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REVIEW ARTICLE

HYDROXYCHLOROQUINE PROPHYLAXIS - VINTAGE WINE FOR EVERY NEW BOTTLE?

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ABSTRACT

Chloroquine and Hydroxychloroquine have been in use for more than 75 years with a satisfactory safety profile, for malaria and autoimmune disorders. They have been repositioned for the prophylaxis or treatment of emerging viral disorders like SARS Co-V, MERS and now in SARS-CoV2, which ,in a few months, has caused havoc globally. Drugs used in earlier epidemics are being tried again and HCQ is in the forefront for its anti-viral and immune modulatory properties. It has shown good results in vitro and few observational studies, but there are objections that it has not been proved in randomised control trials, which was difficult in the initial pandemonium .However, more than 150 trials have now been registered and the picture will be clearer. HCQ has stood the test of the controversies and must be the most trending molecule currently-whether the fanfare from the President of America or it's temporary suspension and re-inclusion in the WHO Solidarity trial! The apex body of India, ICMR, has stood by it through thick and thin and has expanded its advisory for the prophylactic use of HCQ in SARS-CoV2.It has also started a trial for evaluating its use in prophylaxis. Most of the reports so far are related to its therapeutic utility, but the doses used globally are variable. This article reviews the profile of HCQ and its status in preventing infection or severity of SARS-CoV2 and focuses on its prophylactic role.

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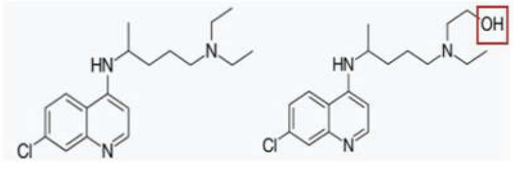
INTRODUCTION

No FDA approved drug may have experienced the ups and downs and none may have gone through the cycle of hope, hype, temporary rejection and transient acceptance that Chloroquine (CQ)/ Hydroxychloroquine (HCQ) have gone through. Chloroquine (CQ)was first synthesised in 1938 and the first trial began in the U.S in 1944. The FDA approved Hydroxychloroquine(HCQ) in 1945 and it was synthesised in 1946. It was supposed to be 40 % less toxic than Chloroquine.[(1)]Fig. 1. CQ been used to treat malaria and HCQ mainly as an immune-modulator in Rheumatoid Arthritis, Systemic Lupus Erythematosus, Antiphospholipid antibody Syndrome and Sjogren's . HCQ has been used for years at a stretch in these patients with reasonable safety. It has been safely used during pregnancy. It has also been used in trials in Diabetes as an adjuvant drug to decrease blood sugar levels. It even has anti-thrombotic properties. [(1)] We had used it for shorter periods after the Chikungunya epidemic of 2006 to treat some patients who had persistent arthralgia/arthritis, with good relief. It has antiviral properties, is relatively cheap and was easily available till this COVID -19 pandemic, when suddenly it's demand sky-rocketed locally and globally.



Chloroquine *Hydroxychloroquine*

Fig. 1 Structure of CQ & HCQ

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On the basis of in-vitro tests, observational studies and reports, in the prevention of, or decreasing the severity of infection by SARS-CoV2, it is postulated that HCQ works by-

1.Its antiviral effect by increasing the pH of the endosomes and lysosomes of the host cells, making enzymes needed for viral entry and transport ineffective. Its immune-modulator effects that decreases cytokines and may prevent the cytokine storm that occurs in severe cases of COVID 19 with multi-system damage.

Background

Limitations of performing controlled clinical trials when no alternatives are available: In a potentially life-threatening infection like SARS-CoV2 , that sweeps over the world like wildfire, can one hold back whatever is available (even if it is only shown to be useful by experience and in observational settings) when nothing else is at hand? Especially in the setting of a dangerous contagious pandemic spreading from humans to humans where there is a high morbidity and mortality, is it ethical and moral to deprive someone of whatever advantage he may potentially get from the preventive properties of a drug like HCQ?

