Review Article

# A Progressive Approach on Lowering COVID-19 Transmission

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## Abstract

Coronavirus disease 2019 (COVID-19) is rapidly expanding in the major parts of the world like U.S.A, Spain and Europe and to respond to it, various countries are using national lockdown strategies and uncompromising restrictions on international travels with the intension to prevent the local transmission. Real time RT-PCR was believed to be "gold standard" but proved to be error prone, pointing out the need for an accurate diagnostic tool like ELISA based assay of immune responses. Several drugs have proved efficient against COVID-19 namely, Favipiravir, Remdisivir, Chloroquine and Monoclonal Antibodies. Most of the countries have been very effective in enhancing the survival rates using such diagnostic tools and treatment methods like Convalescent Plasma therapy. This review paper discusses the overall comparison of the severity of cases in the most affected parts of the world, variations in symptoms, effects of medical history, major treatment methods and measures taken in India to control this global pandemic effectively with least mortality rate (3%) when compared with the countries like U.S.A and Spain. Even when a higher recovery rate (22.5%) was reported in India, enhancing self-resistance by maintaining personal hygiene and distancing from public gatherings are necessary to avoid community spread of the epidemic.

**Keywords:** Convalescent Plasma therapy; ELISA based humoral assay; Indian Strategy; Effect of Past Medical History; Major drugs in use.

## Abbrevations

COVID-19 – Corona Virus Disease – 2019

CrPC- Criminal Procedure Code

ELISA- Enzyme-Linked Immunosorbent Assay

H1N1- Hemagglutinin Type 1 and Neuraminidase Type 1

IgG- Immunoglobulin G

IgM- Immunoglobulin M
IL-6- Interleukin-6
LFIA- lateral-flow immunoassay
mRNA- Messenger RNA
Real time RT-PCR- Real time ReverseTranscription-Polymerase Chain Reaction
rRNA- Ribosomal RNA
SARS-CoV2- Severe Acute Respiratory Syndrome-Corona Virus 2
TNF-α- Tumour Necrosis Factor- α
W.H.O- World Health Organisation
MERS- Middle East respiratory syndrome coronavirus

## Introduction

During the past 4 months, a devastating epidemic, coronavirus disease 2019 (COVID-19) has been reported and is rapidly expanding in U.S.A, Spain, France, Italy and Europe with the initial confirmed cases being identified in Wuhan, China. Due to the alarming levels of spread, severity and inaction, on March 11, 2020, the Director-General of W.H.O, categorised COVID-19 as a global pandemic [1, 2]. The onset of the disease was characterised by signs of dry cough, high fever and breathing difficulty while the affected COVID-19 patients with a medical history of cancer, diabetes mellitus, hypertension and cardiovascular disease were in most critical situation [3].

Initially the determination of presence of COVID-19 infection was carried out using real-time RT-PCR kits and it is a technique used to quantify mRNA in the given sample and was believed to be the "gold standard" method which was very sensitive and specific. While in the case of total RNA quantification, the technique determines no variation in rRNA/mRNA ratio [4]. Even when the technique was widely accepted, there were many false positive and false negative results reported in various tests thus pointing out a need for better detection techniques of the target gene of interest [5].

