International Research Journal of Pharmacy and Medical Sciences
ISSN (Online): 2581-3277

SARS-CoV-2: Peculiarities of the Virus, the Evolving Scenario of its Diagnosis and the Scientific Journey of Hydroxychloroquine as a Potential Drug for COVID-19

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***Abstract*—** *The COVID-19 pandemic, originated in December 2019, is that of an unprecedented one. Since the outbreak in China, about 3 million cases of this infection have been diagnosed and over 200 thousand fatalities till the time of writing this article. There is yet no effective drug or vaccine in picture to help humanity combat this virus, SARS-CoV-2. The past couple of decades have witnessed endemic outbreaks in the form of MERS-CoV and SARS-CoV. During this existential challenge, it is crucial to know about the peculiarities and action of this virus within the host and also the diagnostics in place for detection of presence of this virus in the host. This article introduces a general overview of the virus, its comparison with previous endemic-causing coronaviruses, and its pathophysiology. It then explains the diagnostics that are developed and currently in practise- their principles and management strategies in India planned to increase their manufacturing- to test patients for COVID-19. This article also includes the scientific journey of Hydroxychloroquine through trials as a potential therapeutic option for COVID-19, citing methodology and results from crucial clinical trials around the world that speak for it as a therapy or otherwise.*

***Keywords*—** *COVID-19; Diagnosis; Hydroxychloroquine; RT-PCR; Rapid Antibody test; SARS-CoV-2.*

I. INTRODUCTION

Several local health facilities reported numerous patients with fatal pneumonia due to an unknown pathogen in late December 2019. It was later found that they all were epidemiologically linked to the Huanan seafood wholesale market in Wuhan, Hubei Province China. Consequently, the viruses were successfully isolated from several laboratories and by transmission electron microscopy imaging solar corona like virions were observed[1]; further identified as a betacoronavirus; categorizing it along with SARS-CoV (severe acute respiratory syndrome coronavirus) and MERS-CoV (middle east respiratory syndrome coronavirus). Formerly named as 2019-nCoV, the novel coronavirus was later renamed as SARS-CoV-2 by the Coronavirus Study Group of the International Committee on Taxonomy of Viruses[2]. On 31st January 2020, WHO declared the outbreak as a public health emergency of international concern, a Global Alert. In a mere time of about two months, the virus stayed successful in visiting 6 continents including 66 countries[3]. According to Johns Hopkins University, as of 26 April 2020, COVID-19, the pandemic disease has led to a global death toll of 203,331 and the number of confirmed cases is 2,908,527. Pharma giants, Biotechnology and Microbiology sector, Doctors, Academia, Policymakers, Governing and Regulatory bodies and institutions, are all working towards framing and implementing protocols for prevention of the rapid spreading febrile respiratory illness. The prophylaxis and anaphylaxis for COVID19 are still under development. On March 20, 2020, the WHO announced a global clinical trial called "Solidarity" for COVID-19 treatments comparing four therapies against the standard of care. The Indian Council of Medical research on 3rd April 2020, declared that India will also be participating in the Mega trial. [4] Vaccines for COVID-19 are said to hit the commercial markets around eighteen months from now. The world thus awaits a potential therapy that can help fight the fatal pandemic.

II. THE 21ST CENTURY CORONAVIRUS OUTBREAKS

SARS-CoV and MERS-CoV have been the well-associated viruses with the novel virus that the world has encountered in the 21st century. The process of understanding the trend in the current emergency can be speeded up if proper conclusions are drawn from a comparative analysis of these coronavirus outbreaks. A short relative study that highlights the similarities and differences in the disease progression, epidemiology and some other factors between the ß-coronaviruses is shown in Table I.

III. KNOWING THE VIRUS

SARS-CoV-2 or severe acute respiratory syndrome coronavirus 2 is the seventh coronavirus infecting humans causing COVID-19, the coronavirus disease. Other coronaviruses being HKU1, NL63, OC43 and 229E, cause mild symptoms; and SARS-CoV and MERS-CoV which have caused major outbreaks in 2003 and 2012 respectively.[5,22] SARS-CoV-2 is an enveloped virus (which makes it susceptible to deactivation by micellar action of soap water) with the membrane (M), nucleocapsid (N), envelope (E), spike (S), and hemagglutinin as primary structural proteins.[23] The virus particle is somewhat pleomorphic and spherical with the diameter ranging from 60 to 140 nm with characteristic spikes about 8 to 12 nm in length.[1]

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TABLE I. Comparison between SARS-CoV, MERS-CoV, and SARS-CoV-2[5-21]

S. No.	Criteria	SARS-CoV	MERS-CoV	SARS-CoV-2
1	Genetic similarity with SARS-CoV-2	79% identity to SARS-CoV	only 50% identity to MERS-CoV	88% sequence identity to two bat SARSr- CoV
2	Human cell Receptor	ACE2	DPP4 (CD26)	ACE2
3	Human cells primarily infected	Non-ciliated bronchial epithelial cells and type II pneumocytes	Non-ciliated bronchial epithelial cells and type II pneumocytes	Cells of subsegmental bronchial branches especially type II pneumocytes
4	Disease	Severe acute respiratory syndrome- SARS	Middle East respiratory syndrome- MERS	Severe acute respiratory syndrome (COVID-19)
5	Primary reservoir	Horseshoe bats	Bats	Not yet proved
6	Transmission from host	Infected civets and raccoon dogs to humans	Infected dromedary camels to humans	Not yet proved
7	Symptoms	A dry cough, a fever, diarrhea in the first or second week of illness	Fever, cough, and shortness of breath, severe respiratory illness	A fever, a dry cough, shortness of breath, phlegm production, fatigue
8	Therapy*	Subcutaneous (SC) injections of interferon (IFN)α, alfacon-1; Ribavirin with Lopinavir; Levofloxacin; Azithromycin; Ribavirin (oral/IV); Lopinavir/Ritonavir ± corticosteroids	Convalescent plasma (CP); intravenous immunoglobulin (IVIG), monoclonal antibodies; Ribavirin (with or without IFN, or corticosteroids); oral Lopinavir/Ritonavir	In clinical trials, Remdesivir (previously gave promising results in animal studies for MERS, and SARS); Lopinavir/Ritonavir; Interferon-beta-1a; Chloroquine and hydroxychloroquine
9	First cluster	China, November 2002	Saudi Arabia, September 2012	China, December 2019
10	Countries affected	Spread to 26 countries before being contained after about four months multiple outbreaks	Spread to 27 countries	Spread to every continent except Antarctica
11	R0**	2-5	2-5	1.5-3.5
12	Case fatality rate	9.6%	34.3%	1.38% to 3.4%
13	Median incubation period	5 days, 2-10 up to 14 days	2-14 days	5.1 days, can be up to 1-14 days
14	Total no. of cases and the death toll	8,439 confirmed cases and 812 deaths	2,519 2,519 confirmed cases and 866 deaths	More than 2 million people infected and 190,000 deaths as of 28 April 2020

