoV, gamma CoV, and delta CoV [10]. The SARS-CoV2 virus belongs to the beta CoV category and shares

Figure 1: Structure of respiratory syndrome causing human SARS-CoV2 virion.

Figure 2: Genomic structures of SARS-CoV and SARS-CoV2. Both the viral genomes comprise of the 5'-untranslated region (5'-UTR); open reading frame (orf) 1a/b (blue box) which encodes the non-structural proteins (nsp) for replication; structural proteins (green boxes) i.e. spike (S), envelop (E), membrane (M) and nucleocapsid (N) proteins; the accessory proteins (red boxes) such as orf 3, 6, 7a, 7b, 8 and 9b in the SARS-CoV-2 genome; and the 3'-untranslated region (3'-UTR). Adapted from Shereen., *et al.* 2020.

82% nucleotide identity with the SARS-CoV virus and 50% to the MERS-CoV [11]. The genomic analysis further indicates that SARS-CoV2 strains probably evolved from bats, like the SARS and MERS CoV, and could have reached humans through intermediate hosts [12,13].

SARS-CoV2 genome

The genome of SARS-CoV2 is ~30kb with a 5'-cap and a 3'-polyA tail. The genome consists of the open reading frames ORF 1ab, the structural genes, and the ancillary genes (Figure 2). The *orf1a/b*is the largest gene that encodes the polyproteins pp1a/pp1ab which are processed by internal viral proteases into 16non-structural proteins (nsps) to form the replication-transcription complex (RTC). The structural genes code for the S, E, M, and N proteins whilst the ancillary genes code for the proteins necessary for replication [14].

Viral entry and life cycle

Similar to SARS-CoV, the SARS-CoV2 viral entry into the host cell is facilitated by the binding of spike glycoprotein to the angiotensin-converting enzyme 2 (ACE2) receptor on the human airway epithelia [12,15]. ACE2 is also highly expressed in the lung parenchyma, kidney, stomach, small intestine, and colon implying other sites of infection and routes of viral shedding.

The spike protein comprises of two subunits, S1 responsible for the binding to ACE2 and S2 which allows fusion of the viral and host cell membranes. The C-terminal domain of the S1 subunit comprises the receptor-binding domain (RBD). More specifically, the 394-glutamine residue in the RBD region is recognized by the lysine 31 residue on the human ACE2 receptor. The activation and fusion of the S protein require a proteolytic cleavage at the S1/S2junction by the host protease, furin, which induces an irreversible conformational change [16]. The difference between SARS-CoV and SARS-CoV2 has been identified in the RBD amino-acid sequence and the presence of the furin cleavage site flanked by three O-linked glycans [17]. Few studies demonstrate that these modifications in the spike protein have resulted in a higher binding affinity towards ACE2, which in turn attributes to the higher transmission rate of the virus [18-20]. Moreover, the expression of ACE2 receptor in the lung has been found to increase with age, explaining the higher risk of infection among the geriatric population [21].

After fusion of the viral envelope with the cell membrane, the SARS-CoV-2 releases its RNA into the host cell. The genomic RNA is then used as a direct template and translated into pp1a and 1abreplicaseproteins, which are subsequently cleaved into ma-

ture nsps by the viral proteinases. Nsps consist of enzymes such as RNA-dependent RNA polymerase (RdRp), a3C-like serine proteinase (3CLpro), papain-like proteinase (PL2pro), superfamily 1-like helicase (HEL1), etc. They make up the replication-translation complex (RTC) which aids viral replication. The RTC complex then produces a set of nested minus-strand subgenomic RNAs (sgRNAs) via discontinuous transcription (Figure 3) [22,23]. The transcriptional termination and consequent acquisition of a 5'-leader RNA take place at transcription regulatory sequences which are located between the ORFs.

Figure 3: Life cycle of SARS-CoV-2. The virus attaches to ACE2 receptor on host cells via the S protein. This causes conformational changes in the S protein leading to envelope fusion with host cell membrane through endosomal pathway. The positive genomic RNA is released and is translated into viral replicase polyproteins pp1a and 1ab. These are further cleaved into small products by viral proteinases. The viral polymerase produces subgenomic mRNAs by discontinuous transcription, which are finally translated into viral proteins. The proteins and positive strand are then assembled into virions and released by exocytosis.

The four structural proteins are crucial for the assembly and infectiousness of the virions. The S proteins make up the homotrimer spikes on the viral envelope enabling host cell attachment via ACE2. The M protein consisting of three transmembrane domains promotes membrane curvature giving shape to the viral structure. The E protein is known to be involved in viral assembly and release, whereas the N protein plays a role in encapsulating the genome into virions. The nucleocapsid N protein comprises two domains and has been reported to be suppressors for interferons (IFNs) and RNA interference, enhancing the viral replication capacity [24,25].

The viral proteins and genomic RNA are assembled into virions in the endoplasmic reticulum (ER) and Golgi and finally transported via vesicles to be released out of the cell (Figure 3).

Mutations

RNA viruses have an exceptionally high mutation rate which contributes to viral adaptation [26]. Characterization of viral mutations is necessary to evaluate viral drug resistance, immune escape, and pathogenesis. In SARS-CoV2, 13 variation sites have been discovered in ORF1ab, S, ORF3a, ORF8, and N regions. The positions 28144 in ORF8 and 8782 in ORF1a showed a high mutation rate of 30.53% and 29.47%, respectively [27].