Chloroquine is an anti-malarial drug while it has also been reported as a potential broad-spectrum antiviral drug due to its immune-modulating activity like suppressing the secretion of TNF-α and IL-6 [6-8]. It prevents the viral infection by increasing the pH required for virus fusion and also interferes the glycosylation of cellular receptors. Being used for more than 70 years, chloroquine is a safe and cheap drug and is clinically potent against SARS-CoV2. Hydroxychloroquine, an analogue of chloroquine has high clinical safety profile and it exhibited anti-SARS-CoV activity in vitro studies [9]. In a study conducted on 36 COVID-19 patients, azithromycin added to hydroxychloroquine exhibited significantly a higher rate of virus elimination [10]. Similarly, Chloroquine phosphate is a potent drug against COVID-19 associated pneumonia and the National Health Commission of the People's Republic of China advised it for the treatment [11-15]. Remdisivir is a mono phosphoramidate prodrug and a broad-spectrum antiviral agent which is an adenosine analogue. In a recent study, Remdisivir inhibited SARS-CoV-2 replication in Vero-E6 cells and several other studies showed that it is effective against Bat-CoV, MERS-CoV and human-CoV in primary human lung cells replication [15-17]. Homoharringtonine also called as Omacetaxine mepesuccinate, is a cephalotaxine ester and is known to induce differentiation and apoptosis in some cancer cell types and has established clinical activity against haematological malignancies [18, 19]. Favipiravir is another version of Fujifilm Toyama Chemical's influenza drug Avigan, and it was used to

treat Ebola patients in the African continent where the overall fatality was high in the infected children [20-23]. Monoclonal antibody is known to enhance natural immunity against cancer cells and the therapeutic applications of it for COVID-19 is widely discussed but no monoclonal antibody has been declared for its potency [24-26]. Tocilizumab is a monoclonal antibody currently used to treat rheumatoid arthritis and giant cell arteritis [27-30]. In a study from Tongji Hospital in Wuhan, China, a total of 15 patients with high IL-6 levels, several doses of Tocilizumab was recommended to reduce mortality rate [31]. Similarly fixed dosage of Tocilizumab administered against COVID-19 was successful in a patient who was also suffering from multiple myeloma [32, 33].

This paper discusses the statistical records of the effect of past medical history, percentage of various symptoms manifested in COVID-19 patients based on the publicly available data of the new daily confirmed cases reported from various countries and methods of diagnosis and treatment and a note on Indian strategy of lowering transmission of the infection. The structure, mechanism of action and research update in relation to the epidemic are stated in a tabular form for the top 5 drugs that are used by the countries to treat the patients.

## The Effect of Past Medical History in Various Age Groups

Several reports from China and America state that a majority of affected patients were males [34-36]. The records from Italy show that male sex and age were independent risk factors while all the cases from Germany reported on 30$^{th}$ January 2020 were males with no mentionable medical history [37, 38]. In most of the countries, high fever, dry cough, dizziness, fatigue, shortness of breath and diarrhoea were most common symptoms reported at the time of admission to the hospitals. Gastrointestinal disorders like diarrhoea, nausea etc. were also reported [39, 40]. The percentage of past medical history in various age groups is represented in figure 1 while percentage of various symptoms manifested in COVID-19 patients is depicted in figure 2.