*no approved specific vaccine or treatment for SARS, MERS; COVID-19 is yet explored
**subject to change according to the zone and time

The novel coronavirus is a single-stranded virion with a positive-sense RNA genome and belongs to a large family of zoonotic viruses called Coronaviridae, the largest family of the order Nidovirales. SARS-CoV-2 belongs to the betacoronavirus genera of the Orthocoronavirinae subfamily; the others being alpha, gamma, and delta coronavirus. CoVs are typically harbored in birds and mammals and are commonly found in bats, camels, cattle, cats and other animals.[6] The virus was first detected in Wuhan, China, from where it spread to the world over two months.[1] The high contagion of the virus can be explained by its transmission. As stated in a correspondence titled "Aerosol and Surface Stability of SARS-CoV-2 as Compared with SARS-CoV-1" in 'The New England Journal of Medicine,' on March 17, 2020, at NEJM.org., SARS-CoV-2 can survive in aerosols for 3 hours and up to 72 hours on hard surfaces like stainless steel and plastic. It can be transmitted through respiratory droplets or nasal secretions as well as via fomites. The Imperial Group has determined the R_o value or the basic reproduction number of the virus as 1.5-3.5.[24] Analysis of 21 reports retrieved by CEBM (Centre for Evidence Based Medicine) showed that 5% to 80% of people testing positive for SARS-CoV-2 may be asymptomatic infecting many more people, the exact track of which may even remain unnoticed. The virus dwells in the upper respiratory tract and can lodge itself in the lungs, further leading to the respiratory syndrome. According to WHO, the virus can cause severe illness and breathing difficulty in one out of every six infected people.

IV. THE ORIGIN

'Proximal Origin of SARS-CoV-2', a study published by Nature Journal, suggested three theories that could possibly explain the origin of SARS-CoV-2.[5]

a. Natural selection in the animal host before zoonotic transfer: Bats likely served as a reservoir (since bat coronavirus RaTG13 is 96% identical to the humans i.e. SARS-CoV-2) but their spikes or S protein (spike-like glycoprotein on the envelope of the virus) [25] do not bind efficiently to ACE-2. Some Pangolins coronaviruses exhibit strong similarity to SARS-CoV-2 in RBD (Receptor Binding Domain) and the RBD was found to be optimized for binding to human-like ACE-2. Although both coronaviruses have some strong similarity with SARS-CoV-2, neither of them has polybasic cleavage sites as found in the human coronavirus.
b. Natural selection in humans following zoonotic transfer: It may be possible that natural selection took place in favor of SARS-CoV-2 between initial zoonotic event and acquisition of polybasic furin cleavage probably a period of unrecognized transmission in humans.

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c. Selection during passage in human population: The virus might have acquired the mutations in RBD during adaptation to the passage from one cluster to other in human population.

V. Characteristic Features of SARS-CoV-2 Genome

The study also stated some notable features of the virus that might be the reasons we need novel therapies for the infection. Mutations in Receptor Binding Domain is one of the two key structural peculiarities of SARS-CoV-2. Structural and biochemical studies have proven that the RBD of SARS-CoV-2 has a high affinity for ACE-2 (angiotensin-converting enzyme-2) receptor. High-affinity binding of S (Spike) protein of SARS-CoV-2 is said to be a consequence of natural selection on a human or homologous ACE-2 that led to the rise of a new optimal binding interaction. This could be explained by computational studies which showed that the high binding affinity of SARS-CoV-2 for ACE-2 is not an ideal interaction and RBD sequence is different from that of SARS-CoV to be optimal for receptor binding. Researchers evidently thus propose that SARS-CoV-2 is not a product of purposeful manipulation; however, the assumption may be disproved upon appraisal of new theories.[5] The second characteristic feature is the polybasic furin and O-linked Glycan. Furin aka PACE (paired basic amino acid cleaving enzyme; an endoprotease responsible for recognition of the cleavage site sequence Arg-Xaa-Lys/ Arg-Arg, and catalyzing the hydrolysis of the precursors with basic amino acids Arg-Arg or Lys-Arg) allows effective cleavage at the junction of two subunits of S protein i.e. S1 and S2. The S1 domain mediates binding to the cognate host cell receptor and the S2 domain mediates the fusion events, between the viral membrane and host cell membrane. [6] The furin protein with oxygen-linked glycans determines the host range and infectivity of SARS-CoV-2.

The functionalities of SARS-CoV-2-specific-conserved-genes hold considerable significance in its virulence. While the E gene, majority of the which is localized at the site of intracellular trafficking, i.e. the ER, Golgi, and ERGIC, (ER-Golgi intermediate compartment) where it participates in CoV assembly and budding [26], governs the expression of the envelope protein which is one of the four structural genes of SARS-CoV-2, the RdRp gene (RNA Dependent RNA Polymerase gene)[27] is implicated in RNA polymerization or modification once the viral genome enters the host cell and is a subpart of the long chain of the alliance of 16 protein-expressing genes (NSPs or non-structural proteins) i.e. ORF1ab. Another structural gene, responsible for the expression of nucleocapsid protein is the N gene that keeps the viral envelope stable. Localization of N protein to the endoplasmic reticulum (ER)-Golgi region has advocated a function for it in assembly and budding. It is also involved in CoV replication cycle and the cellular response of host to viral infection.