The objection to the use of HCQ is that it is not supported by randomised controlled trials (RCT),which is the gold standard for approving a drug or procedure.[3,4] It is a study in which the participants(subjects) are allocated at random (by chance alone),to reduce bias, to receive one of several clinical interventions. One of these interventions is the standard of comparison or control, which may be a standard practice, a placebo, or no intervention at all. The drug or intervention being studied is compared with the control by applying an appropriate statistical test.[3,4] In case of SARS CoV2, where no standard drug available, "control" would mean-placebo or no drug.In a recent analysis on behalf of the Oxford COVID team, the authors assessed the trial designs specified in national and international registry of CQ/HCQ trials by downloading the ICTRP COVID 19 Database .They included every trial that mentioned CQ/HCQ and found that 158 trials pertaining to these drugs have been registered this year, of which 50 are related to the prophylactic use of HCQ.[5]

The unusual clusters of cases of pneumonia that occurred in December 2019 were disclosed by China on the 31st of December. China shared the genetic sequence of the Coronavirus 12 days later, on the 12th of January, 2020.On 22 January, human to human transmission was accepted to be occurring, but WHO declared the Coronavirus (when it had spread to 114 countries)as a pandemic on the 11th of March, 2020. Till mid-March or even later, the emphasis was on fomites as a mode of spread and masks or covering the nose and mouth were not promoted for the general public, though respiratory etiquette was. Experts mainly focused on stressing the importance of sanitizing hands and surface, which is of course important. It took some time to acknowledge that droplet spread by the nose is the most important route of transmission and the use of masks by the general public was necessary to prevent the spread and protect the wearer. In a short time, the virus spread its tentacles all over the world and as of today, the 7th of June, 2020, (in 5 months), has infected more than 70 lakhs persons globally and killed more than 4 lakhs.[10]

At the outset, we reiterate that this article is about the preventive aspects of HCQ in SARS-COV 2 and not the therapy of established COVID-19; however, researchers are often seen clubbing the two together. Where toxicities were observed in the setting of multiple drugs being given to critical patients, the whole blame has been pinned on HCQ/CQ. For ex, serious patients of COVID-19 would get HCQ, Lopinavir-Ritonavir and Azithromycin in rather high doses and all of these can lead to QT prolongation. Covid19 itself is known to produce myocarditis. However we observe that only CQ/HCQ is blamed for any adverse reactions seen. This appears a little intriguing, to say the least. Many of the newer drugs that are being promoted, like Favipiravir or Remdesivir have not yet undergone randomised controlled clinical trials in SARS-COV 2 , as are being demanded of HCQ .They have significant side effects, are costly and not easily available. Above all, they are not indicated for prophylaxis.

HCQ shows good results in vitro against SARS CoV-2 and clinicians in China and France had been quite enthusiastic about the response they got in small groups of patients in February-March 2020. However, the major objection to its use has been that there are no RCTs to show the efficacy of the drug for prophylaxis or treatment of the current or earlier viral infections. The acute emergency with which a pandemic due to a life threatening virus like SARS CoV or Cov-2 strikes and explodes is such that randomised clinical trials with placebos are difficult to carry out. In a disaster situation, where there is no cure or vaccine in sight, if any available drug is considered to be remotely of use, it is not ethical to hold it back for carrying out a randomised controlled trial. The truth about the cheap and seemingly unglamorous Hydroxychloroquine has been that it has always been considered, but never really been accepted nor written off. That it could not be rejected in spite of many objections by many experts, is an indirect proof of the hope and faith many experts have in it. How useful it is- needs to be proved. It is an underdog that has hung on and is still among the first group of drugs to be tried whenever a new virus emerges.

Covid-19 and HCQ:- **Reports** India is exporting HCQ to 55 other countries and is India's drug of choice for prophylaxis and treatment.

Small uncontrolled or non-random controlled trials have shown variable results with the use of HCQ in treating COVID-19.

- A report from China says that repositioning of the role of HCQ in SARS-CoV 2 infection was considered after their scientists observed good in- vitro efficacy and 20 trials were started. Results of the first 100 COVID patients showed good results and the drug was included in the treatment protocol.[2]
- A study of 36 patients in France in March 2020 in confirmed cases of COVID, in which 600mg of HCQ was given daily to 20 patients and nasopharyngeal swabs were tested daily, reported that viral loads on the 6th day were significantly lower as compared to controls from another setting where HCQ was not used. Addition of Azithromycin augmented this result. [7]
- The President of the USA recently admitted that he has taken HCQ prophylaxis and had earlier, on the 8th April, thanked India for supplying it to the US, when lakhs of

Americans were infected by the SARS CoV-2 and thousands were dying of COVID-19.[8]