An early phylogenetic analysis of SARS-CoV2 genomes from patient samples around the world has revealed three main variants, namely A, B, and C [28]. They have been distinguished based on amino acid sequences. The virus reported from the Hubei province, China has been designated as the parental A-type. Type B is a derivative of A, with two mutations, and C is a mutational variant of B. Apart from China, type A has been observed in patients from the US and Australia. Certain sub-variants of type A have also been observed, especially in Americans who have been in Wuhan [28]. The type B SARS-CoV2 is also suggested to have emerged in Wuhan and have spread majorly inEast Asia. Whereas, the C variant has been more predominant in Europe, commonly reported in the early patients from France, Italy, Sweden, and England. It is also evident in Hong Kong, Singapore, Brazil, and California.

More recent data suggest that all three strains might be co-existing [29]. Scientist argues that phylogenetic analysis has helped trace and establish the infection routes. Moreover, the possibility of a difference in epidemiological and clinical presentations of these mutational variants cannot be ruled out. However, the significance of such phylogenetic deviations is yet to be completely unfolded. It is crucial to take into account these genetic modifications while designing diagnostics, treatment, and vaccines.

COVID19 epidemiology in India

The COVID trajectory in India is slow in comparison to Europe and the US. The numbers have been steadily rising and have

crossed the 9,432,075marks, however, India still hasn't seen widespread community spread in most of the states.

The first three cases were reported between Jan 30 and Feb 3 in the southern state of Kerala. All were students who had returned from Wuhan, the epicenter of the outbreak. They were hospitalized and treated in isolation wards till they recovered. New cases began to appear after a month, in the first week of March in tourists and individuals with travel history. Since then more cases were confirmed across the country, mainly foreign tourists, citizens returning to the country, and their contacts.

In the initial phase of the epidemic in India, the states of Maharashtra, Kerala, and Delhi were the hotspots with a greater number of cases. The states in eastern India did not report any cases. By early April, the virus had spread to most of the states in India. No cases have been reported yet in Lakshadweep, which could be due to the lack of testing. The current statistics show a high concentration of cases in the western state of Maharashtra and Karnataka. The local health authorities have implemented strict tracing, testing, and isolation measures to minimize rapid spread.

Demographical data reveals that the highest number of positive cases fall under the age group 20-40 years and are males (Figure 4). According to the Ministry of Health and Family Welfare (MoHFW), India, 8.61% of the cases is between 0-20 years (including infants), 41.88% between 21-40 years, 32.82% between 41-60 years, and 16.69% cases above 60 years of age.

The total fatality rate in India is 1.5% and the recovery rate stands at 77.32% as of Oct 31st, 2020. The death rate is significantly on the lower side compared to countries like Italy, the USA, and China. This could be because most of the patients are among the younger population. The highest number of deaths have been recorded in Maharashtra however, the death rate is highest for the state of Punjab due to co-morbidity and lack of awareness.

Early nationwide lockdown and containment measures put forward by the Government of India have contained the virus spread by a substantial degree. The doubling rate of the number of infected cases in mid-August was 25.5 days and that has increased sharply to 70.4 days.

Moreover, the majority of the COVID19 patients (~80%) in India were found to be asymptomatic or mildly symptomatic (theprint.

Figure 4: Number of the coronavirus (COVID-19) cases in India 2020, by age group. Note: India; as of July, 2020. Source(s): COVID-19 Tracker India; Various sources (crowdsourced patient database, government reports); ID 1110522.

in, August24, 2020). Though this means a much higher risk of disease spread, it might also contribute to herd immunity. Conversely, there have been few cases of relapse of the disease after recovery in the country (newindianexpress.com, 16 Sep 2020). Such an incidence may be a consequence of faulty tests, low host immunity, or the emergence of different serotypes of the virus. Studies are underway in South Korea and Japan where there have been significant cases of reinfection or relapse.

COVID19 with underlying Health Conditions

Various reports and studies press on the fact that the majority of the patients that require intensive care are those with co-morbid health conditions [30-32]. Little is known at this point about the molecular pathologies involved in the disease progression of COVID19 patients with pre-existing health conditions or co-infections.

Patients with hypertension and type 1 and 2 diabetes are often treated with ACE inhibitors and angiotensin II type I receptor blockers (ARBs). These medications are known to result in an up-regulation of ACE2 expression which might lead to the severity of disease in these patients [33]. However, there is no scientific evidence yet to prove that the withdrawal of these drugs will improve the prognosis [34,35].

Diabetes is a metabolic disorder that involves a condition of chronic inflammation which could be an underlying reason for increased mortality quotient. Furthermore, elderly diabetic patients develop other complications such as cardiovascular diseases (CVDs) and kidney ailments putting them at very high risk. Another study states the possibility of the onset of acute diabetes in progressive COVID19 as ACE2 is expressed in pancreatic B-cells [30,36,37].

Though pre-existing cardiovascular conditions have been associated with mortality in COVID19, there are also theories that the pathophysiology of the viral infection might contribute to the development of cardiovascular diseases in a patient [38]. However, further investigation is required to establish the exact relationship between diabetes and CVDs in COVID19 pathophysiology.

Likewise, acute kidney injury has been reported in 3% to 9% of the critical COVID19 cases, a leading cause of fatality [39]. Renal tubular cells highly express ACE2 suggesting the kidney as a site of infection for the SARS-CoV2 virus [12]. It is hypothesized that kidney impairment could be an outcome of viral cytopathy or sepsis and cytokine storm. The current treatment mainly involves supportive care, general antivirals, and hemodialysis if necessary [39]. On the other hand, COVID19 infection in chronic kidney patients is deadly [40]. Hence, rigorous screening and isolation measures are essential in dialysis centers and hospitals with in-patients for kidney diseases. The International Society of Nephrology and Kidney International has laid out diagnostics and treatment protocols for patient care in the light of kidney disease (https://www.theisn.org/).