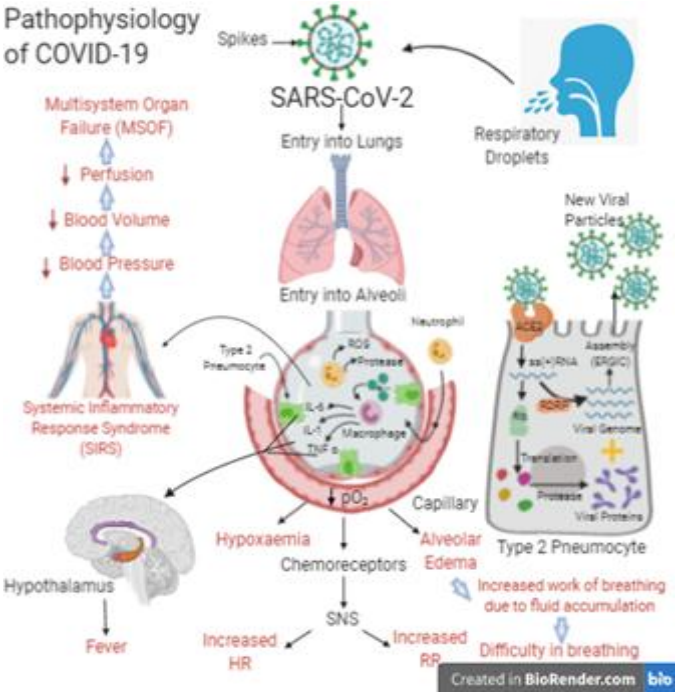
SARS-CoV-2 has a genome of about 29.9 kb and shares 79.5% and 96% identity with SARS-CoV and bat coronavirus, respectively.[28] The virus is said to exhibit genetic camouflage preventing its genes being attacked by host genes.[29] It might be molecular mimicry or structural camouflage of proteins and nucleic acid.[30] A genomic study revealed 98 nucleotide mutations at 93 sites over the entire genome among different SARS-COV-2 strains. Around 58 such mutations caused the change in amino acids, indicating a result of neutral evolution.[32] According to a study, the elicitation of a heterotypic response blocking S gene-mediated entry of SARS-CoV-2 into host cells accords with the genomic as well as the structural conservation of the spike protein along with the relative glycans shields of both SARS-CoV-2 and SARS-CoV. This insinuates that resistance against one virus of the Sarbecovirus subgenus can plausibly provide immunity against related viruses.[25] The mutations in S glycoprotein might induce its conformational changes, probably leading to alterations in the antigenicity of the virus. Characterization of the amino acids involved in conformational changes of the protein structure might aid in developing a new therapeutic strategy for COVID-19. [32]

VI. Pathophysiology of Covid-19

SARS-CoV-2 was reported to enter cells via binding to ACE2, followed by its priming by TMPRSS2 protease enzyme, just like the SARS-CoV.[27, 28] The entry of the virus into the host cell was initially thought to be involving direct fusion with the plasma membrane. A recent study reveals that the process of entry of SARS-CoV-2 is a pH and receptor-dependent endocytosis process. [29] Translation of viral proteins by the host cell machinery immediately takes place starting with large overlapping open-reading frames ORF1ab. The resulting protein is RdRp, the viral RNA-dependent RNA-polymerase, which along with polymerization, is also involved in generating sub genomic mRNAs. These comprise RNAs encoding the nucleocapsid protein N, the envelope glycoproteins E (small envelope protein), M (membrane protein), the S-protein and 8 proteins of unknown function. The assembly of the viral contents occurs in the ERGIC (ER-Golgi Intermediate Compartment). The E proteins are processed in the ERGIC and transported to the budding compartment. The membrane-protein associates with the helical N, E and S proteins. [34] The release of new viral particles is an endosome driven event. Endosome fuses with acidic intracellular lysosome leading to cell lysis thereby releasing new viral particles. [26, 33]

A study by Chen et al. refers to “cytokine storm” that is responsible for the weakening of the adaptive immune system against SARS-CoV-2 infection.[35] The immunological study gave three key findings. 1) T cell depletion and CD4+ T cell dysfunction, 2) CD4+ and CD8+ T lymphopenia (very low in severe conditions), and 3) Increased overproduction of IL-6, IL-2R, IL-10 and (TNF)-α (prominently high in severe conditions). COVID-19 patients asymptomatically spread the illness even before diagnosing themselves as positive for SARS-COV-2 presence due to the delayed emergence of signs and symptoms. The collapse of the anti-viral immunity of the host body further causes respiratory distress and rapid complications that may include fatal pneumonia. This might be one of the several reasons that make SARS-CoV-2, a deadly virus.

According to a Chinese study,[36] percentage of cases dealt with mild symptoms (non-pneumonia and mild pneumonia); patients with severe conditions about 14% of cases, experienced dyspnea, a high respiratory frequency (≥ 30/min, normal range: 12-20/min), low blood oxygen saturation (SpO2 ≤ 93%, normal range: 95-100% for healthy lungs), a low PaO2/FiO2 ratio or P/F [the ratio between the blood pressure of the oxygen (partial pressure of oxygen, PaO2) and the percentage of oxygen supplied (fraction of inspired oxygen, FiO2)] < 300 (normal: about 500), and/or lung infiltrates > 50% within 24 to 48 hours. In 14% of cases, patients with critical disease suffered from respiratory failure, septic shock, and/or multiple organ dysfunctions (MOD) or failure (MOF).[38]



Some laboratory abnormalities that were encountered in COVID-19 patients as reported by a study, precise with other findings, have lymphopenia as the hallmark of the pandemic disease; especially the peripheral CD4 and CD8 T cells are found to be substantially reduced. CD8 cells are also found to contain a considerable number of cytotoxic granules. Neutrophil count, lactate dehydrogenase (LDH), alanine aminotransferase (ALT), aspartate aminotransferase(AST), total bilirubin, creatinine, cardiac biomarkers, D-dimer, prothrombin time (PT), procalcitonin (because of bacterial superinfection; higher in ICU patients than the ones not in ICU), C-reactive protein (CRP) are found to have elevated values whereas, lymphocyte count (hallmark) and albumin levels are decreased significantly.

The symptoms of the disease can take longer to develop. Most infected people show either mild or no symptoms for up to 2 weeks, which makes it difficult to control the infection and hence for proper surveillance of the COVID-19, efficient management strategies are a must.

