ScienSage

Journal of Advanced Scientific Research

Available online through http://www.sciensage.info

ISSN
0976-9595
Review Article

DECONTAMINATION AND TREATMENT METHODS TO WARD-OFF SARS-COV-2 INFECTION: WHAT OPTIONS DO WE HAVE?

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ABSTRACT

COVID-19 caused by SARS-CoV-2 virus is a rapidly spreading disease that has now reached many parts of the world. Its treatment or medicine is not yet available and hence it has been threatening and affecting the world in a variety of ways. There is an immediate need to pay heed towards its effective prevention of spread and to find its treatment, so that it can be brought under control. In addition to various prevention measures like social distancing, maintenance of hygiene, etc., there is a need to decontaminate various surfaces and environments to slow down this epidemic. Simultaneously, the conventional and unconventional methods of treatment for this disease have to be searched or developed. The present article's main aim is to collect and comprehend the possible modes of decontamination and treatment strategies for this viral particle. It also gives basic information about the virus, its structure and life cycle. It is a meta-analysis like study which gives a brief idea about what different scientific studies suggest about the applicability and utilization of a particular strategy in decontamination and treatment of this virus.

Keywords: Coronavirus, SARS-CoV, MERS-CoV, COVID-19, Epidemiology

1. INTRODUCTION

Microorganisms have an intricate relationship with human since beginning of life on earth. Microbes affect a variety of aspects of human society in direct and indirect ways which may be beneficial or harmful. Basic classification of microbes includes categories like bacteria, fungi, protozoa, algae and viruses. Among all these categories, the most unique one is supposed to be the viruses. These are considered to be nucleoprotein structures which behave as non-living particles outside their host. These are obligate parasites which can show properties of a living organism inside their host. These cause many diseases in plants, animals, humans and can even eat away bacteria. These have been responsible for causing a number of diseases ranging from mild flu to fatal conditions like cancer in humans and animals. Apart from age old diseases like small pox, AIDS, viruses have been causing novel infections and diseases such as SARS, bird flu, Ebola virus disease, and many more which have affected lakhs of people all over the world and taking a huge toll also. There have been significant numbers of epidemics and pandemics due to viruses especially in recent decades. According to the WHO, viral diseases continue to raise serious health issues and many viral epidemics have been recorded like severe acute respiratory syndrome coronavirus (SARS-CoV) in 2002-03, and H1N1 influenza in 2009, Middle East respiratory syndrome coronavirus (MERS-CoV) in 2012 (Saudi Arabia) [1]. One of the most recent pandemics that has threatened the whole globe is that of SARS-CoV-2 outbreak that led to spread of Corona virus disease-2019 popularly called COVID-19, which began in wet market at Wuhan (China) in December 2019 [2]. The SARS-CoV-2 is a member of Corona virus family and is quite similar to related viruses found in pangolins and bats [3]. Both SARS-CoV and SARS-CoV-2 that are closely related to each other, originated in bats, which serve as reservoir hosts for these two viruses [4-7]. Palm civets and raccoon dogs are considered as the intermediate hosts for zoonotic transmission of SARS-CoV between bats and humans [8-10] while intermediate host for SARS-CoV-2 remains unknown [11]. Recent research suggests that a mutation that occurred in November 2019 in the spike of SARS-CoV led it jump in to humans [1].

Corona viruses comprise subfamily *Orthocoronavirinae*, in family *Coronaviridae* and are known to cause respiratory tract infections in human ranging from mild (common cold, flu) to fatal (SARS, MERS etc.). These are enveloped viruses with positive sense single stranded (ss) RNA genome along with a nucleo-capsid with helical

symmetry. Its genomic size range is one of the largest among RNA viruses consisting of 26 to 32 kilobases [12]. These have characteristic spikes on their surface that are club-shaped, giving them an image of solar coronaand hence they are named so [13]. These viruses mainly infect mammals and birds causing many fatal diseases that specifically effect the farming industries [14, 15]. These infect humans also and cause mild diseases like common cold [16, 17] and dangerous diseases like pneumonia, bronchitis, SARS etc. [18]. Considering the novel SARS-CoV-2 corona virus, there are many similarities and differences between this newly emerged (for humans) virus and the other corona viruses [19]. It is likely to be more contagious than other corona viruses that cause flu, influenza etc. because it is new for the human immune system, making it able to cause more cellular damage and produce more inflammatory cells [19]. According to the WHO, there is difference in the speed of transmission of both these viruses. The median incubation period of influenza, that is, the time from infection to beginning of symptoms appearance, and its serial interval (the period between successive cases), both are shorter than that of SARS-CoV-2 virus [20]. The serial interval for SARS-CoV-2 is 5-6 days while that for influenza virus, this serial interval is 3 days. This implicates that influenza can spread faster than SARS-CoV-2 virus [20]. Also, the reproductive number that is the number of secondary infections produced from one infected person is between 2-2.5 for COVID-19 virus, higher than that of influenza [20]. For SARS-CoV-2 virus, about 80% of the infections are asymptomatic or mild, 15% are severe where oxygen is needed and rest 5% are critical infections that need ventilation [20]. These fractions of critical and severe infections would be higher than those for influenza [20]. While it will take some time to completely understand the mortality due to COVID-19 (crude mortality ratio is 3-4%), it appears to be higher than that for influenza (especially seasonal influenza) [20]. Another very recent study that compared the 7 lungs obtained while autopsy from COVID-19 patients with 7 lungs obtained while autopsy from patients who expired due to acute respiratory distress syndrome (ARDS) secondary to H1N1 (influenza A) infection with 10 age matched uninfected control lungs [21]. In both cases where patients died of respiratory failure, the histological pattern in peripheral lung was found to be diffused alveolar damage with perivascular T-cell infiltration [21]. But lungs of COVID-19 patients showed an additional distinctive vascular feature that consisted of severe endothelial injury along with presence of intracellular virus and destroyed cell membranes [21]. Pulmonary vessels of COVID-19 patients exhibited widespread thrombosis, microangiopathy with alveolar capillary microthrombi being nine times more prevalent ($p<0.001$) in COVID-19 lungs in comparison to those of influenza [21]. Also, the new vessel growth mainly through intussusceptive angiogenesis in the COVID-19 patients' lungs was 2.7 times ($p<0.001$) of that in influenza patients [21]. All these findings suggest that vascular angiogenesis is a distinctive feature in the pulmonary pathobiology of COVID-19 not present in equally severe influenza virus infection [21].

2. STRUCTURE, COMPOSITION AND OTHER IMPORTANT PROPERTIES OF SARS-CoV-2 VIRUS

The SARS-CoV-2 virus, like other coronaviruses, are spherical particles with spikes made of protein protruding from their surface [22]. Angeletti et al. analyzed transmembrane helical segments in ORF1ab encoded 2 (nsp2) and nsp3 and have suggested that the position 723 has become a serine instead of a glycine residue and position 1010 has now been occupied by proline instead of isoleucine [23]. The disease relapses are actually associated with the mutations [1]. Talking about its genome on the basis of full-length genome sequences, it shows 79.6%sequence identity to SARS-CoV, and 96% similarity to a bat coronavirus at whole genome level [24]. Comparing SARS-CoV-2 with its previous counterpart SARS-CoV, it is observed that both infect and replicate in human lung tissues and are similar in cell tropism, with both types of viruses targeting alveolar macrophages and type I and II pneumocytes [25].