COVID19 co-infection with other respiratory infections is still under investigation. Co-infection with influenza virus has been reported in China and Iran [41,42]. However, early studies did not show the significant deterioration of clinical condition [41]. Meanwhile, Brazil is facing a rise in dengue infections along with COVID19 cases [43]. Health officials highlight that a co-infection is can result in a health crisis in the country. India is the country with the highest tuberculosis (TB) burden that needs to ensure co-infection is prevented. Enhanced surveillance and multiplex panel diagnosis for common infections is important in such a scenario.

Another area of great health concern is COVID19 infection in the backdrop of pregnancy and childbirth. Limited data suggest that pregnant women with COVID19 have the same risk level as an adult (https://www.cdc.gov/). Most of the pregnant women presented mild symptoms, unlike SARS and MERS which had resulted in severe illness with complications. Nonethelessit is important to take all necessary precautions to prevent an infection. Direct vertical transmission from mother to child has been reported in some severe cases [44]. In another study from Wuhan, involving pregnant women with mild symptoms, no viral transmission was reported from mother to baby during childbirth [45]. Serological tests revealed a possible positive transfer of IgM and IgG through the placenta to the new-borns. The presence of IgA in breast milk has not been reported yet.

With new data emerging by the day, it is difficult to conclude clinical outcomes and treatment protocols for COVID19 with any underlying conditions. The current recommendations are mostly preventive and supportive care.

Diagnostics

Contact tracing and accurate diagnosis of people suspected to have had an exposure are essential in determining the course of the COVID19 pandemic. For this reason, there is a pressing demand, globally, to develop quick and reliable screening and testing kits. In light of rapid and widespread transmission, several points that need to be factored in when designing a diagnostic test include:

- Detection method - direct (the virus itself) or indirect (host antibodies)
- Fast turnaround time
- Throughput i.e. the number of tests that can be performed at a time
- Cost-effectiveness and requirement of trained personals and high-end equipment
- The intended use, for example, large-scale screening, monitoring symptomatic patients, point-of-care testing, or treatment selection.

The incubation period of the SARS-CoV ranges from 5 to 14 days and reports show that pre-symptomatic and asymptomatic transmissibility has been high. The viral load is known to peak during the first week of infection and subsequently decrease during week two [21,46]. At the same time, IgG and IgM antibodies against the viral spike protein and nucleocapsid are known to appear in the

blood of the patient. In 3-6 days, IgM is formed and in 8-10 days, IgGs are generated which confers long-term immunity. Hence, it can be said that IgM is suggestive of a recent infection whereas IgG indicates an earlier exposure and the indication of recovery. Some of the diagnostic tests that are currently available, detects viral RNA, viral antigen, or the presence of antibodies in host blood.

Nucleic acid-based diagnostic tests

The molecular diagnostic methods mainly refer to nucleic acid amplification tests (NAAT) where viral RNA is amplified and then detected or quantified. Sample collection is a crucial step in this process. Typically, nasopharyngeal and/or oropharyngeal sample is collected, preferably using flocked swabs. A nasopharyngeal swab is supposed to contain a higher viral load and therefore is considered to be more sensitive [47]. Additionally, lower respiratory tract specimens, such as sputum, endotracheal aspirate, and/or bronchoalveolar lavage fluid can also be tested, especially for patients with more severe symptoms. That said, detection rates in each sample type have been demonstrated to vary from patient to patient and also along the course of the disease [48,49]. The swabs are transferred into a viral transport medium, to preserve the nucleic acid integrity, and then shipped to the laboratory for analysis. In any case of delays, the sample must be stored at -20°C or -70°C or shipped in dry ice.

Qualitative/quantative Reverse transcription-polymerase chain reaction (qRT-PCR)

RT-PCR is the current standard to detect the SARS-C0V2 virus. The samples taken in VTM go through an RNA extraction procedure followed by target amplification in an at hermal cycler machine equipped with provisions of fluorescence-based detection. There are many primers and probes (Fluorescence Taq Man chemistry-based) combination have been validated and developed by different laboratories in the public domain including, WHO, CDC-USA, CDC-China, HK, and Berlin protocol. The primers are usually designed to identify the *Orf1b RdRp* gene (RNA-dependent RNA polymerase gene) or the E (envelope protein gene) and *N* gene (nucleocapsid gene). RNase P has been used as an internal control.

Although qRT-PCR is an accurate and sensitive method, there can be chances of false negatives or false positives. The quality of sampling dictates the presence of viral RNA. If inadequate, it can result in a false negative outcome. Cross-contamination while sample processing is also probable leading to false positives. Besides, positivity for viral RNA does not necessarily correlate100% to the live virus load in the patient and hence the transmission risk can be misinterpreted. Another drawback of the PCR test is the turn-around time, which could be days depending on the transportation of the sample, laboratory setting, and automation. Hence to ensure the accuracy of PCR tests, it is important that sample collection and processing are carried out only by trained professionals properly and safely.

Several other technology platforms are also being explored to develop sensitive and accurate tests that can overcome the gaps in a PCR test. Rapid point-of-care molecular tests such as cartridge-based assays and antigen detection tests are making tremendous advancements [38,50].

Reverse transcription loop-mediated isothermal amplification (RT-LAMP)

RT-LAMP can be used as a cost-effective nucleic acid test as it does not require expensive equipment [24]. RT-LAMP techniques four primers and amplification can happen at a constant temperature of 60-65°C. RNA extracted from swab specimen along with the reagents can be added into a test-tube and the results (positive or negative) can be either visualized by turbidity/colorimetric substances or under the UV light by adding DNA binding fluorescence dye. The results are obtained in less than an hour making it an option for point-of-care diagnostics. RT-LAMP can also be multiplexed to detect more than one target at a time [51].