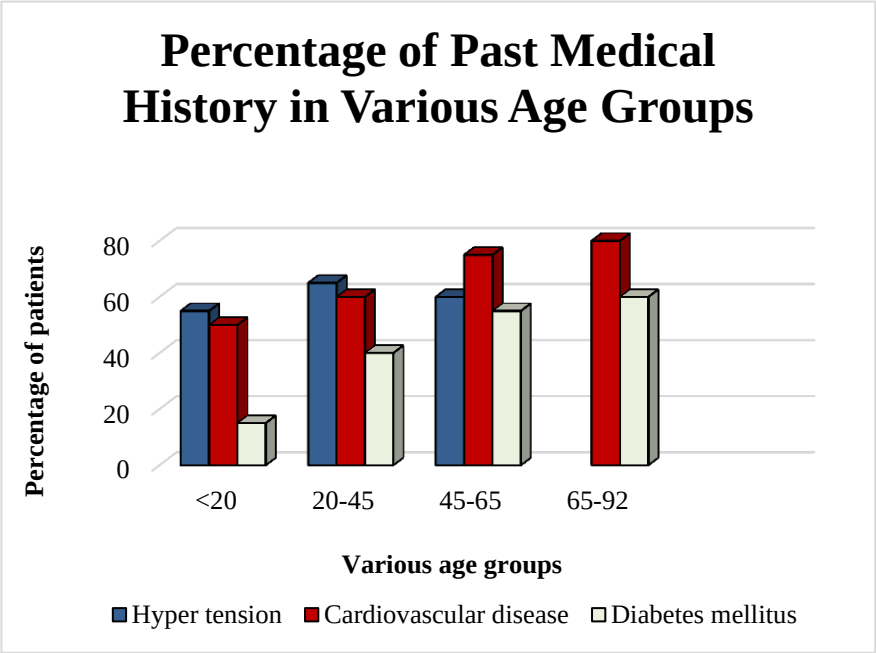



**Figure 1:** Graphical representation of percentage of past medical history in various age groups like hypertension, cardiovascular disease, and diabetes mellitus. People suffering from cardiovascular diseases and hypertension were highly vulnerable when subjected to COVID-19 and they should take utmost care and precautionary measures against it; based on the reports [34-40]

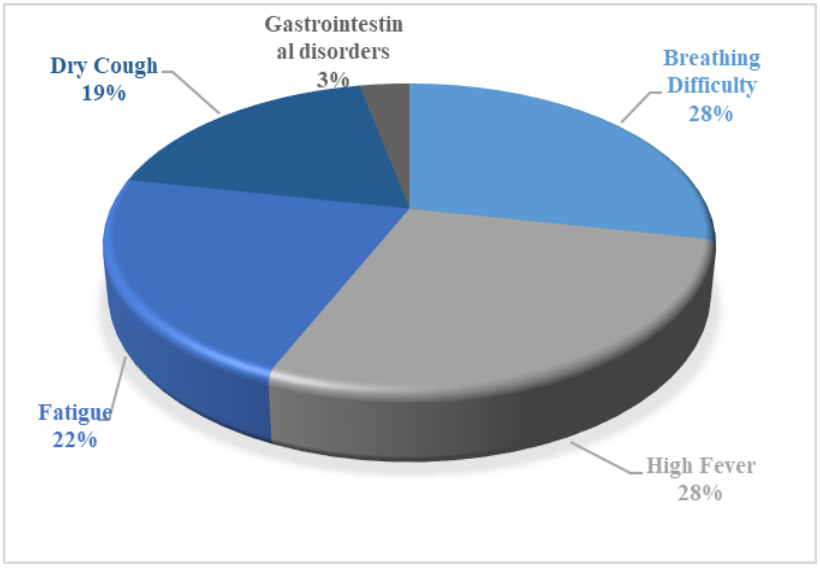



**Figure 2:** Representation of percentage of various symptoms manifested in COVID-19 patients; the majority of the COVID-19 patients had raised body temperature and respiratory related symptoms. Gastrointestinal disorders were least reported; based on reports [34-40, 67-69]

## Detection Methods

### Real-time RT-PCR in COVID-19 detection

From all the available tests, the "gold standard" for rapid, sensitive and specific detection is the Polymerase Chain Reaction (PCR) method while for the detection of COVID-19, reverse transcriptase-PCR (RT-PCR) was widely accepted and regarded as an early detection technique but false-negative and false-positive results were reported in several cases [41-44]. Real time RT-PCR uses primers for attachment and polymerisation at the target site and therefore any mutation in the viral RNA sequence might produce false results. Hence, rapid evolution, slow mutations and wide genetic diversity are believed to be the reasons for these false results and targeting multiple genes and performing amplification could solve this ambiguity.