These spikes attach on to the human cells and then undergo a structural change allowing the viral membrane to fuse with the cell membrane. Its spikes bind to receptors called angiotensin-converting enzyme 2 (ACE2) on human cell surface [22]. Researchers have found that the spikes of SARS-CoV-2 virus are 10-20 times more likely to attach human cells as compared to the previous SARS virus that spread in year 2002 and therefore SARS-CoV-2 can spread more easily from human to human than the previous SARS virus [22]. Another study shows that SARS-CoV-2 S (spike glycoprotein) uses ACE2 to enter the cells and this spike glycoprotein of both SARS-CoV-2 and SARS-CoV have similar affinities towards human ACE2, indicating an efficient spread of SARS-CoV-2 among humans [11].

According to the same study, the SARS-CoV-2 S glycoprotein has a furin cleavage site at the boundary between S1/S2 subunits which is processed while biogenesis and makes this virus different from the SARS-CoV and other SARS related CoVs [11]. Also, it was found in another study that SARS-CoV-2 binds ACE2 with more affinity than SARS-CoV and therefore, it is more infectious than SARS-CoV [26]. The same study suggests that the spike protein of SARS-CoV-2 has significantly lower free energy than that of SARS-CoV and therefore SARS-CoV-2 is more stable and may exist at higher temperature than SARS-CoV [26]. Some studies suggest that SARS-CoV-2 outbreak is different from the 2003-SARS outbreak. It exhibits higher transmissibility but lower mortality than 2003-SARS [27-29]. It has also displayed efficient intra-familial spread [30].

Another strong reason behind high transmissibility and asymptomatic presentation of SARS-CoV-2 is that it infects the human lung tissues and replicates in them more efficiently than SARS-CoV. Within 48 hrs interval, SARS-CoV-2 is reported to generate 3.20 times more infectious virus particles than SARS-CoV [25]. Not only this, SARS-CoV-2 is reported to upregulate only five out of thirteen pro-inflammatory cytokines/chemokines while SARS-CoV upregulates 11 of them [25]. In addition to this, SARS-CoV-2 is found not to induce type I, II and III interferons (the anti-viral molecules) significantly in the infected human lung tissues [25].

3. LIFE CYCLE OF THE CORONA VIRUS

Talking about the life cycle of typical coronaviruses, it is known that initial attachment of the virion on host starts with interaction of S protein and its receptor. This interaction is not only the primary determinant of corona virus infection in the host but it also determines tissue tropism of the virus. Specifically, the SARS-CoV uses angiotensin converting enzyme 2 (ACE2) as its receptor to gain entry inside a human cell [31]. After the receptor binding, the virus accesses host cell cytosol through acid-dependent proteolytic cleavage of S-protein by a cathepsin, TMPRR2 or other protease and then there is fusion of cellular and viral membranes and release of genome of the virus in to the cytoplasm [31].Further, translation of the replicase gene from virion genomic RNA takes place and the replicase gene encodes 2 open reading frames (rep1a and rep1b) which express 2 co-terminal polyproteins (pp1a and pp1ab). Both these polyproteins (pp1a and pp1ab) have nsps(non-structural proteins)-1-11 and 1-16 respectively. These polyproteins are eventually cleaved in to individual nsps [32] by 2 or 3 proteases. Many nsps assemble to form replicase-transcriptase complex (RTC) to develop a suitable environment for RNA synthesis, finally leading to RNA replication and transcription of sub-genomic RNAs [31]. After viral RNA synthesis, translation and assembly of viral replicase complexes take place [31]. As a result of viral RNA synthesis, both sub-genomic and genomic RNAs are produced. Sub-genomic RNAs act as mRNAs for accessory and structural genes present downstream of replicase polyproteins [31].

After replication and formation of sub-genomic RNA synthesis, translation of viral structural proteins viz. S, E and M takes place and these proteins are inserted in to the ER (endoplasmic reticulum). These proteins further move along the secretory pathway in the ER-Golgi intermediate compartment (ERGIC) [33, 34]. Here, the viral genomes get encapsidated by N-protein and bud inside membranes of ERGIC that contain viral structural proteins to form matured virions [35].All these proteins perform their respective functions. For instance, the M protein directs protein-protein interaction needed for corona virus assembly [31]. Upon expression of M protein with E protein, virus like particles are produced [36], while the N protein enhances the virus like particles formation [37]. S protein on the other hand gets incorporated in the virions at this step but it is not needed for viral assembly [31]. M protein combines with the nucleocapsid and this promotes viral assembly completion [38]. After assembly, virions are transported to surface of cells in vesicles and are released through exocytosis. In many corona viruses, the S protein which is not involved in assembly to form virions moves towards the cell surface where it carries out cell-cell fusion among infected cells and their surrounding uninfected healthy cells, to form large multi-nucleated cells. This allows the virus to spread in the infected organism without getting detected or neutralized by antibodies specific for the virus [31]. Its symptoms include sore throat, shortness of breath, cough [39], nasal obstruction, muscular pain, fatigue, and shortness of breath, head ache with or without unilateral or bilateral pneumonia as observed in chest X-ray or CT scan [40]. However, many cases remain asymptomatic showing no marked features of the infection [41].Its world case fatality rate recorded on 30th May 2020 was around 6.18% (in India it was 2.86%) [42].

4. COVID-19: Why this disease reached pandemic level

COVID-19 spread all over the globe in a very short time. It was declared a pandemic by World Health Organization on 11th March 2019 [43]. There are many factors responsible for its world-wide spread:

- Delay in knowing/accepting that it is a human-to-human transmissible disease through cough, sneeze, droplets of saliva etc.;
- Delay in identification of gravity of this disease at an early stage;
- Delay in application of lockdown;
- Delay in prohibition of emigration and immigration at national, international and state level;
- Many infected individuals going undetected due to their being asymptomatic;
- Asymptomatic individuals spreading disease to others as they acted as carriers;
- Specific characters of the virus unlike other viruses due to which it spread and flourished well in both hot climate countries as well as cold climate countries;
- Easy and fast transmission through infected surfaces, fomites, droplets and even air. It is considered to be transmitted through air up to many feet because it is known that gas clouds produced by exhalation can travel up to 27 feet [44].
- It infects anybody of any race, sex, age, region. However, it is reported that it affects more males than females [45].
- Difficult detection due to its symptoms being similar to other flu like diseases which are not serious etc.

All these factors have been responsible for its world-wide impact making it a global concern.