Antiviral agents are currently being explored in trials. Some of these antivirals work by blocking the entry of the virus into the host cells, some block the viral replication, while some delay the response of the immune system. [37] Antiviral agents may not be a standalone factor to stop the cytokine storm, pulmonary destruction and respiratory distress in COVID-19 patients who present late after infection. Targeted immunomodulation reduces the cytokine storm which may ameliorate pulmonary inflammation and likely improve mortality.[35] Further studies on viral factors driving immune dysregulation may provide insights into shaping vaccine responses toward defensive immunity. It is still a long go through many phases of clinical trials of different drugs against the standard of treatment until we find a potential therapy to combat the deadly pandemic disease of COVID-19.

VII. Diagnostic Aspect of the Pandemic in India

Until a potential therapy and/or a vaccine come into the picture, the only way to control the contagion is rapid and accurate diagnostics and strategies like strict lockdown and quarantine. This article discusses the prevalent diagnostic techniques, their development and manufacturing, and maneuverings adopted by the Indian health and research body for proper superintendence and surveillance of the pandemic in the nation.

On 12th January 2020, SARS-CoV-2 genome sequence was published on NCBI Genbank portal. Up to February 24, 2020, 129 full-length genomes were posted on the GISAID and NCBI Genbank (virological.org), which had limited sequence alterations between posted sequences of different patient samples by different laboratories. The highly specific region of SARS-CoV-2 was found soon after the genome went public on the portal. This could serve as potential target genes for nucleic acid tests, although 60-90% part of SARS-CoV-2 genome sequence is highly conserved resembling other coronaviruses. RdRP, E and N genes in this region are the unique sequences that do not match with any other known beta coronaviruses. RT-PCR is based on the detection of these unique sequences of the virus and is now the most accurate and widely used nucleic acid test for the diagnosis of SARS-CoV-2. [6] Other than RT-PCR, Rapid antibody test is the chief screening method employed in India.

The Prevalent Diagnostics: Most countries use RT-PCR assay for diagnosis. Immunoassays, molecular assays, serological tests, rapid antibody tests and other screening methods have been developed by scientists to supplement the gold standard frontline test for COVID-19 detection. While some are in development, many have been approved by different regulatory bodies like FDA (Food and Drug Administration), HSA (Health and Safety/Science authority), MFDS (Ministry of Food and Drug Safety), NRA(National Regulatory Authority), TGA (Therapeutic Goods Administration), WHO EUL (World Health Organization Emergency use Listing Procedure), EUA (Emergency Use Authorization). Some diagnostics have been developed for research use only.[39] There are about 500-600 US FDA and/or CE approved

diagnostic kits, most of which are RT-PCR and antigen tests based on IgG and IgM.[39]

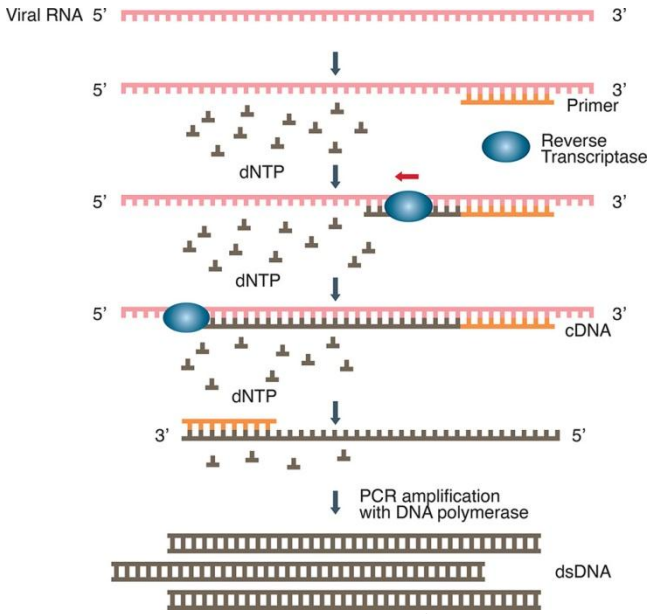
The RT-PCR or Real-Time Reverse Transcriptase-Polymerase Chain Reaction is the 'gold standard' frontline test for early detection of SARS-CoV-2. The National Task Force at ICMR recommends RT-PCR test and Point-of-care molecular diagnostic assays for diagnosis of COVID-19 among individuals who are symptomatic and asymptomatic with direct or high-risk contacts of confirmed cases, on the basis of available evidence and revised testing strategy. In India, besides RT-PCR, other ICMR approved screening tests are Rapid Antibody test, TrueNat™ beta CoV and the CBNAAT.[49] The Rapid antibody test is exercised to assess the ubiquity of the infection within a specific area and is not a sole means of diagnosis of COVID-19. It detects the antibodies IgG and IgM generated as an immune response. The positive samples are reconfirmed with the help of TrueNat™ beta CoV by carrying out a separate confirmatory assay for SARS-CoV-2, for which the throat or nasal swabs are collected in a viral transport medium (VTM) with virus lysis buffer. It has been found that the buffer can neutralize Nipah and h1n1 virus; results of stability of SARS-CoV-2 RNA henceforth are anticipated from ICMR- NIV (National Institute of Virology), Pune. Until then TrueNat™ beta CoV screening test is permitted only to be performed with specified Biosafety levels (BSL 2 or BSL 3) in setups at laboratories.[59] The CBNAAT or Cartridge Based Nucleic Acid Amplification Test is an FDA approved test that has a principle like that of RT-PCR. It is carried out using Cepheid Xpert Xpress SARS-CoV2 for use only under Emergency Use Authorization (EUA) and any lab with functional RT-PCR testing and BSL-2 can carry out CBNAAT without ICMR approval. It is primarily a diagnostic method for Tuberculosis and its drug-resistant forms in Eastern and Central countries. The WHO suggests that countries would need to develop targeted approaches for COVID-19 testing in TB patients, including those with the prior disease.[55]

The RT-PCR Test:

COVID-19 RT-PCR or real-time reverse transcription-polymerase chain reaction test uses the TaqMan fluorogenic probe-based chemistry applying 5' nuclease activity of Taq DNA polymerase hence facilitating the detection of a specific PCR product as it accumulates during PCR cycles. The RNA is isolated from upper and lower respiratory specimens such as bronchoalveolar lavage, nasopharyngeal or oropharyngeal swabs or wash/aspirate, sputum and nasal or upper or lower respiratory tract aspirates (recommended). The swabs are placed in separate sterile tubes with viral transport media (VTM) for preanalytical storage before the assay. The COVID-19 real-time PCR assay- Multiplex Multiplexed assays contain primer/probes sets specific to different SARS-CoV-2 genomic regions and primer/ probes for phage MS2 as internal process control, for nucleic acid extraction. The WHO suggests the following protocol for the workflow of RT-PCR.[41]