- China and Iran decided to use HCQ in preference to CQ as they thought it was less toxic .The postulation was that patients with extensive lung involvement may not have adequate levels in the lungs, though HCQ levels in tissues are much higher than in the plasma. Also, HCQ takes some time to build up. Hence a higher dose may be required .[9]
- A very interesting theory put forth by the author is that if HCQ is started as prophylaxis, it would be present in tissues before the virus reached and may be ready to counter it.[9]
- A recommendation from a group in China in March ,for using HCQ rather than CQ because it is safer and as effective, in preventing uncontrolled cytokine release, suggested that controlled trials were needed, but the drug is time tested for its safety and should be used.[11]
- In a report published in the BMJ on 15 May 2020, the first RCT in China of 150 COVID subjects failed to show a difference in viral conversion in treated and untreated subjects at 28 days and significant gastrointestinal side effects were observed.[12]
- In a French study in March,181 COVID subjects divided randomly in control and HCQ group did not show any statistical difference in the numbers of those who needed ICU care or died within 21 days of admission. [12]
- On the 22nd of May, Lancet published an observational study in which HCQ has been reported to be not useful in the treatment of Covid-19 .The authors, (in which data from 671 hospitals from 6 continents of 96032 patients was said to have been reviewed) concluded that benefit of CQ or HCQ was not seen, but decreased in-hospital survival and increased risk of ventricular arrhythmias was seen.[13] Many knee-jerk reactions followed this study .
- The Australian HCQ ASCOT trial was suspended and the investigators paused patient recruitment.[14]
- The article in Lancet has come under fire from more than a hundred medical experts and scientists for queries about the authenticity of the data mentioned. Conflict of interest of the study are being pointed out. The scientists wrote an open letter to the editor and the authors, but many of their queries remained unanswered.[35]Some countries stated that the data mentioned about them in the study reported by Lancet, was not given by them and was incorrect, hence the design of the trial, lack of proof of data etc were questioned.[14,34]A negative report against HCQ, which has been in use for many decades, when the world needs it badly, was inconceivable to the Indian authorities, who swung into action.
- ICMR wrote to WHO, especially about the difference in doses used in different countries. The total dose given in India for treating a COVID -19 patient is 2400 mg over 5 days whereas in many countries, it is nearly 9600 mg in 11 days-4 times the quantity used by India .ICMR feels that the much lower dose used by India is adequate and the high dose given in other countries may be responsible for the adverse effects reported.[15,16]
- HCQ is one of the drugs in the randomized trial RECOVERY, for treatment of COVID-19 cases, in the U.K. After the advisory by WHO, HCQ was reevaluated by an independent committee and it was concluded that HCQ is safe enough to be continued in the trial.[15]
- Lancet has followed up with a few corrections in the report. However, this led to an out- of- the blue negative opinion about HCQ in COVID-19 and France banned the use of the drug.[17]
- In a recent correspondence on 22 May, in Lancet, that was published on the same day as the other negative article, the use of HCQ for high-risk Covid-19 contacts in India has been lauded as a prudent approach. The authors say that the drug has a reasonable time-tested safety profile as far as cardiac or G-6 PD deficiency is concerned.[18] Given that there is no available, affordable preventive therapy or vaccine, the drug should be used as prophylaxis for high risk groups.
- Around this controversial time, Dr. Anthony Fauci commented that HCQ is not an effective treatment for COVID-19, has adverse effects and though he is not sure whether it should be banned, he advocated caution with its use.[19]He is the editor of Harrison's Principles of Internal Medicine, Director of National Institute of Allergy and Infectious diseases ,chief advisor on policy matters of health to the U S government and his opinion matters. He has stated that a vaccine will be available in the next few months.[19]Of course, a vaccine is the near ideal solution, provided it is adequately effective and within reach of all. Till then, what do we have?
- This was followed by FDA questioning the safety of HCQ,(though it had cleared the drug way back in 1945)adding that using HCQ in combination with Azithromycin may aggravate the QT prolongation. [20]
- WHO has sadly, been rather sceptical about the drug and its uncommon side effects are being highlighted repeatedly. On the 18th of March, WHO had launched the international Solidarity trial, to evaluate the role of various drugs in treating Covid-19.After the negative report in the article in Lancet, WHO suspended the arm of the Solidarity trial that was testing the role of HCQ, on the grounds of safety concerns. WHO says that though CQ & HCQ are licensed products and any other country can decide whether or not to use them, the drugs have a lot of toxicity and are not yet confirmed to be of use in COVID 19 with randomised controlled clinical trials.[21]
- WHO, in the latest interim guidelines for management of COVID-19, issued on May 27, 2020 has recommended that Chloroquine and Hydroxychloroquine along with some other drugs) should not be administered as treatment or prophylaxis for COVID -19, outside the context of clinical trials. In the remarks, WHO says that CQ and HCQ, with or without Azithromycin can cause QT prolongation and together cause cardio toxicity .[21]
- On the other hand, Indian Council of Medical Research (ICMR) has been expanding the domain of the prophylactic use of HCQ – recently it has advised healthcare workers who are not directly involved in COVID care and even frontline workers like police and paramilitary personnel to take it – of course, with consent and screening. ICMR has advised that there should not be any complacency and all other precautions like covering the nose and mouth and hand sanitisation, social distancing, should be rigorously followed. ICMR has also started a multi-centric observational study of HCWs working in dedicated COVID hospitals regarding the use of HCQ.[22,23]
- The World Health Organisation (WHO) has again done a flip-flop and decided to resume the hydroxychloroquine