5. POSSIBLE STRATEGIES TO CONTAIN SARS-CoV-2 VIRUS SPREAD

For the beginning of spread of COVID-19 to it reaching the epidemic and then pandemic level, one of the main reasons has been its ubiquitous presence. In whichever region the virus reached, it contaminated almost everything like surfaces, air, currency, metals, cardboard, plastics, eatable etc. In addition to developing effective treatments for this infection, the strategy to reduce it affecting more people therefore lies in decontaminating all these things, which is of course, not an easy task. Also, a single type of decontamination procedure cannot be applied on each and every thing. One cannot wash currency notes with warm-water soap solution! Another challenge is that decontamination has to be done on a large scale so the method applied should be such that it can decontaminate large areas and surfaces in a short time keeping in view the involved cost also. So, conclusively we have to work on two major fronts: one is large scale decontamination of surfaces and objects and the other is searching a treatment for this viral infection.

There are many methods of decontamination that are applied in different industries and are also available in literature based on experimental studies proving their decontaminating properties. These may be physical or chemical methods discussed in following sub-sections-

5.1. Physical methods

5.1.1. UV rays

The ultraviolet (UV) light is an ionizing electromagnetic radiation that isinvisible to human eye, having wavelength range of 10nm-400nm, shorter than the wavelength of visible light. UV light is of different types like UVA (320nm-400nm), UVB (280nm-320nm) and UVC (200nm-280nm), with UVA having longest wavelength and lowest energy to UVC having the shortest wavelength and highest energy [46]. These rays have the capacity to damage DNA to different levels. UVA is very much less absorbed by DNA and RNA and hence it induces much less damage. UVC is absorbed by both DNA and RNA bases, and can lead to photochemical fusion of 2 adjacent pyrimidines to form covalently linked dimers which become non-pairing bases [47]. UVB also causes such induction of formation of pyrimidine dimers, but with about 20-100 times less efficiency than UVC [47]. UVA is very weakly absorbed by DNA and RNA, but causes additional genetic damage by production of reactive oxygen species that causes oxidation of bases and breaks in strands [48]. Due to such effects of UV light on nucleic acids, these have been used in microbial disinfection and there have been certain experimental studies based evidences that prove that these rays can be used in inactivation of different microbes like bacteria, viruses, phages etc. Sunlight is also a good source of UV light and its virucidal effects have been demonstrated. Lytle and Sagripanti (2005) have suggested in their article that sunlight or specifically solar UV radiation behaves as the main natural virucide in the environment [49]. UV rays destroy viruses by chemically modifying their genetic material (DNA and RNA). The most effective UV wavelength for inactivating of viruses is 260 nm [50], which lies in the UVC range. DNA and RNA are also

damaged by UVA and UVB but with lesser efficiency [51]. McDevitt et al. (2012) have also suggested in their study that air disinfection through upper-room 254nm germicidal UVC light in public buildings could reduce influenza transmission through air-borne route [52]. According to Darnell et al. (2004) had also suggested that the SARS-CoV was inactivated by UV light at 254 nm. As far as the recent SARS-CoV-2 virus is concerned, Cascella et al. (2020) have suggested that like other CoVs, it is sensitive to ultra-violet rays [1].Keil et al. (2020) have also proved through their experimental study that riboflavin and UV light can decrease the SAR-CoV-2 titre in both plasma and platelet products to a below detectable limit in the tissue culture and their data suggests that this combination of riboflavin and UV light could reduce the theoretical risk of SARS-CoV-2 getting transmitted through transfusion [53]. Cadnum et al. (2020) used UVC in decontaminating the N95 respirators with *S.aureus*, MS2 and Phi6 (RNA virus) as test microbes and found that UVC could help in decreasing contamination of N95 respirators but their efficacy is lesser in comparison to high-level disinfection cabinets which are able to disinfect for a longer time [54]. Hamzavi et al. (2020) have also proposed to repurpose phototherapy devices like UVB units to serve as platform for UVC germicidal disinfection in a time when it has become necessary to reuse the disposable N95 filtering facepiece respirators, as the supplies are rapidly dwindling [55]. Thus, many studies involving corona viruses have shown that UV rays can inactivate them with a few evidences involving SARS-CoV-2. However, a recent Chinese study done by Yao et al. concluded that there is no association between transmission of COVID-19 and UV radiation [56]. More concrete evidences thus are required to reach a well-established conclusion.

5.1.2. Heat

Previous studies involving SARS-CoV have shown that these viruses are more stable at low temperature and low humidity. For instance, Chan et al. (2011) had suggested that SARS-CoV in dried form over smooth surfaces retained its viability for over five days at 22 to 25°C and relative humidity of 40 to 50%, but they rapidly lose their viability (>3 log10) at higher temperature olike 38°C and higher relative humidity of more than 95% [57]. Another previous study by Darnell et al. suggested that SARS-CoV virus get inactivated upon heat treatment of 65^0C for more than 4 minutes with incomplete loss of infectivity even maintaining this temperature for 60 minutes while upon treating it with 75°C for 45 minutes, it got completely inactivated [58]. Latest studies have also some suggestions which indicate the inactivating effect of heat on coronaviruses including SARS-CoV-2. According to Cascella et al. (2020), the SARS-CoV-2, like other CoVsis sensitive to heat [1]. As per a recent correspondence by Chin et al., the SARS-CoV2 virus is quite stable at 4°C, but it is sensitive to heat [59]. When SARS-CoV-2 is incubated in virus transport medium at a final concentration of ~6.8 log unit of 50%tissue culture infectious dose per ml, for up to 14 days, it was found to be highly stable at 4°C and there was only a 0.7 log unit reduction of infectious titre on day 14 [59]. However, when incubation temperature is raised to 70°C, the time for virus inactivation was decreased to 5 minutes [59]. Cascella et al. have also suggested that SARS-CoV-2, a member of βCoVs category, is sensitive to heat like other CoVs [1]. Some studies have however suggested limited effectiveness of dry heat in inactivation of bacteriophages (MS2 and phi6) in comparison to MRSA used for decontamination of N95 respirators [54]. Another study suggested on the basis of RBD-ACE2 binding for SARS-CoV-2 is much more sensitive to temperature than SARS-CoV, and therefore it is expected that SARS-CoV-2 infectivity would decrease much faster than SARS-CoV when temperature increases [26]. In the same study however, on the other hand (as mentioned above) it is said that due to lower free energy of spike protein of SARS-CoV-2 than SARS-CoV, the former is more stable and is able to endure higher temperature than the later [26]. So it is obvious that the scientific community groups have different opinions regarding the effect of heat in inactivating SARS-CoV-2 and more elaborate experimental studies are needed to prove either of the two.