Next-generation sequencing (NGS)

is a highly sensitive technique to confirm the presence of viral RNA in a nasopharyngeal/oropharyngeal swab. Apart from detection, NGS can also reveal the genome sequence of the virus. This helps to identify any mutations in the virus and can be used to track virus evolution. NGS is more of a research and development tool rather than a regular diagnostics method.

CRISPR (clustered regularly interspaced short palindromic repeats) –Cas

CRISPR based assays are also under development. Broughton and colleagues have validated a rapid detector assay that employs RT-LAMP followed by CRISPR-Cas12 detection in a lateral flow format with visual detection [52]. Additionally, Hou's group is devel-

oping a similar SHERLOCK assay using Isothermal Recombinase Polymerase Amplification (RPA) and subsequent CRISPR-Cas13 directed detection [53].

Serological diagnostics

Serological tests are quick and less complex that recognizes IgM or IgG antibodies against the SARS-CoV2 virus, typically in the blood of the patients. These tests being faster are expected to play a very important role in population surveillance which allows prompt containment measures and hence, management of the pandemic.

Seroconversion during a viral infection usually happens between days 7 and 11 post-exposure. A negative antibody result does not always equate to no infection. One of the studies demonstrates that detection of IgM can identify early onset of the disease, with sensitivities comparable to RT-PCR tests (by day 5 after exposure) [54] however many instances of the low sensitivity of rapid cards have been reported. Antibody tests are useful in analyzing the stage of infection and are more relevant as point-of-care testing that helps with clinical decisions (Figure 5). RT-PCR tests may show false negative due to decreased viral shedding but antibody profile can reveal the exact disease phase.

Figure 5: Variation of the Levels of SARS-CoV-2 RNA and Antigen, IgM and IgG after infection.

There is growing interest in the development of rapid serological tests and some of the *in-vitro* diagnostics platforms are described below.

- **Enzyme-linked immunosorbent assay (ELISA):** ELISA is a high sensitivity and specificity assay that can be multiplexed to qualitatively or quantitatively detect proteins. ELISA is widely being utilized to detect anti-viral antibodies in patient blood as well as detection of viral antigens like S-protein in swab samples/blood. A multiplex approach to detect viral antigens and blood IgM/IgG in a simultaneous test can give valuable clinical information on viral load as well as phase of infection. The sensitivity of such a test needs to be evaluated. Moreover, ELISAs can also be designed to identify levels of other important clinical markers such as C-reactive protein and IL-6 that help decide treatment protocols. Though ELISAs have high throughput, conventional ELISA requires laboratory equipment and trained technicians limiting their use for surveillance.

- **Chemiluminescent Immunoassays (CLIA):** It is a variant of ELISA that uses an automated platform to detect light signals. Few automated CLIA platforms have been approved to detect IgM/IgG levels in the blood (Table 2). Results can be obtained in ~30minutes and automation gives it an upper hand in terms of throughput.
- **Lateral flow Immunoassays (LFIA):** LFIA is one of the most trending areas of development for COVID19 diagnostics. It is a simple and easy-to-use portable test format that gives a visual result in <15 minutes (Figure 6).

Several IgG and IgM detection LFIA kits are already available in the market. This technique can also be employed to detect viral

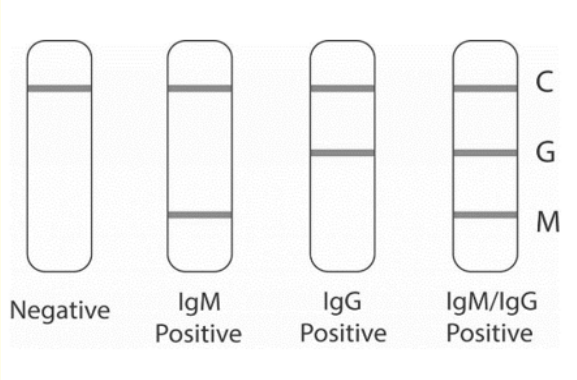

Figure 6: Illustration of a COVID19 rapid card test result by BioMedomics Inc. C- control, G- IgG, M- IgM.

antigens (mainly S protein) in blood, saliva samples, or respiratory swab fluids. A multiplex test for antibodies and viral antigens from different sample types from the patient will be of greater clinical significance. They can be readily used for surveillance at airports, schools, offices, and hospitals. Such rapid tests are crucial to screen positive cases and to determine population immunity.

Test Results			Clinical interpretation of the patient
RT-PCR	IgM	IgG	
+	-	-	Maybe in the window period of infection
+	+	-	Early-stage of infection
+	+	+	The active phase of infection
+	-	+	Late or recurrent stage of infection
-	+	-	Early-stage of infection or false positive for RT-PCR
-	-	+	Recovered
-	+	+	Recovery phase or false positive for RT-PCR

Table 1: Interpretation of molecular and serological tests.

Clinical biomarkers and ancillary tests

In patients with moderate to severe clinical symptoms, chest radiography is obligatory to diagnose pneumonia. Ground glass opacities in the lung was observed in more than 70% of the COVID19 patients after the onset of symptoms [27]. Some of the other clinical features to look out for include decreased albumin, elevated C-reactive protein, and elevated lactate dehydrogenase levels, lymphopenia, higher erythrocyte sedimentation rates, and increased bilirubin and creatinine levels [38]. Also, host inflammatory response markers and organ dysfunction markers are often used for patients with severe symptoms to determine the mode of therapeutic intervention and the clinical outcomes [4,38].