### Early Humoral Response to COVID-19

When existing diagnostic methods were highly error prone, the potential of antibody testing for COVID-19 grabbed attention. IgM is the first immunoglobulin secreted during an infection while IgG is produced in maximum and is maintained in the body after an exposure to the antigen. Early Humoral (antibody) Response tests involves testing of IgA, IgM, IgG or the entire sets of antibodies response against the SARS-CoV-2 using ELISA-based assay on the viral nucleocapsid protein [45]. In several studies which were antibody based, it was reported that the positive ratio in IgM antibody test was significantly higher than in RT-PCR detection and that a combined IgG/IgM test seemed to be more effective than testing a single antibody. ELISA and LFIA methods were used and showed 99% sensitivity and with reference to the pandemic, ELISA tests would be safer. In a comparison between IgG and IgM tests, IgG tests were more efficient and accurate [43, 46]. As per W.H.O., ELISA tests are highly specific and sensitive. On April 2nd 2020, the United States of America, approved rapid serological tests made by Cellex© which could give results in 15 minutes' time, while ELISA kit based on both spike protein and nucleocapsid protein was used for antibody detection in China due to its reduced cost and higher accuracy. Also screening patients with gold standard PCR followed by antibody based tests were also recommended [47-50].

## Treatment Methods

As of 12th May 2020, there is no vaccination available but there are certain drugs that are used to reduce the symptoms, there by increasing the chances of recovery and enhances the self-resistance. Convalescent Plasma therapy is preferred by most of the countries and clinical studies showed a higher survival rate. The main way to boost personal immunity is to maintain personal hygiene, a healthy lifestyle and adequate nutritional intake. But the specific mechanism of the virus remains unknown, and no specific drugs have been developed. At present, it is important to control the source of infection, cut off the transmission route, and use the existing drugs and means to control the progress of the disease proactively. Remdisivir, Favipiravir, Hydroxychloroquine, Chloroquine and Homoharringtonine are under clinical studies and these drugs reduce the symptoms of the infection effectively. A drug repurposing model of using a defined combination of these drugs is also in its budding state. Several monoclonal antibodies are also tested for their interaction to COVID-19 membrane proteins which could prevent cell invasion and proliferation of the viral particles. 5 top drugs that are reported to be effective after referring to a total of 16

research papers, their mechanisms of action, structures and latest advancements in relation to COVID-19, are tabulated and represented in table 1.

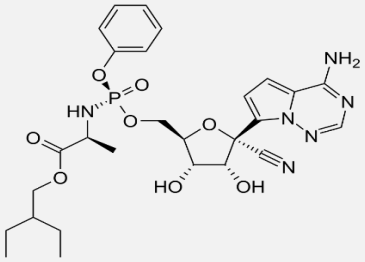
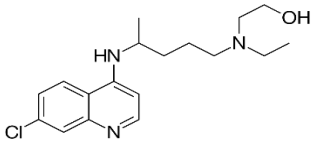
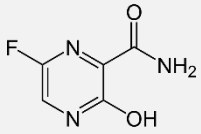
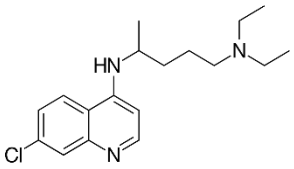
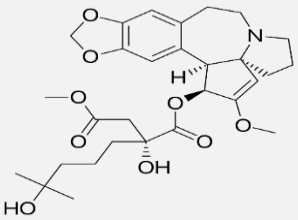
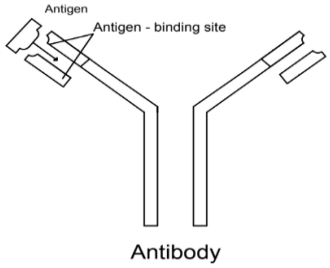
| No | Name | Structure | Mechanism | Research update in relation to COVID-19 |
|---|---|---|---|---|
| 1. | Remdisivir |  | Evades exonuclease proofreading activity of RNA [16] | Faster time to clinical progression and a combination of Remdisivir and chloroquine was recommended [51, 52]. |
| 2. | Hydroxychloroquine |  | Exact mechanisms are not known | Reduces pneumonia with least toxic effects [53, 54]. |
| 3. | Favipiravir |  | Inhibits viral RNA polymerase [55, 58] | Quicker viral clearance and improvement in chest imaging [56, 57] |
| 3. | Chloroquine |  | Reduces cytokine production [59] | Suppressing symptoms reducing severity level [60] |
| 4. | Homoharringtonine |  | Affinity to the A-site cleft in the large ribosomal sub-unit, preventing translation and leads to apoptosis of cells [61] | From 3 viral inhibitors, hexachlorophene, nitazoxanide and homoharringtonin, homoharringtonin was found to be most effective [62] |
| 5. | Monoclonal Antibody |  | **Tocilizumab:** Inhibits the binding of Interleukin-6 to its receptor [63] | The higher lymphocyte count and C-reactive protein levels returned [64, 65] |