5.1.3. Ultra-sound (Ultrasonic waves)

Ultrasonics is proven to be inactivating and destroying viruses in addition to other microbes like bacteria and yeast [60]. These lyse the cells to release the material present inside the virus particle or intracellular material in bacteria [60]. A study demonstrated the effect of high-frequency ultrasound and visible light together on two viruses namely phiX174, a ssDNA virus and MS2, a ssRNA virus, with the effects being differential [61]. In this study, high frequency ultrasound (582, 862 and 1142 kHz) were used with and without visible light and different viral concentrations prepared by diluting the viruses (bacteriophages) in phosphate buffer saline

solution to a titre of 103-104 pfu/mL [61]. The virus inactivation data obtained was a first order kinetic expression with inactivation being faster at lower frequencies (582 and 862 kHz) [61]. Also, the inactivation of MS2 was faster than that of phiX174. In case of MS2, the simultaneous use of ultrasound and visible light was more effective than ultra-sound alone (synergistic effect) but that in case phiX174 phage actually hindered the inactivation [61]. Since the SARS-CoV-2 is also an RNA virus, it is possible that use of ultrasound may destroy it. These waves may be used in decontaminating the surfaces where these viral particles may be present. There have been studies suggesting the use of ultrasound for decontamination of fresh fruits and vegetables [62], water [63] etc.

5.1.4. Infrared rays, Near-infrared rays and visible light

The Infrared rays are electromagnetic waves whose wavelength range is from 700 nm to around 1mm. These radiations along with the near infrared rays have been used for diagnostic purposes to detect viral infections like dengue [64], foot and mouth disease among cattle [65] etc. These have a heating effect and hence their utilization in decontamination of objects has been elucidated in few studies. To some extent however, their use in treatment of viral infections has also been searched upon. In the year 2011, Samim et al. synthesized gold nano-rods that show a surface plasmon resonance band at near infrared region due to which these were able to produce heat upon irradiation [66]. Thus, these rods have the capacity to be utilized as thermal therapeutic agents to damage bacterial cells, viruses, cancer cells, and DNA selectively [66]. Previous studies have also suggested such use of photothermal nanotherapeutics [67, 68].

There are also evidences showing that blue light is able to inactivate many viruses including coronavirus that causes common flu [69]. In the same article it is said that red and near infrared light are able to reduce respiratory disorders similar to those associated with corona virus infection, in experimental animals [69]. In patients, red light is shown to alleviate chronic obstructive lung disorders as well as asthma [69]. These can be very cost effective methods to develop low cost devices that can sanitize equipment, reduce infections, general environment etc. [56]. Hence efforts should be made in this direction to explore clinical value of light rays like Infrared, red, near infrared etc. [69].

5.2. Chemical methods

There are certain chemical reagents which are effective in decontaminating surfaces, equipment, etc. even of SARS-CoV-2. Darnell et al. (2004), had tried inactivation of SARS-CoVs using formaldehyde (37%) and glutaraldehyde (8%) diluted upto 1000 and 4000 times and they found that inactivation using these chemical agents is dependent on temperature [58]. For instance, both formalin (dilute formaldehyde) and glutaraldehyde were unable to inactivate virus (SARS-CoV) at 4°C even after incubating them for 3 days [58]. At 25°C and 37°C, formalin was able to inactivate most of the virus after 1 day incubation, however, some viral particles remained infectious on day 3 also [58]. Glutaraldehyde on the other hand, was able to completely inactivate the virus by 2nd day of incubation at 25°C and by 1st day of incubation at 37°C [58]. So, on this basis it can be speculated that these chemicals can also inactivate SARS-CoV-2 virus also, if proper conditions are provided.According to Cascella et al. (2020), SARS-CoV-2, like other SARS-CoVs, can be inactivated by application of lipid solvents like ethanol, ether (75%), peroxyacetic acid, chlorine containing disinfectants, chloroform (but not chlorohexidine) [1]. Peracetic acid and hydrogen peroxide may be effective against SARS-CoV-2, as these are quite successful in decontaminating N95 respirators contaminated with MRSA, Phi6 and MS2 bacteriophages [54].

5.3. Anti-viral drugs including anti-HIV drugs

Certain anti-HIV drugs have been under consideration to be used as a treatment for COVID-19. In a latest randomized, controlled, open-label trial a total of 199 hospitalized adult patients having confirmed SARS-CoV-2 infection, no benefit was observed by treatment with lopinavir-ritonavir beyond standard care, and the study suggests further requirement of future trials involving severely ill patients to confirm or exclude the possibility of a treatment benefit [70]. Another promising candidate compound to act against SARS-CoV-2 is remdesivir (GS-5734), that is a nucleotide analog prodrug which is currently under clinical trials for treatment of Ebola virus infection [71]. It is known that Remdesivir inhibited the replication of MERS-CoV and SARS-CoV in tissue cultures and has also shown efficacy in animal models [71]. Also, a combination of HIV-1 (human immunodeficiency virus type-1) protease inhibitors lopinavir/ritonavir (LPV/RTV) and IFN-β (Interferon-β) was found to be effective in patients having SARS-CoV infection and this combination also improved the clinical

parameters in mice and marmosets infected with MERS-CoV [71]. So, it is expected that these drugs (Remdesvir, LPV/RTV-IFN-β) may be effective against SARS-CoV-2. Another candidate compound is a guanosine analogue called Ribavirin which is an anti-viral compound used in treatment of many viral infections including viral hemorrhagic fever, hepatitis C, etc. [71]. Initially after giving promising results in MERS-CoV rhesus macaque model [72], the data obtained for ribavirin combined with IFN (IFN-α 2a or IFN-β1) have been conflicting [73]. In addition to this, ribavirin decreases the hemoglobin concentration which is an undesirable side-effect. These factors bring down its potential as an anti-viral agent in SARS-CoV-2 case [71].Chloroquine and hydroxychloroquine are inhibitors of viral entry and they do so by increasing the endosomal pH needed for the fusion of virus with the cell [74]. These also interfere with glycosylation of cellular receptors of SARS-CoVS (ACE-2) [74]. Nitazoxanide and Ivermectin are both immunomodulators, with Nitazoxanide interfering with host-regulated mechanisms (pathways) involved in replication of virus, amplification of cytoplasmic RNA sensing and type-1 IFN pathways and Ivermectin inhibiting the nuclear import of viral and host proteins by inhibiting importin-1 heterodimer [74]. Both these drugs are also under consideration for SARS-CoV-2 therapy. Nelfinavir is an HIV-1 protease inhibitor which can be considered but it has not been studied in humans for SARS-CoV-2 yet [75, 76]. Some more examples of drugs under consideration with regard to COVID-19 are favipiravir (a guanine analogue), sarilumab (IL-6 receptor antagonist), tocilizumab (recombinant humanized monoclonal antibody acting as IL-6 receptor antagonist) etc.[77]. In case of severe pneumonia and critically ill children, lopinavir/ritonavir and hydroxychloroquine trial should be considered [78] as suggested by Sankar et al. (2020). However, on the basis of a systematic review done by Ford et al. (2020), it is not certain whether anti-retrovirals like LPV etc. prevent SARS-CoV-2 infection or improve clinical outcomes among patients who are at higher risk of acquiring COVID-19 [79].