I. First-line screening assay- E gene assay
II. Confirmatory assay- RdRp gene assay
III. Additional confirmatory assay- N gene assay

The viral RNA is reverse transcribed to cDNA and amplified. As the probe anneals to a specific target sequence located between the forward and reverse primers, it eventually degrades in the extension phase due to the 5' nuclease activity of Taq polymerase and the probe (dye) that separates from the quencher dye, thereby generates a fluorescent signal. [42]



Source: Assay Techniques and Test Development for COVID-19 Diagnosis

The first RT-PCR test was designed and developed by WHO in January 2020, soon after the virus was identified. It requires 4-6 hours to complete and costs about Rs 4500. Different laboratories and institutes are developing faster and more accurate RT-PCR tests for early detection of the deadly coronavirus.

RT-PCR technique can provide quantitative as well as qualitative analysis with a significantly small amount of RNA required for gene expression studies. Along with its high specificity, the technique is sensitive to contamination and a robust and well-documented method, making it suitable for an unrestricted and reliably accurate diagnosis. Though RT-PCR is the key diagnostic for COVID-19, it is apprised that the agent detected may not be the definite cause of disease. It cannot provide information on other diseases or symptoms and does not exclude co-infection of other microbes.[41] Hence, negative results do not impede SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be consolidated with patient history, clinical investigations, and epidemiological data. Since the test relies upon capturing and detecting the virus, it is possible to miss patients who have cleared the virus and recovered from the disease, thus indicating only currently infected patients as positive for SARS-CoV-2.[43] The virus may only be detectable in sputum or nasopharyngeal swab but not necessarily at both locations at the same time in an infected person, which clearly indicates the likelihood of false-negative results. This is because of the varied distribution of the virus across the respiratory tract that differs individually. [42]

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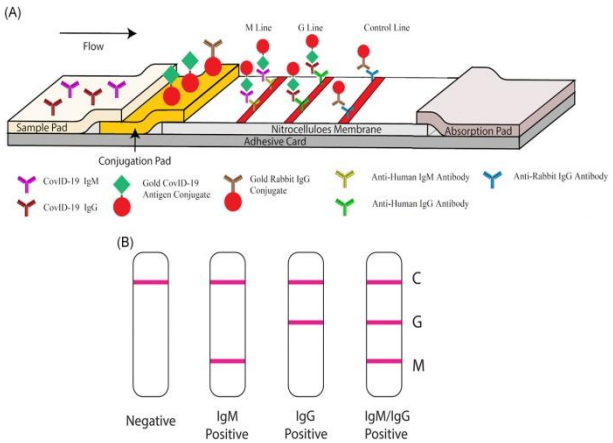
The Rapid Antibody Test

The Rapid Antibody test is based upon the principle of an immunochromatographic assay and determines the antibodies produced by the body as an immune response to infection. It can be performed using the serum, plasma or venipuncture of whole blood specimens. The qSARS-CoV-2 IgG/IgM Rapid Test Cassette detects antibodies against the SARS-CoV-2 virus. The test comprises of 1) SARS-CoV-2 recombinant conjugates and rabbit IgG-gold conjugates; and IgG test line with anti-human IgG, 2) an IgM test line with anti-human IgM, and 3) control line C with rabbit IgG immobilized on a nitrocellulose strip. When a specimen is followed by assay, the buffer is added to sample well, IgG and/or IgM antibodies, if present, will bind to SARS-CoV-2 conjugates (agglutination) which relocates through nitrocellulose membrane by capillary action. When the complex meets the threshold coinciding with the line of immobilized antibody (anti-human IgG or anti-human IgM), it is arrested as a Burgundy colored band, thereby confirming a reactive or positive test result.[44]



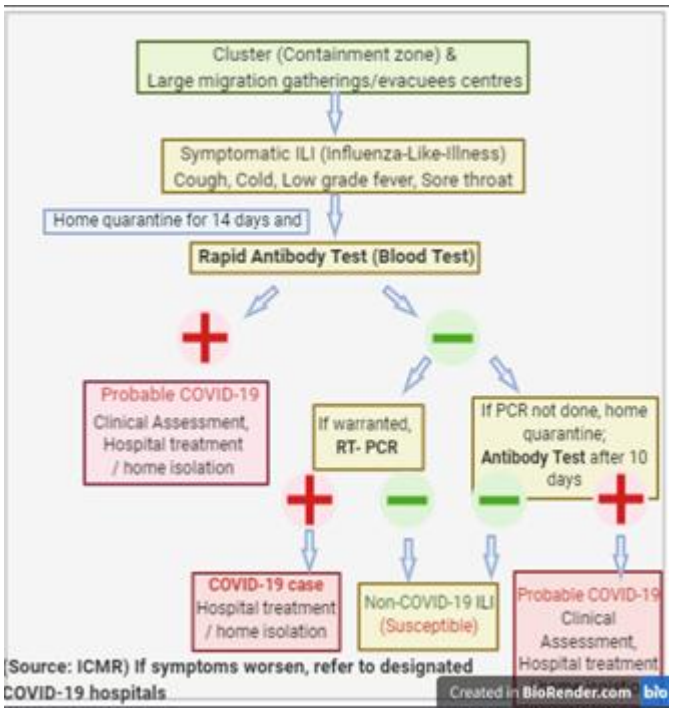
Source: Development and clinical application of a rapid IgM-IgG combined antibody test for SARS-CoV-2 infection diagnosis Zhengtu Li, et.al. 2020.[44]

Serological or hematological testing for detection of the antibodies IgG or IgM is aimed to identify clusters in the global spread of COVID-19 thus serving the epidemiologic and disease surveillance purposes. The test result is typically available within 30 minutes and costs about Rs 400 per test. It comes positive after 7-10 days of infection and remains positive after several weeks after infection. IgM appears in blood within 3-5 days of infection whereas IgG may take as long as 1-2 weeks. Qualitative detection of antibodies indicative of SARS-Cov-2 infection is used as an aid for diagnosis of SARS-Cov-2 infection furthermore, non-reactive or negative test results do not rule out COVID-19 infection.[44]