(HCQ) part of its Solidarity Trial, a global effort to find a treatment for Covid-19, from the 4th of June, 2020, after The Lancet issued an "expression of concern" over the study it had published on the 22nd of May ,that had questioned the effectiveness of HCQ. The WHO had earlier suspended the HCQ segment of its trial based on this study.[24]

- On the 5th of June, 2020, the "Recovery" randomised control trial in UK, that is studying the role of various drugs in the therapy of COVID-19 came to the conclusion that Hydroxychloroquine is not useful and halted that arm of the trial. However, as per ICMR guidelines, in India, HCQ continues to be used for treatmentof COVID-19. This article is related to the prophylactic, preventive use of HCQ , which continues .WHO also suspended the therapeutic use of HCQ on the 16thof June , as it's "Solidarity" trial also failed to show benefit. However, this does not apply to pre or post exposure preventive use of HCQ.[25]
- Following these results, the USFDA has revoked the emergency use of HCQ that it had permitted. However, many researchers including ICMR have decided to continue their trials, especially for prophylactic ,preventive use.[26]

Current Status of HCQ in India: The Joint national group and the National Task force (NTF) for COVID-19 (Indian Council of Medical Research) reviewed the use of HCQ for prophylaxis of SARS-CoV-2 infection in high risk population based on the emerging evidence on its safety and efficacy.[23]

- In-vitro testing of HCQ for antiviral efficacy at NIV, Pune, showed reduction of infectivity /log reduction in viral RNA copy (viral load)of SARs-CoV2.[23]
- The data on assessment of HCQ prophylaxis among 1323 HCWs indicated mild adverse effects such as nausea (8.9%), abdominal pain (7.3%), vomiting (1.5%), hypoglycemia (1.7%) and cardio-vascular effects (1.9%).However, as per the data from the Pharmacovigilance program of India, there have been 214 reported instances of adverse drug reactions associated with prophylactic HCQ use. Of these, 7 were serious individual case safety reports with prolongation of QT interval on ECG in 3 cases.[23]
- A retrospective case-control analysis at ICMR has found that there is a significant dose-response relationship between the number of prophylactic doses taken and frequency of occurrence of SARS-CoV-2 infection in symptomatic healthcare workers who were tested for SARS-CoV-2 infection.
- Another investigation from 3 central government hospitals in New Delhi indicates that amongst healthcare workers involved in COVID-19 care, those on HCQ prophylaxis were less likely to develop SARS-CoV-2 infection, compared to those who were not receiving it. The benefit was less pronounced in healthcare workers caring for a general patient population.[27]
- An observational prospective study of 334 healthcare workers at AIIMS, out of which 248 took HCQ prophylaxis (median 6 weeks of follow up) in New Delhi also showed that those taking HCQ prophylaxis had lower incidence of SARS-CoV-2 infection than those not taking it.[27]
- In light of all of the above, the Joint Monitoring Group and NTF have now recommended the prophylactic use of HCQ in frontline workers like healthcare workers, police and paramilitary forces, for 7 weeks. In clinical practice HCQ is commonly prescribed in a daily dose of 200mg to 400mg for treatment of diseases such as Rheumatoid Arthritis and SLE for prolonged treatment periods with good tolerance. With available evidence for its safety and beneficial prophylactic drug against SARS COV-2 during the earlier recommended 8 weeks period, the experts further recommended its use beyond 8 weeks on weekly dosage with strict monitoring of clinical and ECG parameters which would also ensure that the therapy is given under supervision. Based on the available evidence, it has been opined that HCQ is relatively safe, when certain contraindications are avoided, and has some beneficial effect as a prophylactic option.[23]