Tests for cytokine release syndrome (CRS)

There is accumulating evidence pointing towards excessive cytokine production as one of the leading causes of COVID19-related mortality. When compared to mild cases, the cases with pneumonia and ARDS presented an increase in inflammatory cytokines including IL6, IL10, IL2, TNF, and IFN-gamma [55]. Such hyper-inflammatory response also termed as cytokine storm can result in plasma leakage, vascular permeability, disseminated intravascular coagulation, and finally organ failure. Hence, blood cytokine tests, mainly a spike in interleukin-6 (IL-6) has been proposed as a marker for respiratory failure due to lung inflammation [56]. This could indeed be crucial in preventing fatal outcomes in several patients.

In the currently available tests, RT-PCR gives a more accurate read-out during the acute phase of the illness, whereas the determination of antibody levels is more appropriate during the chronic phase. From a clinical standpoint, a combination of RT-PCR and serological assessment is more significant for COVID19diagnosis and treatment decisions (Table 1).

Researchers are actively investigating other prospective point-of-care multiplex techniques that can perform molecular nucleic acid and immunological tests. An automated and portable single platform rapid assay in this regard can greatly influence the management of the pandemic.

Diagnostic kits from many companies are currently available in India. Some of them are indigenous however many RT-PCR tests and antibody rapid tests are being procured from the USA, Singapore, and China. The make-in-India diagnostics development and manufacturing efforts are rapidly progressing too. Mylab's RT-PCR testing kit was the first to be approved by CDSCO/ICMR for commercial usage in India. Several other Indian companies and research institutions are also working on RT-PCR, antibody rapid tests, RT-LAMP, and CRISPR based tests [57]. Some of the ICMR approved diagnostic kits are summarized in tables 2 and 3.

Disease management and Therapeutic Strategies

The current line of treatment for COVID19 focuses on supportive and symptomatic care as a specific anti-viral drug or vaccine is yet to emerge. Mild cases Majority of hospitalized patients may require oxygen support to manage respiratory distress. In severe cases, mechanical ventilation or extracorporeal membrane oxygenation support may be required (www.who.int). Antibiotics and antifungals can be prescribed to treat co-infections. Management of septic shock and organ function support is vital in critical cases.

With the healthcare sector being overwhelmed around the world, and given the urgency, scientists are looking into drug repositioning and accelerated drug development. Several candidates are already in clinical trials. In India, the Indian Institute of Medical Research (ICMR) has issued guidelines on the treatment protocols

Some commercially available COVID19 Diagnostic kits		
S. No	**Name of Company**	**Name of the Kit**
1	Altona Diagnostics	RealStar SARS-CoV-2 RT-PCR kit 1.0
2	MY LAB	Patho Detect
3	Seegene	Allplex 2019-nCoV assay
4	SD Biosensor	nCoV Real-Time Detection kit
5	KILPEST (BLACK-BIO)	TRUPCR SARS-CoV-2RT-qPCR kit version 2
6	Huwel Lifesciences	Quantiplus CoV detection kit ver 2.0
7	BGI	Real-Time Fluorescent RT-PCR Kit for detecting 2019-nCoV
8	ABI (Applied Biosystems)	TaqMan2019-nCoVControl Kit v1
9	Medsource Ozone Biomedicals	COVID-19 RT-PCR kit
10	Helini Biomol-ecules, Chennai, India	Helini Coronavirus [COVID 19] Real-time PCR kit
11	ADT Biotech Sdn-Bhd, Malaysia	LyteStar 2019 nCoV RT-PCR kit 1.0
12	OSANG Health Care	Gene Finder COVID-19
13	Cepheid	Xpert Xpress SARS-CoV-2
14	Biogenomics (India)	BIO COVID ID/ COVID-19 qualitative PCR detection kit v. 2
15	Merril Diagnostics	Meril COVID-19 One-step RT-PCR Kit
16	Gene Matrix	NeoPlex COVID-19 detection kit
17	IIT Delhi	Covid 19 Probe-free Real-Time PCR Diagnostic Kit
18	Cosara Diagnostics	SARAGENE™ Corona Virus (2019 NCV) Test kit
19	Labcare Diagnostics	Accucare COVID One step RT-pCR kit
20	NextGen *Invitro* Diagnostics Pvt Ltd.	COVSCAN SARS-CoV2 RT-qPCR kit

Table 2: RT-PCR based kits approved by ICMR/CDSCO for the detection of SARS-CoV-2 RNA by Real-Time PCR. Out of 45 kits evaluated by ICMR, the following 21 kits are approved.