**Table 1:** The structure, mechanism of action of the drugs that are used to treat the infection and latest research update of it in relation to COVID-19. Monoclonal antibody is a promising drug for COVID-19 treatment as it prevents cytokine storm which is responsible for sudden death of patients. Hydroxychloroquine effectively cures pneumonia while a combination of effective drugs is also recommended for enhancing survival rates.

## Convalescent Plasma therapy

Several studies reported that convalescent plasma therapy in COVID-19 infected patients has considerably reduced hospital stay and mortality rate. In the case of 2009 severe influenza A H1N1 infection, plasma therapy reduced the mortality rate by 95% and W.H.O. recommended this treatment method for Ebola virus and there were no adverse effects reported [66, 67]. Analysis in 1703 influenza-pneumonia patients who received an infusion of influenza-convalescent human blood products showed a reduction of 21% in the overall mortality rate [68]. For an effective passive antibody therapy, a sufficient amount of the antibody must be administered and when it is given to an infected person, this antibody will be in the circulatory system, reaches tissues and protects against the antigens. In a study of 5 COVID-19 patients, who were treated with convalescent plasma from the donors with IgG and IgM antibodies, it was observed that very high specificity towards the virus was established [69]. On 25th April 2020, reports from India about four COVID-19 patients who received plasma therapy in the capital city showed better chances of recovery and the government decided to conduct it on more patients in the coming days and several hotspots like USA and Italy are looking forward for it.

## Side effects reported from various studies

Although several drugs are available none of them are approved for the treatment of COVID-19. The major negative effect of hydroxychloroquine and choloroquine is that they may bind with melanin of retinal epithelium thus damaging the photoreceptor cells and inner layer of retina [70]. While in a study conducted on 1446 consecutive patients there was no specific association between hydroxychloroquine use and mortality rate [71]. Similarly, several fatal adverse effects were reported in 66% of remdisivir treated patients under study, while Favipiravir is an approved drug for influenza in Japan and China and is under investigation process for the use against COVID-19 [72]. Indian Institute of Chemical Technology (IICT), Hyderabad has started the production unit for favipiravir and Drug Controller of India allowed clinical trial of Favipiravir with a maximum treatment duration of 28 days. Homoharringtonine has the major risk of developing myelosuppression, haemorrhage, hyperglycaemia, and fatal harm [73]. Treatment with monoclonal antibodies might induce allergic reactions, diarrhoea, fever, chills etc. while convalescent plasma therapy have not yet reported any potential side effects.

## A Note on the Indian Strategy of Lowering Transmission

India has taken necessary steps and precautions to control the spread of the disease by opening control rooms in the same month of the outbreak in Wuhan, China and imposed Section 144 of the CrPC with a complete lockdown till May 3rd. This helped the country to limit the death toll and community spread was avoided in many densely populated states and decided to conduct Convalescent Plasma therapy on

more patients in the coming days. In India, multiplex PCR panels were used for diagnostics in the onset of the infection but now if no pathogen was identified the samples were referred for several serological testing. The Indian Council of Medical Research (ICMR) approved 176 labs, to conduct diagnostic tests and the U.S. Food and Drug Administration has approved such kits for diagnostic testing for COVID-19.