A few facts about HCQ – CQ has been used for malaria treatment and prophylaxis extensively.1 With a single oral dose of HCQ of 200 mg in a healthy male volunteer, the mean peak blood concentration was 129.6 ng/ml, reached in 3.26 hours, The half- life was long -22.4 days! This was because of the extensive tissue uptake.[1]

How safe? Gastritis, headaches are relatively common but simpler side effects.[1]

Retinopathy-Quite rare. HCQ is administered for 2-5 years in cases of Rheumatoid Arthritis and Lupus. If a person is taking more than 6.5 mg/kg/day then visual field testing is recommended at baseline and every year. As HCQ prophylaxis for COVID is expected to be at a much lesser dose and for a much shorter duration, the development of retinopathy is unlikely , though of course it needs to be monitored clinically and by perimetry.[1]

Cardiac toxicity- A prolongation of QT does occur in some individuals due to HCQ, even with a single dose; hence it is advisable to get an ECG done before starting the prophylaxis. Since the half- life is more than 3 weeks, one can consider giving a smaller loading dose (200 mg) instead of 400 mg twice a day for prophylaxis. Since there is a wide variation in the body weight of different persons, instead of a blanket dose, it may be advisable to give the drug as a dose per kilogram of body weight. The EC50 for achieving the dose needed for malaria prophylaxis is a loading dose of 400 mg twice a day, followed by 400 mg next day and 400 mg weekly afterwards, for 2 weeks after the risk of exposure is over. A similar regime has been advised by ICMR for the prophylaxis of COVID 19 in selected individuals. The QT interval is measured from the beginning of the QRS complex to the end of the T wave in Lead 2/V5/V6, whichever is the longest and QTc calculated by nomogram. (Less than 430 msecs is normal. More than 450 msecs in males and 470 msecs in females is abnormal)[28]

The real concern is of cardiac arrhythmias –actually the drug has been around for nearly 75 years and Chloroquine that is supposed to have more side effects has been used extensively for malaria prophylaxis in soldiers and for shorter periods in tourists.[1]It has been used for treating malaria or even suspected cases of malaria at primary health care levels and generally found to be safe. It has also been used for shorter periods in hepatic amoebiasis, with good results. We have used it extensively and the only common side effect we saw was gastritis.

Many drugs can increase the QT interval and have an additive effect if given together[28]

Antibiotics, mainly Macrolides like Azithromycin, Clarithromycin. Metronidazole, Quinolones like Moxifloxacin, Gatifloxacin, Sparfloxacin. Antipsychotics –Haloperidol, Chlorpromazine, Risperidone Citalopram. Antidepressants-Amitriptyline, Imipramine, Doxepin, Fluoxetine Fluconazole, Cisapride, Quinidine, Procainamide Antivirals: Lopinavir, Ritonavir, to name a few. Hence before starting HCQ, a thorough drug history should be taken. However, if a person has a history of cardiac disease (which is likely, especially in the elderly) or if other medications are being given for other purposes then one should be cautious, may start with a lower dose, observe and then decide. There are few anecdotal reports of sudden deaths in COVID patients who were getting Chloroquine as therapy in a higher dose along with Azithromycin – which can also prolong the QT. COVID is known to produce myocarditis and it is likely that multiple factors may have led to the death, but the fact is that only HCQ is being blamed. This does not mean that adequate care and caution is not warranted. The point to be noted is that, in prophylaxis for COVID, HCQ is given alone and in a smaller dose as compared to its use for therapy of a patient of COVID, hence the risk would be minimised.