S. No.	Kit Detail
1.	SARS-CoV-2 Antibody test (Lateral flow method): Guangzhou Wondfo Biotech, Mylan Laboratories Limited (CE-IVD) M R Roofs Private Ltd Abbott Laboratories Cadila Healthcare Limited (ZydusCadila)
2.	COVID-19 IgM IgG Rapid Test: BioMedomics (CE-IVD)
3.	COVID-19 IgM/IgG Antibody Rapid Test: ZHUHAI LIVZON DIAGNOSTICS (CE- IVD)
4.	New Coronavirus (COVID-19) IgG/IgM Rapid Test: Voxtur Bio Ltd, India
5.	COVID-19 IgM/IgG Antibody Detection Card Test: VANGUARD Diagnostics, India
6.	Makesure COVID-19 Rapid test: HLL Lifecare Limited, India
7.	YHLO iFlash-SARS-CoV-2 IgM and IgG detection kit (additional equipment required): CPC Diagnostics
8.	ACCUCARE IgM/IgG Lateral Flow Assay kit: LAB-CARE Diagnostics (India Pvt. Ltd)
9.	Abchek COVID-19 IgM/IgG Antibody Rapid Test: NuLifecare
10.	One Step Corona Virus (COVID-19) IgM/IgG Antibody Test: ALPINE BIOMEDICALS
11.	COVID 19 IgM/IgG Rapid Test Kit; Medsource Ozone Biomedicals (ver 2.0)
12.	Immuno Quick Rapid Test for Detection of Novel Coronavirus (COVID-19) IgM/IgG Antibodies: Immuno Science India Pvt. Ltd
13.	Standard Q Covid-19 IgM/IgG Duo test – One Step Rapid Antibody test: SD Biosensors
14.	COVID-19 IgG/IgM Rapid Test Kit Rafael Diagnostic: BMT Diagnostics

Table 3: Rapid antibody-based test approved by ICMR/CDSCO. Out of 23 rapid tests following 14 are approved however not recommended by ICMR for diagnosis of COVID-19.

and prescription of drugs for COVID19. Several clinical trials, approved by the Central Drug Standard Control Organization (CDSCO) are already in progress to study effectiveness and treatment response in Indian patients.

Antiviral drugs

Several drugs that have demonstrated *in-vitro* antiviral properties and were used for earlier human CoV diseases are being considered for prophylaxis and treatment. Most of them target viral replication or viral entry into the host cell. The toxicity, safety, and efficacy of these drugs in the light of COVID19 are yet to be established. A few of them are discussed below.

- Lopinavir-ritonavir, a protease inhibitor, was used for the treatment of HIV and was initially suggested for COVID19 based on previous studies during the SARS and MERS epidemics. Although this drug was prescribed for COVID19 treatment in China, there are recent reports that say itis not effective against SARS-CoV2 [58].
- Remdesivir (GS-5734), a nucleoside analogue that inhibits RNA polymerase, is a broad-spectrum antiviral drug used against several RNA viruses. Pre-clinical studies have displayed promising results and trials are now underway to evaluate the efficacy and safety in COVID19 patients.

Chloroquine, an anti-malarial drug is currently being investigated as a potential COVID19 medication. The antiviral activity of hydroxychloroquine has been described earlier against SARS-CoV and is touted as an optimistic drug candidate. The mechanism of action is likely to be a hindrance of viral fusion by elevation of endosomal pH and alteration in the terminal glycosylation of ACE-2 [59-61]. Another *in-vitro* experiment has shown that a combination of remdesivir and chloroquine has a higher inhibitory effect [62]

Interferons such as beta-1a (IFN-B1a, used for multiple sclerosis) and alpha-2b (IFN-A2b) are also being currently studied as a prospective therapeutic agent [63].

ICMR has advocated against the use ofIFN-B1b and Ribavarin due to their reported toxicity. Oseltamivir (used to treat swine flu) is not recommended as well, due to a lack of efficacy studies against coronaviruses. That said, ICMR further sanctioned the use of Lopinavir-ritonavir in extreme cases with severe respiratory pain, low circulatory strain, or organ dysfunction [64].

Anticytokine therapy

Various monoclonal antibodies which are cytokine blockers used in autoimmune diseases and other inflammatory disorders are now being tested to suppress cytokine storms in critically ill patients. Tocilizumab, an IL6 receptor blocker has been proposed as a likely therapeutic intervention for COVID19 induced CRS [21,65,66]. Other anti-cytokine drugs under consideration include Sarilumab (IL6-R blocker), Baricitinib (Janus Kinase (JAK) Inhibitor), Anakinra (recombinant Hu IL1-R antagonist), and Leronlimab (anti-chemokine receptor CCR5). These mAbs could be lifesaving drugs to patients that present CRS. Conversely, these therapies pose a risk of co-infections and even viral reactivation. Therefore, isolation and care are mandatory.

Sri Aurobindo Institute of Medical Sciences (SAIMS) in Indore, India is the first institute to have initiated trials with tocilizumab (TCZ) for critically ill COVID19 patients. As of April 20, 2020, out of the five patients being treated, two have recovered significantly.

Convalescent Plasma therapy

Convalescent plasma collected from recovered individuals that contain anti-SARS-CoV antibodies has been proposed as a short-term strategy to confer immunity. Apart from use as a prophylactic drug for health workers and susceptible individuals, it can also be used to treat critically ill patients to improve survival [67-69]. Reports from China showed beneficial outcomes in patients, including a drop in viral load and pulmonary lesions, after convalescent plasma therapy.

Other than China, countries like South Korea, the UK, and the USA has tested this therapy. The ICMR will soon be initiating clinical testing of plasma therapy for critically ill patients in different states of India including Kerala, Punjab, and Gujarat.

Biologics and immunotherapies

Numerous small molecules, peptide drugs, and mAbs are being designed to specifically interrupt the function of certain viral proteins, especially viral entry via attachment of S protein. Peptidemimetics for RBD and S2 domain have shown significant inhibition of viral entry in *in-vitro* models for SARS-CoV. In similar lines, several peptides and monoclonal antibodies are being developed that can target S-ACE2 interaction [70,71]. Viral nsp proteins are another major class of therapeutic targets as they are involved in viral RNA synthesis and replication. Among the 16 nsps, 3CLpro, PLpro, RdRp, and helicase are in focus due to the presence of an enzyme active site [72]. RNA interference therapy is also in the pipeline that targets viral genes to prevent gene expression or translation.