As represented in the figure 3, the overall mortality rate in India is low compared to the other countries, i.e. only 3% and this was achieved in India by unprecedented measures to contain further transmission of COVID-19. In case of various states in India, lockdown that was into action for 21 days which was later extended till May 2nd and from all confirmed cases, 26.24% are in Maharashtra. Union Ministry of Health and Family Welfare and its robust surveillance system is closely monitoring more than 9.5 lakh suspected Covid-19 cases across the country. Also, travel history of all patients with respiratory symptoms and made any international travel or even contacted with any sick person were diagnosed and kept for home quarantine or in isolation in hospitals regardless of a negative test result.

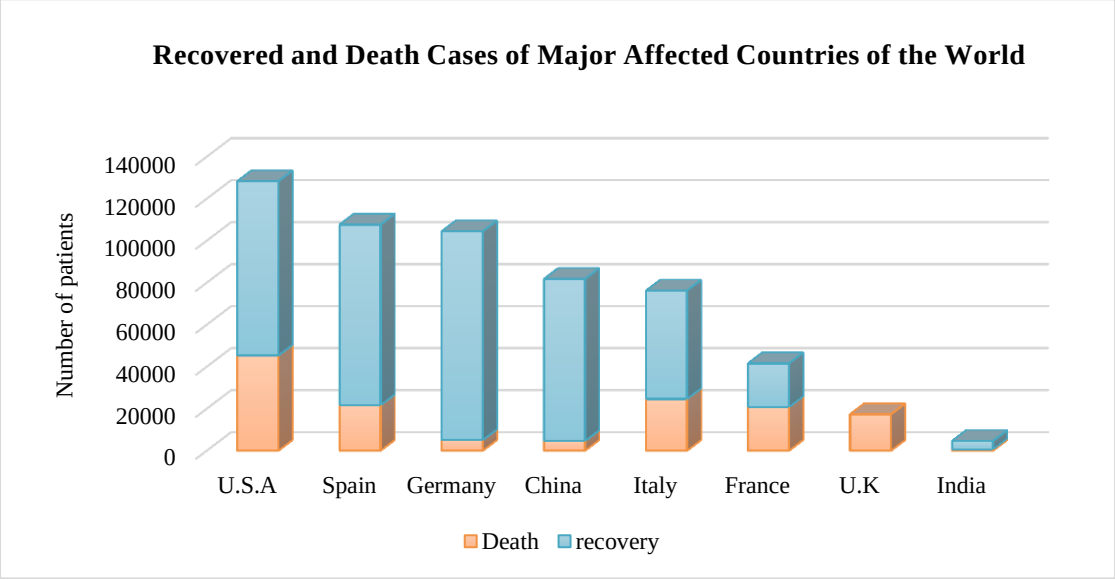



**Figure 3:** Graphical representation of total number of confirmed deaths and recovered cases from most affected countries of the world and is compared with records from India; based on daily reports of W.H.O. Mortality rate is very low in India when compared to other countries and this is achieved by better diagnostic tools, effective treatment protocols and imposing restrictions on travel and person to person contacts. A huge increase in the number of patients is observed in the United States of America, Spain and Germany while the rate of recovery was lower when compared to other countries.

From the everyday count of reports from various parts of the world, it is clear that India is in a better position than many other countries in case of total number of cases being reported and the recovery of patients as well. However, an aggressive approach and immediate action have to be taken so as to avoid community spread in the states with relatively high population density. Convalescent Plasma therapy should be implemented in more severely affected states of the country to make sure higher survival rate. Also balanced immunity boosting diet, timely medications and even urgent ventillatory support should be made more accessible. Our study has limitations since virus viability was not shown and the sample size was small, due to difficulty to run more tests in a time of emergency. However, the emergency of the outbreak required a fast assessment of the current situation. While the epidemic still shows no sign of ending, further studies are underway to more comprehensively determine the potential of the environment as a transmission medium.

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## Disclosure:

All authors have no potential conflicts of interest.

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