About SARS CoV-2 is a Coronavirus of beta lineage. It is an enveloped, positive stranded RNA virus. After SARS CoV and MERS, it is the 7th Coronavirus to infect humans. The incubation period is usually 3 to 8 days, average 5 days, but can be up to 14 days. . It is mainly spread by droplets and also contaminated objects and surfaces, via hands to the nose or mouth or even conjunctiva.[29] It has club shaped spikes with which it attaches to ACE-2 receptors .Each spike is made of the S glycoprotein and has a stalk and 3 heads, the heads form the RBD(Receptor Binding Domain)

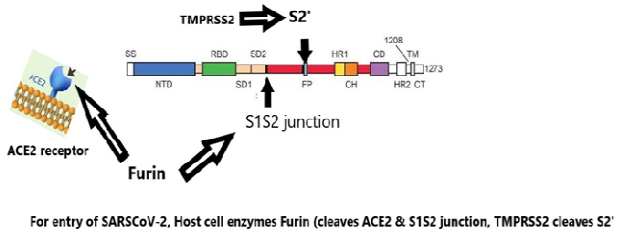

Fig. 2. Cleaving (Priming of spike protein of SARS CoV-2 by Furin and TMPRSS2)

The two functional domains of this 'S' are:Fig. 2

- S1-Receptor binding domain – (RBD).This recognises the host cell receptor i.e. angiotensin converting enzyme 2(ACE2). The terminal part of RBD is RBM, which actually binds to host receptor.
- S2-which contains mediators of fusion of viral and cell membranes.

What does chloroquine do to human cells, to prevent viral entry? A quick revision of the structure and function of a human cell is necessary to understand where and how HCQ works. More than 50 enzymes are synthesised in the rough endoplasmic reticulum in the cytoplasm. Transport vesicles bud from the trans Golgi network (TGN)that is a later Golgi structure that is responsible for sending proteins to their final destination- which may be the cell surface, endosomes, lysosomes, or secretory granules. They carry the enzymes in an acidic environment, which is produced by ATPase proton pump. They are tagged with Mannose-6 phosphate that guides them to travel to the lysosomes.

Endosomes are produced by endocytosis at the plasma membrane. Lysosomes are produced by fusion of transport vesicles and endosomes.

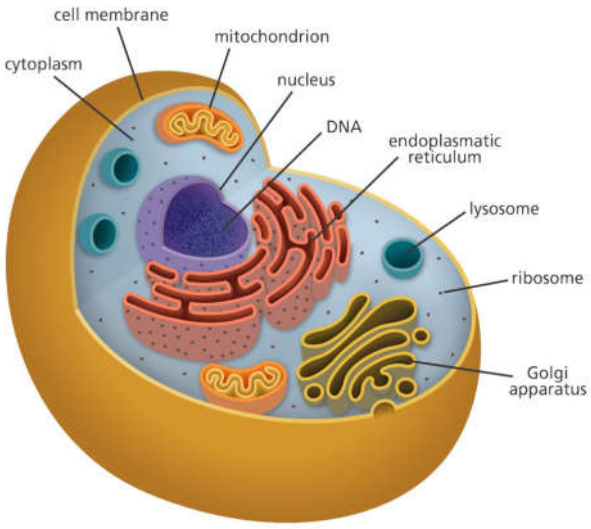

Fig 3. Cell structure

Lysosomes are membrane bound spherical vesicles in the cytoplasm of a cell that act like scavengers and digestive organelles. They store the 50 hydrolytic enzymes in packets (proteases, peptidases, phosphatases, lipases etc). The membrane of the lysosome protects the other parts of the cell from being attacked by its own enzymes. The second protective mechanism that protects the intracellular components from the lysosomal enzymes is by the alkaline pH of the cytoplasm outside the lysosomes, that inactivates the enzymes which need an acidic environment, in case they accidentally leak (Fig 3) The pH of the lumen of the lysosome is 4.5-5 (acidic) whereas that outside, of the cell cytoplasm is 7.2 (slightly basic). The proteases and other enzymes require an acidic pH to function. If the pH becomes more alkaline, the proteases like furin that are vital to cleave the S1-S2 site of CoV-2 spike may not function.12,20."Furin" is a host cell protease produced by the Golgi and stored in endosomes and lysosomes near the cell membrane and produces the final structural changes of S2 that is needed for membrane fusion by activating certain spike proteins in the S2 . The final facilitation for cell entry is carried out by the host cellular enzyme TMPRSS2. (Trans membrane protease serine 2) ,that cleaves the S2'(R797) cleavage site, which is inside the S2 domain of SARS CoV-2.