This test quantifies human SARS-CoV-2 antibodies, IgM (1st line of antiviral defense, prior to adaptive IgG responses) and IgG (significant for long term immunity and immunological memory), generated as an immune response to the infection. Knowledge concerning the immune response to SARS-CoV-2 is insufficient and still unfolding; it is hence unclear how long IgM or IgG antibodies may endure following the infection. This lateral flow assay allows a single patient sample from part of the body where sampling is non-invasive to be tested for the presence of the virus and gives quick results. So far, available tests can only determine if a patient has at some point been infected with COVID-19 and rules out the limitation of RT-PCR assay of not being able to indicate inactive infections. Further testing would be needed to check if a patient is currently infected.[41]

Implementing Bodies and Manufacturing Companies in Action

As of 30 April 2020, according to the Ministry of Health & Family Welfare (MoHFW), a total of 23651 COVID-19 cases, (including 111 foreign nationals) have been reported in 32 states/union territories. 8324 have been discharged with 1074 deaths.[40] Although the long meticulous process of finding solutions to the inevitable pandemic has been fast-tracked almost everywhere; it is still difficult to bring down the number of cases rising significantly day by day. Quick and efficient diagnosis and urgent actions are a must for management of the global public health emergency. Rapid Antibody test kits have been employed for sero-surveillance, as a strategy for areas reporting clusters (contaminant zone) and in large migrations gathering or evacuees' centers.[48] Fig. 2 shows the strategy adopted by the Indian apex health research body for testing and diagnosis.



To suffice for the rising number of COVID-19 cases, efforts are being taken by the central governing bodies to account for enough kits for diagnosis. For uninterrupted supply of reagents and efficient distribution to testing laboratories in this emergency, 16 depots have been established across the nation, modeled into self-contained units by strengthening manpower resources and infrastructure. NIMR (National Institute of Malaria Research), New Delhi

and NIV, Pune along with ICMR, function as central depots.[57] The ICMR with 5 Centeres of Excellence[61] are also constantly evaluating the laboratories for the screening process. As of 29 April 2020, total operational (initiated independent testing) Government laboratories reporting to ICMR are 292, amongst which are 244 RT-PCR labs, 41 TrueNat Test labs and 7 CBNAAT Test labs.[49] As per the cost is concerned, approved Government laboratories charge Rs. 1,500 ($20) for an initial screening and Rs. 3,000 ($40) for an additional confirmatory test. Private laboratories which have been approved screening have been allowed to charge not more than Rs. 4,500 ($60).[47] In a report, ICMR stated that country has capacity of 60,000 tests a week. The daily COVID-19 testing capacity in the country is currently very low as compared to other countries, with about 830,201 tested samples as of April 30 and is expected to reach 1 lakh tests per day by 31st May 2020. As part of its efforts to increase the testing volumes, ICMR approved the use of diagnostics used for testing drug-resistant TB for conducting COVID-19 tests. Although the Government's efforts to scale it up by allowing a good number of private labs to do the test hit a dead-end due to limited availability of kits for the RT-PCR tests, the Union Health Ministry assured that by May 2020, 10 lakh indigenous RT-PCR kits will be available. [55, 56]

To meet the increased requirement of testing material with an increasing number of tests, the current mode of inventory stocking and distribution is scaled up significantly. Globally, Pharmaceutical and Biotech companies like Thermo Fischer Scientific, Quest Diagnostics, Roche Diagnostics, Qiagen, are ramping up the capacity to provide a greater number of test kits and services. According to a leading News report, India is focusing on acquiring medical supplies as priority items from countries like China, South Korea, France and Israel among others as part of its efforts to contend the COVID-19 crisis. In addition to 6.50 lakh, antibody test and RNA Extraction kits sent from Wondfo Biotech Co., Guangzhou and 250,000 from Zhuhai Livzon Diagnostics Inc., China; more 3 lakh Rapid Antibody test kits besides 100,000 RNA extraction kits from MGI Tech Co. from Shenzhen, as cleared by the Chinese customs were imported to India by mid-April for adequate supplies.[49] At least four multinational manufacturers of COVID-19 test kits are gearing up to supply more than a million diagnostic kits to India. The multinational companies Seegene, Thermo Fischer, and Siemens and Altona, were expected to start supplying Covid-19 test kits in early April.[51] The probes for diagnosis of COVID-19 RT-PCR are currently being procured from the USA and are supplied to the government testing laboratories across the nation. The approvals for antibody-based rapid kits were granted to many international Biotech and Pharmaceutical Companies such as BioMednomics (USA), Sensing Self Ltd (Singapore), Biomaxima (Poland), and Wondfo (China).

TABLE II. Work up in the number of diagnostics[51, 52, 53]

	Manufacturing Companies	Work-up in Number of Diagnostic Kits
1	MyLab	Raise from 1,50,000 to 2 million per week
2	Medsource Ozone	First set of kits in mid-April with 300,000 per week
3	3B BlackBio Biotech	Ramp up from 25,000 to 100,000 tests by the end of April
4	Kilpest India	Increase from 500,000 to 1 million tests over 3 months
5	Helini Biomolecules	Scale-up from 25,000 to 100,000 by early May
6	Voxtur Bio	Can produce 10 million tests a month, by the end of April
7	Thermo Fisher Scientific (US-based Company)	Working closely with the Indian government as well as private testing laboratories to support scale-up testing by reagents
8	Seegene (South Korean firm)	Supply of about one million kits to India by the end of April
9	Siemens (Germany)	The first lot of 30,000-40,000 tests to ICMR for free testing under Siemens Fight Covid-19 initiative; nearly 100,000 tests available in India by mid-April

A glimpse of the expected increment in the number of testing kits as promised by some of the key Indian laboratories and International Companies (approved to export medical supplies and health equipment to India)