Vaccines

More than 100 vaccine candidates are already being tested worldwide. The speed and scale of the pandemic are driving scientists and pharma companies to expedite vaccine development like never before. Nevertheless, it may take 12 to 18 months to check safety and efficacy and make it globally accessible. Moreover, coronaviruses being susceptible to mutation, vaccine development schemes also need to factor in the effectiveness of the different types of viral strains.

Various companies and research groups are exploring different technology platforms for COVID19 vaccine development. As of April 11, 2020, three candidates had entered human trials for clinical evaluation(WHO). China's CanSinoBiological Inc. in collaboration with the Beijing Institute of Biotechnology has started phase II trials for recombinant Adenovirus type 5 vector (Ad5-nCoV) which codes for the full-length spike (S) protein. Meanwhile, phase I trial is in progress for Moderna's lipid nanoparticle (LNP)-encapsulated mRNA vaccine candidate, mRNA-1273, that encodes for a prefusion stabilized form of the spike (S) protein. Recently, *In vivo* Pharmaceutical's DNA plasmid candidate, INO-4800(intradermal injection followed by electroporation), and Oxford University's ChAdOx1 nCoV-19(non-viral vector) has entered phase I trial.

In India, several companies including Zydus Cadila, Serum Institute, Biological E, Bharat Biotech, and Indian Immunologicals, have commenced pre-clinical studies on different vaccine candidates. Most of the Indian vaccine efforts also focus on the spike protein.

Preparedness and control measures in India

With a total population of 1.3 billion and densely populated zones, the COVID19 posed a high risk for India. The number of cases had started rising steadily since March 2020 pushing the government authorities to take immediate measures.

The huge task ahead was to contain the virus and check the transmission rate. India is among one the countries that have enforced very strict measures. Containment protocols, awareness programs, and airport screenings were swiftly put in place in many states, since early March. Aggressive contact tracing was deployed to identify susceptible individuals and keep them under surveillance (home-quarantine for 14 days and the next 14 days for self-reporting). As an emergency response international travel was restricted and educational institutions were shut down. Several clusters and hotspots were identified and closed to suppress community transmission. A national lockdown (effective from 24 March to 31 May), quite early on in the course of the pandemic has helped prevent devastating effects.

Although the reported cases in India has been considerably low, the real numbers are suspected to be much higher. The Ministry of Health and Family Welfare (MoHFW) of India, through its various agencies like National Health Mission, Central Procurement, Department of Health Research/ICMR, and National Centre for Disease Control is working to boost the health sector preparedness. MoHFW has already allocated funds for the development and distribution of diagnostic tests, ramping up of surveillance activities, setting up of laboratories, leveraging health infrastructure, and drug development research (https://www.mohfw.gov.in/). The establishment of several dedicated centers for COVID19 and the deployment of a mobile sample collection facility has been paramount in these times. Also, a centralized procurement for drugs and necessary medical equipment has been put in place. MoHFW has also issued guidelines for frontline healthcare staff and training of clinicians on disease management.

To achieve the goal of the test, identify and isolate, it is necessary to expand the network of equipped laboratories [73]. Multiple labs under the Virus Research and Diagnostic Laboratory (VRDL) were selected based on the location and bio-risk assessment, with at least Biosafety Level 2 (BSL-2) facility. Nodal centers were established in each state for coordination of sample collection and shipment. Figures 7 and 8 show the state-wise capacity for testing. The National Institute of Virology (NIV), Pune, functions as the apex laboratory and resource center, providing technical training, workflow designs as well as quality assurance services. It is also required to adhere to biosafety requirements to ensure the health and safety of the technicians involved [74].

India is one of the first few countries to have isolated the SARS-CoV2 virus [75]. Researchers at the NIV, Pune, have successfully isolated two strains. The specimens were obtained from different regions and tested for *E gene, RdRp(1), RdRp(2), and N gene* using RT-PCR. Next-generation sequencing (NGS) analysis revealed two different strains (Type A and C) with a mutation in the spike protein sequence but not in the RBD-ACE2 interface [76]. The latest reports say that all three strains have been currently identified in Indian patients. Continuous genetic analysis is essential to moni-

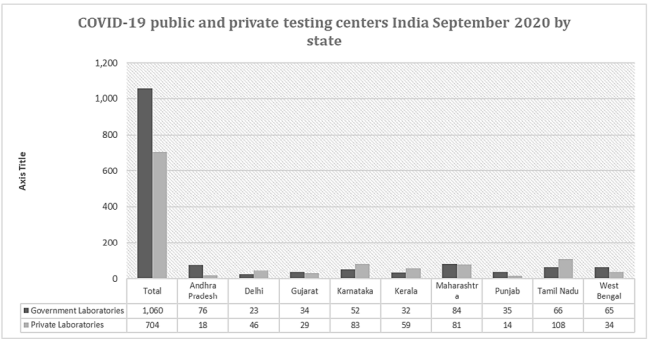

Figure 7: Number of government and private testing centres for the coronavirus (COVID-19) across India as of September, 2020, by state. Source: Indian Council of Medical Research.