5.4. Biological methods

5.4.1. Interferons

Interferons (IFNs) are broad-spectrum anti-viral agents interacting with toll like receptors and which inhibit viral replication [80]. IFNs and their corresponding receptors are sub-set of class-2 α-helical cytokines [81], representing the early elements in innate and adaptive immunity [82]. Initially it was considered that human WBCs upon infection with viruses got induced to produce interferons [83], but later it was found that these are produced by many different types of cells, tissues and animals also [84]. Interferons comprise the 1st line of defense against viral infections in mammals as their system is designed for blocking the spread of viral infection in the body, at times even at the cost of accelerating the death of infected cells [84]. Interferons work by inducing production of many proteins which are not synthesized in resting cells, by activating the Jak-STAT pathways [84]. A viral infection is identified by many cellular pattern-recognition receptors and it itself triggers interferon production by the infected cells [85]. The surface receptors present on the uninfected cells identify these secreted interferons and activate various intra-cellular signaling pathways which induce the expression of IFN-stimulated genes [85]. The proteins encoded by these genes are responsible for inhibiting different steps of viral replication [85]. Viruses have mechanisms to protect themselves against this efficient mammalian system but administration of exogenous interferons as therapeutic anti-viral agents can shift the equilibrium of this host-viral interaction in host's favor [85].

As already mentioned that SARS-CoV-2 induces IFNs less effectively [25], it becomes obviously important to give external support to the host cells by administering exogenous IFNs, which can prove highly effective against this viral infection. Also, it has been seen that SARS-CoV-2 is more susceptible to IFNs in comparison to SARS-CoV as the infection rate of SARS-CoV-2 was significantly reduced upon IFN-α 2b inhalation [86]. Further, study done by Mantlo et al., reveals that SARS-CoV-2 is more sensitive as compared to other human viruses including SARS-CoV [87]. IFNs are therefore, good candidate molecules for SARS-CoV-2 infection. A number of research groups are working in this direction. For instance, some research groups have reported the use of IFN-α (broad spectrum anti-viral drug approved for treating viral hepatitis) in treating COVID-19 (five million units through vapor inhalation twice a day alone or in combination with ribavirin (500 mg 2-3 times per day) and lopinavir/ritonavir (400 mg/100 mg) for 10 days [88-90]. It can be used for prophylaxis against SARS-CoV-2 also [91]. According to Mantlo et al. (2020), treatment with IFN-α or IFN-β at the concentration of 50 IU per ml decreases viral titre by 3.4 logo or 4 log in Vero cells [87]. IFN-β is known to

improve lungs' condition and increase their ability to fight viral infections. It has been reported earlier that lowering in IFN-β production in the body increases the susceptibility to develop severe respiratory disorders occurring due to viral infection [90]. It has also been observed that SARS-CoV-2 suppresses IFN-β production in the body to protect itself from immune response [88]. So, it can be said conclusively that IFNs are very efficient in combating viral infections and can yield good results in case of SARS-CoV-2 also, provided they are not suppressed by this infection and/or administered exogenously.

5.4.2. Vaccines

Many different types of vaccines are under development for SARS-CoV-2. The vaccine technologies under evaluation are recombinant protein subunit vaccine, whole virus vaccine and nucleic acid vaccines [92]. There are also some other emerging approaches to develop therapeutics, vaccines for this virus like the development of peptide-mimetic inhibitors using computers and software [93, 94]. However, the task of developing a vaccine especially against a virus poses a variety of challenges like frequent mutations in viruses, safety concerns of the vaccine, etc. Even when there are sequence-related and structural similarities between the spikes of SARS virus and SARS-CoV-2, 3 different antibodies against the 2002 SARS virus were unable to bind with SARS-CoV-2 spike protein, indicating that this new virus has to be dealt with unique vaccines and antibody based treatment methods [22]. Researchers are working towards vaccine candidates targeting SARS-CoV-2 spike protein and they also expect to use this spike protein to isolate antibodies from recovered individual who were infected with this novel virus [22]. There are many therapeutics under clinical trials in China for SARS-CoV-2 and more than 20 vaccines are under development for COVID-19, there are currently no therapeutics or vaccines for it [20].

Vaccine development is actually a time consuming task, but still many attempts are being made to develop vaccines against SARS-CoV-2. For instance, Ahmed et al. (2020) screened experimentally-obtained SARS-CoV-derived B- and T-cell- epitopes in immunogenic structural proteins of SARS-CoV and identified a set of T- and B-cell epitopes obtained from the nucleocapsid (N) and spike (S) proteins which map to SARS-CoV-2 proteins in an identical manner [95]. According to them, since no mutation has been observed in these identified epitopes (as of 21st Feb. 2020), immune targeting of these epitopes can provide protection against SARS-CoV-2 [95]. According to Walls et al., the SAR-CoV polyclonal antibodies are able to inhibit SARS-CoV-2 spike mediated entry in to the cells and this indicates that cross-neutralizing antibodies targeting conserved S-epitopes can be elicited upon vaccination [11]. In March 2020, the 1st phase I clinical trial of a novel lipid nanoparticle (LNP)-encapsulated mRNA-based vaccine named mRNA-1273 that encodes spike protein of SARS-CoV-2, started in USA and production of such mRNA-based vaccines is a very concrete recent development in this regard [96]. However, development and testing of such vaccines at a fast pace is itself a big challenge [96].

Since sequencing of the SARS-CoV-2 genome has been achieved, many nucleic acid based vaccine candidates have come up, most of which are based on the S-protein coding sequence [97]. Some examples are INO-4800 (A DNA vaccine that can be delivered to human cells and translated into proteins to produce immune responses); mRNA-1273 (a synthetic mRNA strand encoding prefusion-stabilized viral S-protein); stabilized subunit vaccine; ChAdOx1 nCoV-19 (a vaccine composed of non-replicating adenovirus vector); nanoparticle based vaccine particles etc. [97]. All these vaccine candidates are associated with their respective pros and cons and are still under development and approval. Thus, it becomes clear that much work is underway to develop a specific vaccine with no associated adverse effects for SARS-CoV-2. However, much more is needed to be done.

5.4.3. Plasma therapy

Immunoglobulins or convalescent plasma or simply plasma therapy is a well-established method for passive immunization against viral infections [71]. Immunoglobulins or convalescent plasma has been utilized as the last option for improving survival rate of SARS patients, who exhibiting health deterioration even after treatment with methylprednisolone [98]. Many previous studies have shown a shorter stay at hospital along with lower mortality in patients administered with convalescent plasma [99-101]. Convalescent plasma collected from patients recovered from Ebola virus infection was recommended by WHO as an empirical treatment during outbreaks in year 2014 [102]. Similarly, in 2009 H1N1 influenza A pandemic, a study showed a significant decrease in relative risk of mortality among patients who were treated with convalescent plasma [103]. However, there are studies saying that such

benefits are not incurred upon due to plasma therapy. For example, a study done with MERS-CoV showed that sera obtained from recovering patients from the infection did not have sufficient antibody titers for therapeutic use [104]. Also, the appropriate titer of recovery-phase sera antibodies required for efficient therapy against SARS-CoV-2 remains to be determined [71].