However, as it goes without saying, there are few challenges at the ground level concerning the acquisition of supplies and performing diagnostics. While the insufficiency of raw materials and ingredients; high procurement prices of equipment from Europe and East Asia, especially China and Japan; packaging issues, etc., is concerning the manufacturer; there are unprivileged and poor who, in spite of being exposed to the deadly virus, cannot afford the diagnostic tests. On April 8, Supreme Court even ordered private laboratories to offer COVID-19 tests for free, but later clarified that patients who can afford the test should pay and free testing should be only done for the poor. Another major challenge has been the imprecision reported in the imported kits from Guangzhou Wondfo Biotech and Zhuhai Livzon Diagnostics ranging from 6-71% aberration in the accuracy of the test results. Some complaints were even raised about the RT-PCR tests working improperly and thus demanding repeat testing. ICMR even issued a two-day break for validation of the results of the Rapid antibody testing kits by the ICMR expert team in field conditions, the results of which showed wide variation in the sensitivity, notwithstanding the early promise of good performance for surveillance purposes. Consequently, state administrations were advised to discontinue the use of these particular kits and return them to be sent back to the supplying companies.[60]

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Further Research Initiatives

After successful isolation and sequencing of SARS-CoV-2 at ICMR, scientists are engaged in research studies and planning to give perspicacity into the virus and COVID-19 prevention and management. The apex health research body has collaborated with other science and research institutes (CSIR, DST, DAE, ICAR, DRDO, DBT) for developing research solutions to the situation. A National Task Force has been set up with five key divisions focusing upon different aspects of the pandemic, thus initiating organized and efficient research studies. The divisions comprise clinical research, diagnostics and biomarkers, epidemiology and surveillance, operation research, and vaccines/drug research and development. A review committee to formulate national task force studies has been constituted at the national level.[54] A recent call for intent by ICMR, for the study titled "A Phase II, Open-Label, Randomized Controlled Study to Assess the Safety and Efficacy of Convalescent Plasma to Limit COVID19 Associated Complications" suggests that Plasma therapy if proven, can be one of the therapeutic options; however, the results are awaited.[49, 62] Research proposals were also invited with some areas focused on the theme of mission mode translational immunology: Novel diagnostic approaches for patient stratification and risk assessment for the severity of sequelae; Immunogenetics and molecular epidemiology based population studies on COVID-19 – consortium approach Biologics or small molecule-based modulation of the immune system for therapy or prevention; Cell-based approaches for treatment or prevention of COVID-19 and associated disease sequelae: for fast track funding for Translational Immunology and Cellular; Therapeutics approach targeting COVID-19. [63] The Government is also working with global partners on vaccine development. The participation in WHO: Public Health Emergency Solidarity Trial, an international randomized trial of additional treatment in hospitalized patients may hasten the development of potential therapies in India.

VIII. Journey of Hydroxychloroquine Through Trials Around the World

Hydroxychloroquine, an aminoquinolone class of drug is used to prevent and treat malaria caused by mosquito bites. In addition, it is also used as a treatment drug for auto-immune diseases like Rheumatoid Arthritis, Discoid and Systemic Lupus Erythematosus, Juvenile Idiopathic Arthritis. These remain the FDA approved therapeutic indications of HCQ.[64] HCQ comes with an associated risk of precipitating arrhythmias and other cardiac effects in patients, especially in ones with elongated QT interval along with other side-effects of hepatic impairment, renal impairment, severe hypoglycemia, adverse hematological effects, etc.[66]

FDA, as of 9th April 2020, accelerated the development and use of HCQ along with Convalescent Plasma and Hyperimmune Globulin as treatment measures for COVID-19.[65] It has issued an Emergency Use Authorisation (EUA) to permit the use of HCQ-Sulphate to treat adolescents and adults who are hospitalised with COVID-19 for whom a clinical trial is not available, or participation is not feasible.[66]

The use of HCQ for treatment of COVID-19 so far has come in a steady stream of scientific studies, often as 'preprints.' None of the studies that have been released meet the aureate standard for demonstrating a drug's effectiveness- a large scale, double-blinded randomized controlled trial (RCT), though several trials are on their pathway to meet the same. While everybody awaits the results of these trials, this article tries to mention some of the studies that have been released so far that prove HCQ's therapeutic efficacy to treat COVID-19, and otherwise.

On 4th of February 2020, the journal 'Cell Research' published a letter to the editor by Chinese scientists.[67] The report contained the results of their experiments which were designed to assess the efficacy of existing drugs against SARS-CoV-2. They evaluated anti-viral efficacy of five FDA-approved drugs which were Ribavirin, Penciclovir, Nitazoxanide, Nafamostat, Chloroquine and two well-known broad-spectrum antiviral drugs Remdesivir (GS5734) and Favipiravir (T-705) against a clinical isolate of 2019- nCoV in vitro.[67] Their efficacies were evaluated by quantifying the viral copy numbers in the cell supernatant by quantitative real-time PCR (qRT-PCR) and were then confirmed with visualization of viral nucleoproteins (NP) expression through immunofluorescence microscopy. This in-vitro study reported promising results of Remdesivir and Chloroquine as 'highly effective in the control of 2019-nCoV infection in vitro.

This report that claims the apparent efficacy of Chloroquine's inhibition of SARS-CoV-2 is largely in vitro. Researchers have gotten similar promising results with HCQ against various viruses in past in-vitro studies, including SARS-CoV, but have yet to show its in vivo effectiveness in RCTs.

A substantial reason for media coverage of HCQ was the French study which purported to show the action of combination of HCQ along with Azithromycin, a common antibiotic, leading to a significant reduction in viral load for patients with COVID-19. This study, unlike the above-mentioned was a clinical trial, involving actual patients and its publication in the International Journal of Antimicrobial Agents (IJAA) underwent peer review. In this trial, the role of HCQ on respiratory viral loads was evaluated. The patients received 600 mg of HCQ daily from early March to March

16th and their nasopharyngeal swabs were tested daily in a hospital setting.[68] Azithromycin was added to the treatment depending on the patient's clinical presentation.[68] Untreated patients who refused treatment were considered as negative control and the presence or absence of virus at Day-6 post inclusion was considered as the end point.[68]

Twenty patients that were treated in this study showed reduction in viral load compared to control group and much lower average carrying duration than reported of untreated patients. The report also suggested that Azithromycin added to HCQ treatment was significantly more effective for viral load reduction.[68]