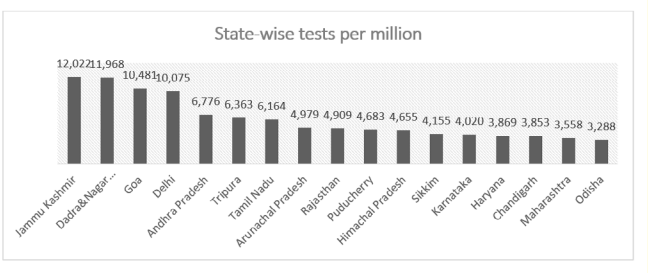

Figure 8: Coronavirus (COVID-19) test numbers across India by state (per million). Source: Ministry of Health and Family Welfare (India).

tor mutations in SARS-CoV2 so that vaccine development is not hindered. Isolation of Indian strains will aid further research on viral structure and mechanisms, also to putting R&D of diagnostics, drugs, and vaccines on the fast track. India is also looking forward to conducting human clinical trials for various drug and vaccine candidates, including convalescent plasma and ayurvedic preparations.

Taking a step further, a group of scientists is studying the coronavirus strains in bat species from seven states across India [77]. Bats are considered as reservoirs of pathogenic viruses and this study aims at assessing the CoV distribution in the bat population in India. The Discovery of such etiological agents can provide leads for the development of diagnostic and preparedness for future emergent viruses.

Discussion

The year 2020 has woken up to a very changed world. The novel beta coronavirus, later termed as SARS-CoV-2, swept across the globe resulting in the first pandemic of the 21st century. This has led to unprecedented health and economic crisis in many countries.

Believed to have originated in bats, this new zoonotic virus jumped species and is speculated to have reached humans via the live animal market in Wuhan. Soon after the viral genome was deduced in early January 2020, numerous research groups worldwide set to work on delineating the biology and molecular mechanisms. The sequence analysis showed that the virus is closely related to the SARS-CoV of the 2002 SARS outbreak. Although less fatal than earlier coronavirus outbreaks such as SARS and MERS, the rate of transmission has been higher. All countries have now implemented aggressive containment and control measures to minimize the spread. Expanding screening and diagnostics measures play an important role in this scenario.

Most of the diagnostic technologies can easily confirm the disease in symptomatic patients but several gaps remain in addressing asymptomatic carriers. Furthermore, cost-effective, rapid, point-of-care tests are the need of the hour for screening in low-resource settings. The RT-PCR is reliable to confirm patients in the window period but has its limitations. The longer turnaround time, the requirement of trained personals and shortage of VTM and assay reagents make it unsuitable for field testing. Serological tests, on the other hand, are fast and easy to use but can miss crucial pre-symptomatic carriers in the window period. In this regard, Reverse Transcription Loop-Mediated Isothermal Amplification (RT-LAMP) might be an alternative testing method but sensitivity and efficacy need to be evaluated. Meanwhile, a combination of active contact tracing, mass screening, and movement restriction can help to an extent. Some countries are also adopting pool testing to increase efficiency versus cost. In India, the ICMR recommends pool testing only in low prevalence areas with a maximum pooling of 5 samples.

Of all the current therapeutic strategies under development, drug repurposing seems to be the best-fit considering results in a

shorter timeline. Many drugs and disease management protocols are being adapted based on the experiences of earlier *betacoronavirus* epidemics. *In-silico* computational methods and artificial intelligence has greatly enhanced the capacity to identify lead molecules targeting viral antigens. Convalescent plasma therapy is quite a promising solution until the vaccine arrives, however, an appropriate facility for blood collection, storage, and cold chain transportation is necessary. Identifying patients with cytokine release syndrome is also extremely important to keep the fatalities low.

When it comes to vaccines, international coordination between scientists, regulatory bodies, policymakers, funders, health institutions, and governments is indispensable to ensure quality and availability in affected regions. The vaccines under development must be able to combat all the strains of SARS-CoV2 as new variants are expected to emerge throughout this pandemic. For this reason, a singular vaccine approach will not suffice. Some experts recommend the use of other vaccines like BCG against respiratory diseases to boost immunity and prevent co-infections. However, there is no solid evidence yet to support this theory. Also, IgG-induced long-term immunity against a secondary infection is also unsure at this point.

India had an initial slow trajectory of COVID19 cases compared to other hard-hit countries like the USA, Italy, and the UK. As the lockdown regulations were partially lifted, the number of cases rose steadily.

The first case reported in India was on January 30th, in Italy on January 31st and in the USA on Jan 21st. The local health authority in Kerala, the state where the case was first reported in India, ensured strict contact tracing and quarantine measures. Subsequently, in March, as other clusters were identified and the numbers started growing, the government of India enforced a nationwide lockdown which checked a COVID19 spurt. Countries like South Korea and Singapore employed stringent testing and tracking by establishing dedicated COVID19 centers which helped keep the numbers low. Once again, this attests that a quick response and early implementation of government policies is a major factor in determining the course of the epidemic.

How will India flatten the curve? Expanding the testing capacity and strengthening the healthcare infrastructure and network will be some of the decisive factors. The figures data show that the numbers are still on the rise. As expected, the easing of restrictions increased the number of cases. The lifting of lockdown was done in phases so that a rapid spike is prevented. Regions have been mapped as containment zones (red and orange), buffer zones, and green zones based on the number of active cases(www.mohfw.gov.in). The green zones, with no active cases, saw some relaxation and reopening of certain activities.

Conclusion

COVID19 is expected to remain for some more time, hence continuous efforts are required to ensure low mortality and flattening of the curve. There are also chances of a second wave if public activities and services are not well planned. Social distancing and the use of PPEs have become a new normal. In India persistent efforts also need to be focused on preventing wide-spread community transmission, especially in rural areas and places with poor sanitization and healthcare facilities. Several government-aided research institutions and pharma companies are at the forefront of developing cost-effective and easy-to-use diagnostic kits and vaccines in India. As a result of the COVID19 pandemic, we can anticipate an improvement in surveillance measures, enhancement of health service infrastructure, and better preparedness for future epidemics.

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