Data from rigorous controlled clinical trials of convalescent plasma are quite few [105] but there are some robust examples are available. Convalescent plasma has been used in COVID-19 pandemic in China and their data suggests clinical benefits along with decrease in viral loads, radiological resolution and improvement in survival [105]. A small sample sized study done by Zhang et al. that had undertaken 6 recovered patients of SARS-CoV-2, who donated convalescent plasma reported that 5 of the 6 samples were weakly positive for IgM antibody by ELISA while they had high titers of IgG. The patients who were treated with this donated convalescent plasma did not need mechanical ventilation for eleven days after plasma transfusion and were later transferred to the general ward [106]. According to Zeng et al., convalescent plasma having an elevated level of SARS-CoV-2 IgG antibodies have been used in clinical therapy and exhibited good effects in China [107]. In another study done in China involving 6 confirmed COVID-19 patients were enrolled and transfused with convalescent plasma [108]. No adverse effects were observed and two of the recipients the virus was eliminated post plasma administration [108]. In two other recipients, the anti-SARS-CoV-2 antibody titers were found to get increased [108]. These results show that convalescent plasma therapy is quite effective and is specific also [108]. In another study conducted in China that involved 10 severely-ill patients receiving convalescent plasma (one dose=200mL) with neutralizing antibody titers above 1:640 and with an additional supportive care and anti-viral agents, many benefits were incurred like improvement of clinical symptoms and laboratory parameters within 3 days after transfusion, rapid increase in level of neutralizing antibody upto 1:640 in 5 cases, while in 4 other cases, maintenance of high level of neutralizing antibody of 1:640 [109]. Also, there was increase in oxyhemoglobin saturation within 3 days with increase in lymphocyte counts and decrease in C-reactive protein [109]. Seven patients who had viremia, the viral load reached undetectable level post transfusion and there were no adverse effects observed [109]. All these observations showed that convalescent plasma therapy is well-tolerated and is able improve clinical outcomes [109]. Most of these studies are based on very less number of patients and therefore more elaborate studies with more number of enrollments of patients are required to reach some concrete conclusions.

5.5. Alternative medicine

There are many forms of parallel therapy strategies in addition to the main stream methods, like Ayurved, Homeopathy, Traditional Chinese medicine etc. There are a few studies that suggest some natural, herbal, alternative medicines for prophylaxis and even treatment of COVID-19. For instance, an invited commentary suggests that some measures like- medicated water, mouth rinse and gargle (turmeric, natural salt, neem, catechu etc.), steam inhalation (with aromatic oils like menthol), nasal application of ghee (butter oil), sesame or coconut oil in nostrils etc. may prove useful in local prophylaxis against SARS-CoV-2 and may act as a complement towards therapeutic management [110]. Ayurveda also suggests many non-pharmacological methods like diet, mental relaxation, sleep, Asanas, Yoga to improve lung health [110]. It also advocates that the daily diet should include pulses, ginger, garlic, mustard seeds, cumin seeds etc., which can help in systemic prophylaxis [110]. The Rasayana therapy is also suggested to have direct importance in prophylaxis and management of SARS-CoV-2 infection [110]. The plant products used in Rasayana therapy are reported to be effective in immunomodulation and restoration of immune haemostasis [111]. Clinically, many botanicals described in the Ayurveda are used in boosting immunity like *Tinospora cordifolia* (Guduchi), *Withania somnifera* (Ashwagandha), *Asparagus racemosus* (Shatavari) etc. [110]. According to Rastogi et al. (2020), the Traditional Chinese medicine's contribution to controlling the COVID-19 epidemic cannot be overlooked and they have proposed a thorough plan in which Ayurvedic methods have been mentioned to handle different COVID-19 patient groups like unexposed asymptomatic group, exposed symptomatic group, patients with mild symptoms, and those with moderate to severe symptoms [112]. So, according to many research groups, these alternative medicines should be brought under trial so that their therapeutic potential can be identified and utilized for treatment of current pandemic.

6. CONCLUSION

SARS-CoV-2 outbreak is global challenge for which every country, big or small, developed or developing, is trying to come up with effective and safe solutions through which its spread can be contained and the infected individuals are treated well. To slow down its spread, many physical, biological and chemical methods have been discussed in above section. All these methods have their own advantages and disadvantages. For example, according to many studies, in current situation anti-viral drugs, remdesvir, LPV/RTV, chloroquine/hydroxychloroquine have shown effective inhibition of SARS-CoV-2 *in vitro* but are associated with severe side-effects due to high dose requirements and narrow therapeutic window [24]. So it becomes important that these adverse effects are reduced and for that matter, repurposing of these drugs with proper formulation is to be done to make them more efficient, safe and free from side-effects [24]. For this purpose, extracellular vesicles (natural carriers in human body playing important role in cell-to-cell communications) can be used as unique drug carriers for targeted delivery of protease inhibitors for treating COVID-19 eliciting lesser systemic side effects [24]. Also, the physical methods discussed above are not applicable on all surfaces and for every potentially contaminated object. So, it has to be made sure that the best and most effective method is used for every specific object and if possible, combination of different methods should be used to obtain the best results.

The first level treatment options include repurposed anti-malarials and anti-virals, plasma transfusion as development of existing or new vaccines is going to take time [113]. Further, careful monitoring, developing strategies, implementation of control measures, creation of proper medical guidelines and ethical laws are also needed as the trajectory of this outbreak is quite unpredictable [113].

With intelligent application of all these methods and more new upcoming ways to contain COVID-19, and with the understanding that which method would yield best results in a given region or country, we will surely be able to slow down COVID-19's progress and save millions of lives world-over.

7. ACKNOWLEDGEMENT

The Author acknowledges Dr. D.S. Bisen, Principal, Government Science College, Pandhurna, Chhindwara for his support in completion of this work.