There were several loop-holes cited in this study such as the down-playing of clinical outcomes, where instead of focusing on whether or not the patients improved, got worse or died, the study based their analysis on whether the virus' presence was being detected in nasal swabs, measuring how long the patients were shedding the virus. Hence, many reports showed that this article was mischaracterizing the drug as a '100% treatment'.[69] Two weeks after the study was published online, the International Society of Antimicrobial Chemotherapy which published the IJAA released a statement saying that the board 'believes the article does not meet the Society's expected standard, especially relating to the lack of better explanations of the inclusion criteria and the triage of patients to ensure patient safety'.[70]

On April 6, a group of Chinese scientists published a study (pre-print) of 62 patients with mild cases of COVID-19 diagnosed and admitted in Renmin Hospital of Wuhan University.[71] Unlike the French-trial, this trial was randomized and had comparable treatment and control groups. Time to clinical recovery (TTCR), clinical characteristics, and radiological results were assessed at baseline and 5 days after treatment to evaluate the effect of HCQ. The findings of this study reported that TTCR, body temperature recovery time and cough remission time were significantly shortened in the HCQ treatment group, two patients of which had mild adverse reaction.[71]

However, in this trial, to prove clinical efficacy the number of patient population was low, and it did not include patients with severe or critical stage of COVID-19. The study authors concluded that their study and its results 'partially confirmed' the potential of HCQ as a treatment and said large scale trials were still needed.

Meanwhile, awaiting a large-scale clinical trial, a French retrospective analysis study of real-life patients to emulate clinical trials inferred that their results did not support the use of HCQ in patients hospitalized for documented SARS-CoV-2-positive hypoxic pneumonia. The data included the collection of routine care of 4 hospitals in France where COVID-19 positive patients were admitted and the trial aimed at assessing the efficacy of HCQ at 600 mg/day.[72] The study included 181 patients out of which 84 received HCQ treatment within 48 hours of admission (HCQ group) and the rest 97 who didn't (Control group). It was seen that 16 patients in HCQ group were transferred to the ICU or died within 7 days vs 21 in the Control group and 24 patients in the HCQ group developed acute respiratory distress syndrome within 7 days, vs 23 in the Control group. Also, 8 patients in the receiving HCQ demonstrated electrocardiogram modification requiring HCQ discontinuation.[72] Hence, their study concluded by reporting no efficacy of HCQ in COVID-19 patients.

However, retrospective analyses are not a substitute for Randomized Clinical Trials. Also, a confounding factor is that the researchers were unknown regarding the physician's rationale behind treating a patient with HCQ or not, hence not giving a substantial idea for the drug's efficacy.

In mid-April, a group of researchers in Brazil posted a preprint indicating their decision to halt clinical trial of Chloroquine at high doses after they observed high incidences of arrhythmias, a well-known side effect of CQ and HCQ, and deaths.[73, 74] The study had a predefined sample size of 440 patients, out of which, 81 were enrolled- 41 to high dosage group and 40 to low dosage group. The results reported viral RNA in 31 out of 41 patients in the high dosage group and 30 out of 41 patients in the low dosage group on Day-4. Lethality on Day-13 was found to be 16 (39%) in the high dosage group vs. 6 (15%) in the low dosage group, also the high dosage group presented with higher occurrences of QTc interval (7 out of 37, 18.9%) vs the low dosage group (4 out of 36, 11.1%).[73, 74] Hence, the paper recommended that HCQ be used only in critically ill patients because of its potential safety hazard.

An accompanying editorial by three American physicians advised that the results 'should prompt some degree of skepticism toward the enthusiastic claims about chloroquine and perhaps serve to curb the exuberant use'.[75]

However, the Brazilian clinical trial was designed to compare the efficacy of two specific dosage forms of the drug, and it did not include a control group who were administered placebo. Trials for a low dose of CQ continue to occur to gauge its efficacy. Also, they were not able to conclude definitively that the cardiac side effects were only due to CQ.

Similar to the retrospective analysis done by the group of French researchers, a retrospective analysis was conducted by researchers in US where they studied the outcomes for 368 patients who were treated for COVID-19 in the United States Veteran Health Administration medical centers until April 11. Patients were categorized into- patients who received HCQ (97), patients who received HCQ+Azithromycin (113) and those who didn't receive HCQ (158).[76] Their study found that patients who received HCQ, with or without Azithromycin did not lessen their need for a ventilator or reduce the risk of death.[76] Their results also stated, compared to no HCQ group, the risk of death from any cause was higher in the HCQ group and that risk of ventilation was the same in HCQ and HCQ+Azithromycin group.[76] They concluded that HCQ, with our without co-administration of Azithromycin, was not found to reduce the risk of mechanical ventilation in patients hospitalized for COVID-19.

As with the French retrospective analysis, this analysis too isn't a substitute for RCTs. A major confounding factor is that the patients who received HCQ in this trial were severely or critically ill. A panel put together by the National Institute of Allergy and Infectious Diseases, led by Dr. A. Fauci, said that

Aditya Mhatre and Sakshi Kasat, "SARS-CoV-2: Peculiarities of the Virus, the Evolving Scenario of its Diagnosis and the Scientific Journey of Hydroxychloroquine as a Potential Drug for COVID-19," *International Research Journal of Pharmacy and Medical Sciences (IRJPMS)*, Volume 3, Issue 4, pp. 7-18, 2020.

there was insufficient data to 'recommend either for or against' HCQ.

On April 24, the FDA issued a 'Drug Safety Communication Warning' against use of HCQ outside hospital setting and stated- "Hydroxychloroquine has not been shown to be safe and effective for treating or preventing Covid-19."[14]

The above-mentioned clinical trials and their shortcomings only highlight the importance of awaiting the results of ongoing, prospective, randomized, controlled studies before widespread adoption of the drug.

IX. Conclusion

The COVID-19 pandemic which is growing at an alarming rate has shown the unprepared side of even the developed countries to fight against this virus. Currently there are no specific vaccines or treatments for COVID-19. Until the proven efficacy of any drug candidate, containment of the virus and prevention of further spread are the only viable options to check the ongoing outbreak. In the absence of approved vaccine or therapy, isolating the infected patients and quarantining the suspected ones are the only effective strategies to control the spread. In times of duress, it is invariably the scientists and companies engaged in Research, Development and Manufacturing of drugs and vaccines who are urged to find a solution with the shortest timeline possible.

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