Thus both furin, followed by TMPRSS2 enzymes (which are necessary for many normal cellular functions) are needed for SARS CoV2 to enter the host cell. These enzymes are manufactured in the Golgi and travel from the Trans Golgi network to be stored in lysosomes that have an acidic pH. Though Furin is said to be capable of functioning at a higher pH of 6 to 8, it needs a lower pH for some substrates, TMPRSS2, like other lysosomal enzymes, needs a pH of 4.5 to 5 to function.[30,31]

HCQ and CQ raise the pH of the lysosomes and the enzymes cannot function properly. SARS-CoV2 cannot enter host cells without cleavage by these enzymes. Thus HCQ can prevent entry of virus into cell, if the host has been receiving it for prophylaxis. When lung cells are infected, infected cells fuse with neighbouringnon-infected cells to form a syncytium and the virus spreads from one cell to another.[30,31]

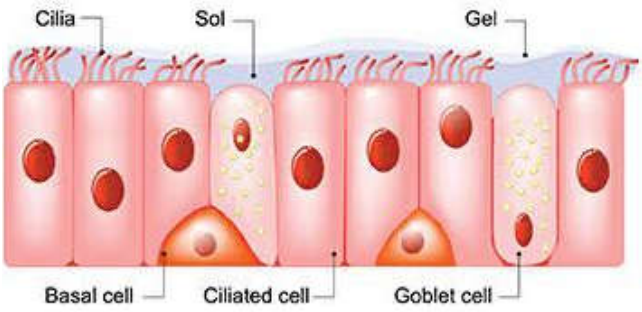

Fig. 4 Anatomy of nasal mucosa

How does SARS Cov-2 enter the human host? ACE-2 receptors are abundant in the nasal (Fig. 4) cavity and upper respiratory tract and the nasal epithelium is the chief portal of initial infection and transmission.ACE2-2 receptors are also found in oesophagus, ileum, colon and superficial conjunctival cells. Thus, SARS CoV-2 has a potential to spread through the nasolacrimal ducts also.[32]Fig.5

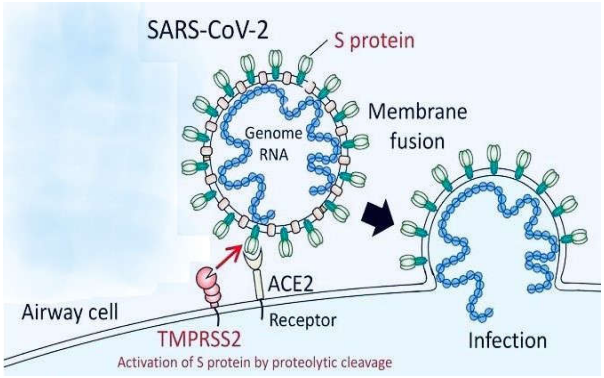

Fig. 5 SARS-CoV-2 host cell entry

How does HCQ act? HCQ acts as an antiviral, by preventing the entry and multiplication of the virus in host cells by increasing the pH of endosomes and lysosomes and may also prevent or minimise the cytokine storm by its immune-modulator effect. HCQ is postulated to act to restrict the entry of the virus into the cell through the ACE-2 receptor and also it's transport in the cell from early to late endosomes. In experimental studies, virions were seen in early and late endosomes, but upon HCQ treatment, the number of virions of SARS CoV-2 in the late endosomal lysosomal protein was much lesser as compared to untreated cells. This suggests that the transport of SARS CoV-2 from early endosomes to late endosomes is blocked by HCQ. HCQ treatment also increased the size and number of endosomes and increased the pH of lysosomes from 4.5 to 6.5. The concentration of HCQ in the lung may reach 200-700 times that in plasma at a safe dosage of 6-6.5 mg/kg/day. HCQ inhibits the entry as well as post - entry stages of SARS CoV-2 in cells, by blocking endosomal maturation at intermediate stages of endocytosis. HCQ has been extensively used in autoimmune diseases and can significantly decrease the production of cytokines.