8. REFERENCES

1. https://www.ncbi.nlm.nih.gov/books/NBK554776/
2. WHO. Coronavirus disease 2019 (COVID-19) situation report-62. March 21, 2020. https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200322-sitrep-62-covid-19.pdf?sfvrsn=f7764c46_2(accessed March 22, 2020).
3. https://www.sciencedaily.com/releases/2020/03/200317175442.htm
4. Ge XY, Li JL, Yang XL, Chmura AA, Zhu G, Epstein JH, et al. *Nature*, 2013; **503**:535-538.
5. Hu B, Zeng LP, Yang XL, Ge XY, Zhang W, Li B, et al. *PLoSPathog.*, 2017; **13**:e1006698.
6. Yang XL, Hu B, Wang B, Wang MN, Zhang Q, Zhang W, et al. *J. Virol.,* 2015a; **90**:3253-3256.
7. Zhou P, Yang XL, Wang XG, et al. *Nature,* 2020; **579(7798)**:270-273.
8. Guan Y, Zheng BJ, He YQ, Liu XL, Zhuang ZX, Cheung CL, et al. *Science*, 2003; **302**:276-278.
9. Kan B, Wang M, Jing H, Xu H, Jiang X, Yan M, et al., *J. Virol.,* 2005; **79**:11892-11900.
10. Wang M, Yan M, Xu H, Liang W, Kan B, Zheng B, et al. *Emerg. Infect. Dis.,* 2005; **11**:1860-1865.
11. Walls AC, Park YJ, Tortorici MA, Wall A, McGuire AT, Veesler D. *Cell,* 2020; **181(2)**:281-292.e6.
12. Woo PC, Huang Y, Lau SK, Yuen KY. *Viruses,* 2010; **2(8)**:1804-1820.
13. Almeida JD, Berry DM, Cunningham CH, Hamre D, Hofstad MS, Mallucci L. *Nature,* 1968; **220(5168)**:650.
14. Lee C. *Virol J.* 2015; **12(1)**:193.
15. Bande F, Arshad SS, Bejo MH, Moeini H, Omar AR. *J Immunol Res.,* 2015; **2015**:424860.
16. Mäkelä MJ, Puhakka T, Ruuskanen O, Leinonen M, Saikku P, Kimpimäki M, et al. *J ClinMicrobiol.* 1998; **36(2)**:539-542.
17. Owusu M, Annan A, Corman VM, Larbi R, Anti P, Drexler JF, et al. *PLoS One,* 2014; **9(7)**:e99782.
18. Zhong N, Zheng B, Li Y, Poon L, Xie Z, Chan K, et al. *Lancet,* 2003; **362(9393)**:1353-1358.
19. https://newsnetwork.mayoclinic.org/discussion/how-the-virus-that-causes-covid-19-differs-from-other-coronaviruses/
20. https://www.who.int/emergencies/diseases/novel-coronavirus-2019/question-and-answers-hub/q-a-

detail/q-a-similarities-and-differences-covid-19-and-influenza
21. Ackermann M et al. *New England Journal of Medicine,* 2020; DOI: 10.1056/NEJMoa2015432.
22. https://www.nih.gov/news-events/nih-research-matters/novel-coronavirus-structure-reveals-targets-vaccines-treatments
23. Angeletti S, Benvenuto D, Bianchi M, Giovanetti M, Pascarella S, Ciccozzi M. *J. Med. Virol.,* 2020; **92(6)**.
24. Kumar S, Zhi K, Mukherji A, Gerth K. *Viruses,* 2020; **12(5)**:E486.
25. Chu H, Chan JF, Wang Y, et al. *Clin Infect Dis.*, 2020; ciaa410.
26. He J, Tao H, Yan Y, Huang SY, Xiao Y. *Viruses,* 2020; **12(4)**:428.
27. WHO. 2020. Novel coronavirus (2019-nCoV) situation reports. World Health Organization. http://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports/.
28. Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y. *Lancet*, 2020; **395**:507-513.
29. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. *Lancet*, 2020; **395**:497-506.
30. Chan JFW, Yuan S, Kok KH, To KKW, Chu H, Yang J, et al. *Lancet,* 2020; **395**:514-523.
31. Fehr AR, Perlman S. *Methods MolBiol,* 2015; **1282**:1-23.
32. Ziebuhr J, Snijder EJ, Gorbalenya AE. *J Gen Virol.,* 2000; **81**:853-879.
33. Krijnse-Locker J, Ericsson M, Rottier PJM et al. *J Cell Biol.,* 1994; **124**:55-70.
34. Tooze J, Tooze S, Warren G. *Eur J Cell Biol.,* 1984; **33**:281-293.
35. deHaan CA, Rottier PJ. *Adv Virus Res.*, 2005; **64**:165-230.
36. Bos EC, Luytjes W, van der Meulen HV et al., *Virology,* 1996; **218**:52-60.
37. Siu YL, Teoh KT, Lo J et al., *J Virol*. 2008; **82**:11318-11330.
38. Hurst KR, Kuo L, Koetzner CA et al., *J Virol.,* 2005; **79**:13285-13297.
39. Han C, Duan C, Zhang S, et al. *Am J Gastroenterol.,* 2020;10.14309/ajg.0000000000000664.
40. Jin X, Lian JS, Hu JH, et al. *Gut*. 2020; **69(6)**:1002-1009.
41. Yu X, Yang R. *Influenza and Other Respir Viruses,* 2020;10.1111/irv.12743.
42. https://ourworldindata.org/grapher/coronavirus-cfr?country=ITA~KOR~OWID_WRL~DEU~ISL~IND.
43. WHO. WHO Director General's opening remarks at the media briefing on COVID-19 – 11 March 2020. https://www.who.int/dg/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19---11-march-2020.
44. https://www.usatoday.com/story/news/health/2020/03/30/coronavirus-social-distancing-mit-researcher-lydia-bourouiba-27-feet/5091526002/
45. Conti P, Younes A. *J BiolRegulHomeost Agents*, 2020; **34(2)**:10.23812/Editorial-Conti-3.
46. https://www.cancer.org/cancer/cancer-causes/radiation-exposure/uv-radiation.html
47. Perdiz D, Grof P, Mezzina M, Nikaido O, Moustacchi E, Sage E. *J. Biol. Chem.* 2000; **275**:26732-26742.
48. Tyrrell JL, Douki T, Cadet JJ. *Photochem. Photobiol. B.,* 2001; **63**:88-102.
49. Lytle CD, Sagripanti JL. *J Virol.* 2005; **79(22)**:14244-14252.
50. Rauth, AM. *Biophys. J.,* 1965; **5**:257-273.
51. Setlow, R. *Biochim. Biophys. Acta,* 1960; **39**:180-181.
52. McDevitt JJ, Rudnick SN, Radonovich LJ. *Appl Environ Microbiol.* 2012; **78(6)**:1666-1669.
53. Keil SD, Ragan I, Yonemura S, Hartson L, Dart NK, Bowen R. Vox Sang. 2020;10.1111/vox.12937.
54. Cadnum JL, Li DF, Redmond SN, John AR, Pearlmutter B, Donskey CJ. *Pathog Immun.,* 2020; **5(1)**:52-67.
55. Hamzavi IH, Lyons AB, Kohli I, et al. *J Am AcadDermatol.,* 2020; **82(6)**:1511-1512.
56. Yao Y, Pan J, Liu Z, et al. *EurRespir J.,* 2020; **55(5):**2000517.
57. Chan KH, Peiris JSM, Lam SY, Poon LLM, Yuen KY, Seto WH. *Advances in Virology*, 2011; **2011**:7 pages.
58. Darnell ME, Subbarao K, Feinstone SM, Taylor DR. *J Virol Methods,* 2004; **121(1):**85-91.
59. Chin AWH, Julie T S Chu JTS, Perera MRA, Hui KPY, Yen HL, Chan MCW, Peiris M, Poon LLM. *The Lancet Microbe.* 2020.
60. https://www.hielscher.com/ultrasonics-for-vaccine-production.htm

61. Chrysikopoulos CV, Manariotis ID, Syngouna VI. *Colloids Surf B Biointerfaces*. 2013; **107:**174-179.
62. Bilek SE, Turantaş F. *Int J Food Microbiol.,* 2013; **166(1):**155-162.
63. Mason TJ, Joyce E, Phull SS, Lorimer JP. *UltrasonSonochem,* 2003; **10(6):**319-323.
64. Chen L, Wang H, Guo T, et al. *J Immunol Methods,* 2018; **456**:23-27.
65. Rainwater-Lovett K, Pacheco JM, Packer C, Rodriguez LL. *Vet J,* 2009; **180(3):**317-324.
66. Samim M, Prashant CK, Dinda AK, Maitra AN, Arora I. *Int J Nanomedicine,* 2011; **6**:1825-1831.
67. Zharov VP, Kim J-W, Everts M, Curiel DT. *J Nanomedicine.* 2005; **1:**326-345.
68. Zharov VP, Kim JW. *Lasers Surg Med,* 2006; **18:**16-17.
69. Enwemeka CS, Bumah VV, Masson-Meyers DS. *J PhotochemPhotobiol B,* 2020; **207**:111891.
70. Cao B, Wang Y, Wen D, et al. *New England Journal of Medicine,* 2020; **382(19):**1787-1799.
71. Martinez MA. *Antimicrob Agents Chemother.,* 2020; **64(5):**e00399-20.
72. Falzarano D, De Wit E, Rasmussen AL, Feldmann F, Okumura A, Scott DP, et al. *Nat Med.,* 2013; **19**:1313-1317.
73. Arabi YM, Shalhoub S, Omari AA, Mandourah Y, Al-Hameed F, Sindi A, et al. *Am J RespirCrit Care Med.*, 2017; **195**:A6067.
74. ŞimşekYavuz S, Ünal S. *Turk J Med Sci.,* 2020; **50(SI-1):**611-619.
75. Agouron Pharmaceuticals Inc. Viracept (nelfinavir mesylate package insert. La Jolla, CA: Agouron Pharmaceuticals Inc; 2005.
76. Barlow A, Landolf KM, Barlow B, et al. *Pharmacotherapy*, 2020; **40(5):**416-437.
77. Lu CC, Chen MY, Chang YL. *J Chin Med Assoc.*, 2020;10.1097/JCMA.0000000000000318.
78. Sankar J, Dhochak N, Kabra SK, Lodha R. *Indian J Pediatr.,* 2020; **87(6):**433-442.
79. Ford N, Vitoria M, Rangaraj A, Norris SL, Calmy A, Doherty M. *J Int AIDS Soc.,* 2020; **23(4):**e25489.
80. Uematsu S, Akira SJ.*Biol Chem.,* 2007; **282**:15319-23.
81. Krause CD, Pestka S. *PharmacolTher.*, 2005; **106(3)**:299-346.
82. Pestka S. *J Biol Chem.*, 2007; **282(28)**:20047-20051.
83. Cantell K, Hirvonen S, Kauppinen HL, Myllylä G. *Methods Enzymol.*, 1981; **78(Pt A)**:29-38.
84. Sen GC. *Annu Rev Microbiol.* 2001; **55**:255-281.
85. Fensterl V, Chattopadhyay S, Sen GC. *Annu Rev Virol.,* 2015; **2(1)**:549-572.
86. Shen K, Yang Y. *World J Pediatr.*, 2020. https://doi.org/10.1007/s12519-020-00344-6
87. Mantlo E, Bukreyeva N, Maruyama J, Paessler S, Huang C. *Antiviral Res.,* 2020; **179:**104811.
88. Lu H. *Biosci Trends,* 2020; **14:**69-71.
89. Dong L, Hu S, Gao. *J. Drug DiscovTher.*, 2020; **14:**58-60.
90. Sallard E, Lescure FX, Yazdanpanah Y, Mentre F, Smadja NP. *Antivir Res.,* 2020; **178:**104791.
91. Lokugamage KG, Schindewolf C, Menachery VD. *bioRxiv.,* 2020. doi: 10.1101/2020.03.07.982264
92. Chen WH, Strych U, Hotez PJ, Bottazzi ME. *Curr Trop Med Rep.*, 2020; 1-4.
93. Ibrahim IM, Abdelmalek DH, Elshahat ME, Elfiky AA. *J Infect.,* 2020; **80(5):**554-562.
94. Robson B. *ComputBiol Med.,* 2020; **119**:103670.
95. Ahmed SF, Quadeer AA, McKay MR. *Viruses,* 2020; **12(3):**254.
96. Wang F, Kream RM, Stefano GB. *Med SciMonit.* 2020; **26**:e924700.
97. Tu YF, Chien CS, Yarmishyn AA, et al. *Int J MolSci,.* 2020; **21(7):**2657.
98. Chen L, Xiong J, Bao L, Shi Y. *Lancet Infect Dis.*, 2020; **20(4):**398-400.
99. Lai ST. *Eur J ClinMicrobiol Infect Dis.*, 2005; **24**:583-591.
100. Cheng Y, Wong R, Soo YO. *Eur J ClinMicrobiol Infect Dis.* 2005; **24**:44-46.
101. Soo YO, Cheng Y, Wong R. *ClinMicrobiol Infect.*, 2004; **10**:676-678.
102. WHO 2014. http://apps.who.int/iris/rest/bitstreams/604045/retrieve.
103. Hung IF, To KK, Lee CK. *Clin Infect Dis.,* 2011; **52**:447-456.
104. Arabi Y, Balkhy H, Hajeer AH, Bouchama A, Hayden FG, Al-Omari A, et al. *Springerplus,* 2015; **4(1)**:1-8.
105. Bloch EM, Shoham S, Casadevall A, et al. *J Clin Invest.*, 2020; **130(6):**2757-2765.
106. Zhang L, Pang R, Xue X, et al. *Aging,* 2020; **12(8):**6536-6542.

107. Zeng F, Dai C, Cai P, et al. *J Med Virol.,* 2020; 10.1002/jmv.25989.
108. Ye M, Fu D, Ren Y, et al. *J Med Virol.,* 2020; 10.1002/jmv.25882.
109. Duan K, Liu B, Li C, et al. *Proc Natl AcadSci U S A,* 2020; **117(17):**9490-9496.
110. Tillu G, Chaturvedi S, Chopra A, Patwardhan B. *J Altern Complement Med.,* 2020; **26(5):**360-364.
111. Agarwal R, Diwanay S, Patki P, et al. *J Ethnopharmacol*, 1999; **67:** 27–35.
112. Rastogi S, Pandey DN, Singh RH. *J Ayurveda Integr Med.,* 2020;10.1016/j.jaim.2020.04.002.
113. Khan T, Agnihotri K, Tripathi A, Mukherjee S, Agnihotri N, Gupta G. *AlternTher Health Med.* 2020; AT6471.