Cytokine storm, a fulminant hyper- cytokinemia, associated with multi-organ failure, was postulated to be the main cause of mortality in the SARS epidemic of 2002 (in which nearly 8000 persons were affected and 800 died) and HCQ was used as one of the immune-modulator drugs with apparently promising results. SARS CoV2 also produces a dangerous cytokine storm due to uncontrolled immune system hyperactivity that attacks the victim's own organs.The patients who need ICU management have been found to have a higher concentration of pro-inflammatory cytokines (Interleukins and tumour necrosis factors) factors) and chemokines (CXCL10,CXCL8,CXCL9,CCL2,CCL3,CCL5, interferons, colony stimulating factors).[33]

Toll –like receptors normally recognise invading pathogens and endogenous material from damaged tissue. However, excessive action of these receptors disrupts the immune homeostasis and sustained chemokine and cytokine production occurs that harms the body's organs. . CQ and HCQ inhibit immune activation by reducing Toll –like receptor signaling, cytokine production and ,reducing CD154 expression in T cells.CD154 is also known as C40Ligand or C40L that belongs to the TNF super-family-this activates T cells and macrophages to produce more cytokines.[6] ICMR has initiated a multi-centric Observational Clinical Trial to study the prophylactic use of HCQ in health care workers in preventing Covid-19 and the side effects of HCQ, in dedicated COVID hospitals. The dose recommended is that which is used in Malaria prophylaxis- 400 mg twice a day on day one, followed by 400 mg on second day and weekly 400 mg for 7 weeks or more. Real-time PCR evaluation of nasopharyngeal swab is carried out at baseline and every four weeks for 3 months and anytime if HCW becomes symptomatic. Blood sample will be drawn at the beginning and at the end of 3 months and tested for antibodies in NIV Pune. The trial will be very useful for evaluating the utility and safety of HCQ in frontline health care workers.

The Future? We are in a very unusual and uncertain scenario that has led to an unprecedented global lockdown for more than 2 months, of a variable degree. India has done rather well overall, barring in a few cities and areas, due to strict enforcement of curfew, but as relaxation starts, the virus may raise its spiky head further. The earliest that a vaccine may be available is 6 months hence. Till then, social distancing, masks and hand hygiene are our only saviours. That is why we and much of the world looks at HCQ with hope that is rather justified. If more evidence based proof can be collected about its prophylactic and therapeutic use, India, who produces more than 70% of the world's HCQ, will have given the humble molecule the glory it deserves. If the ICMR study confirms positive benefits, it may be possible to confidently extend the prophylactic use of HCQ beyond frontline workers, to elders and persons with co-morbidities, who are at a higher risk of severe disease and death due to COVID 19. More research may be warranted to study its minimal effective dose for this purpose ,as tissue levels of the drug reach nearly 500 times the plasma level. Maybe, a lesser dose may suffice and the side-effects, that are mostly dose related, would be grossly decreased. Time will soon tell whether the world accepts HCQ, or whether, as the proverbial Vetal of the Vikram and Vetal mythology, it will stay around, waiting for a new virus to come, to be rejected again. Considering the events that have unfolded so rapidly in this crisis, this time, it appears that it will prove itself. It seems that HCQ is here to stay.

Table 1. Characteristics of Registered Chloroquine and Hydroxychloroquine Studies[5]

	Only HCQ	Only CQ	HCQ and/or CQ	Total
Total	117	25	16	158
Planned Enrolment	122,721	4,740	11,760	137,221
		Study Type		
*Interventional**	116 (99%)	25 (100%)	16 (100%)	157 (99%)
Observational	1 (0.8%)	0	0	1 (0.6%)
		Purpose**		
Treatment	70 (60%)	25 (100%)	12 (75%)	107 (68%)
PREP	38 (32%)	0	3 (19%)	41 (26%)
PEP	12 (10%)	0	1 (6.3%)	13 (8%)
Randomized	117 (100%)	19 (76%)	16 (100%)	153 (97%)
Blinded	53 (45%)	6 (24%)	5 (31%)	64 (41%)
		Control type		
Placebo	45 (38%)	5 (20%)	5 (31%)	55 (35%)
Active	13 (11%)	2 (8%)	3 (19%)	18 (11%)
"Standard of care"	24 (21%)	8 (32%)	8 (50%)	40 (25%)
Inactive therapy	7 (6%)	1 (4%)	0	8 (5.1%)
Non-consented	6 (5.1%)	0	0	6 (3.8%)
No treatment	6 (5.1%)	1 (4%)	0	7 (4.4%)
Not specified	0	2 (8%)	0	2 (1.3%)
Other***	0	1 (4%)	0	1 (0.6%)

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