



## Corona Virus: A Review of COVID-19

Maulshree Bhandari\*, Nayan Kumar, Rohit Kumar, Rishabh Singh, Veerendra Pathak and Jitendra Singh

\*Department of Pharmacognosy, Invertis institute of Pharmacy, Bareilly, (U.P.) - India

Department of Pharmacy, Invertis institute of Pharmacy, Bareilly, (U.P.) - India

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### Abstract

In December 2019, an outbreak of COVID-19, caused by new Corona virus, known as novel Severe Acute Respiratory Syndrome Virus-2 (SARS-CoV-2) In Wuhan city, China. On January 30, 2020 The WHO declared as a public health emergency or characterized as Pandemic. As on May 7, 2020 around world 3,822,951 Cases are confirmed out of which 265,084 peoples died with the virus. Corona virus causes Respiratory infection Pneumonia, Cold, Sneezing, Coughing & it may also causes diarrhea & kidney failure. Corona viruses enter in human cell through type II Pneumocyte of alveoli by attaching to ACE-2 receptor of endothelial. The first people affected with COVID-19 had links to an animal & seafood market. The fact indicates that animals initially transmitted the virus to humans.

**Key words:** Corona virus, MERS-CoV, SARS-CoV, RT PCR, Plasma therapy

### Introduction

#### History & Origin

Corona virus was firstly discovered or identified in 1930s. At that time, Acute Respiratory infection was shown in domesticated chickens. In 1931, a new respiratory infection of chickens in North Dakota was described by Arthar Schalk and M.C. Hawn. Six years later Fred Beaudette and Charles Hudson successfully isolated and cultivated the infectious bronchitis virus which caused the disease. In 1940s, two more animal coronaviruses, mouse hepatitis virus (MHV) and another is transmissible gastroenteritis virus (TGEV), were isolated. Human coronavirus were discovered and isolated by using two different methods in the United Kingdom and United States in 1960s. E.C. Kendall, Malcom Byone, and David Tyrrell isolated a novel common cold virus B814 from a boy, they working at British Medical Research Council in 1960. In 1965, Tyrrell and

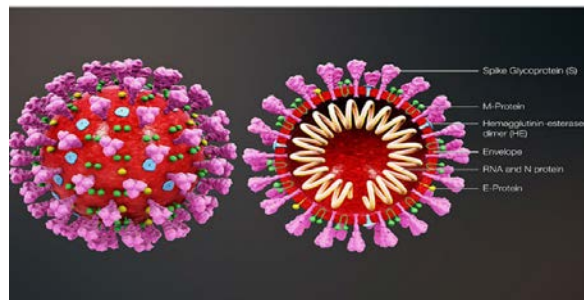
Byone successfully cultivated the novel virus by serial passing through organ culture of human embryonic trachea. The new cultivating method was introduced to lab by Bertil Hoorn. At same time, Dorothy Hamre and John Procknow at the University of Chicago isolated a novel cold virus 229E from medical students, which they grew in kidney tissue culture. The novel virus 229E, like the virus strain B814, when inoculated into volunteers caused a cold and was inactivated by ether. A researcher group at the National Institute of Health was able to isolate another member of this new group of virus using organ culture and named the virus strain OC43. Like B814, 229E, and IBV. Human coronavirus 229E and OC43 continued to be studied in subsequent decades.

**\*Corresponding Author**

**E.mai:** [maulshree.b@invertis.org](mailto:maulshree.b@invertis.org)

Other human coronavirus have been identified, including SARS-CoV in 2003, HCoV NL63 in 2004, HCoV HKU1 in 2005, MERS-CoV in 2012, and SARS-CoV-2 in 2019. [1-4]

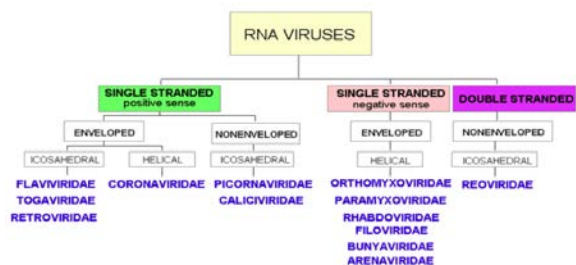
### Microbiology



**Fig. 1: Structure of Corona virus**

Corona virus is a large family, mostly spherical or pleomorphic, positive sense single stranded RNA and covered with club shaped glycoprotein. An average diameter of coronavirus is 120nm, diameter of envelope 85nm and the spikes are of 20nm long. The viral envelope consists of a lipid bilayer in which the membrane (M), envelope (E), spikes (S) and Hemagglutinin esterase.

On average a Coronavirus particle has 74 surface spikes. The Coronavirus surface spikes are homotrimers of the s protein which is composed of S1 and S2 subunit. Inside the envelope there is Nucleocapsid, which is formed from multiple copies of the Nucleocapsid (N) protein, which are bound to the positive sense single RNA genome. The lipid bilayer envelope, membrane proteins and Nucleocapsid protects the virus when it is outside the host cell. Coronavirus belonging to family Coronaviridae and have four sub types such as alpha, beta, gamma and delta Coronavirus. Each of subtype has many serotypes, some of them affects human or animals such as pig, birds, cats, mice and dogs. [5-10]



**Fig. 2: Classification of RNA virus**

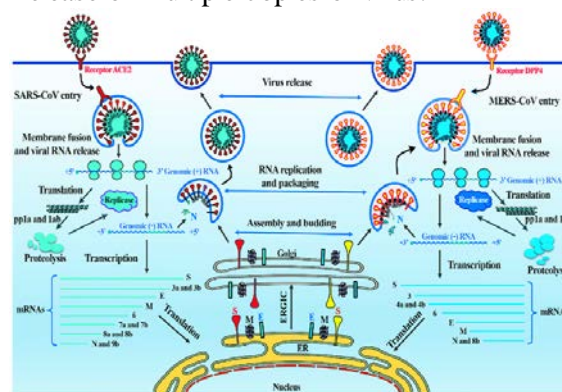
### Mode of transmission

Respiratory infectious can be transmitted through droplets of different sizes; When the droplet particles are  $>5-10\mu\text{m}$  in diameter they are referred to as respiratory droplets and when droplets are  $<5\mu\text{m}$  in diameter they are referred to as droplet nuclei. According to current evidence, COVID-19 is primarily transmitted between people through respiratory droplets and contact routes. Droplet transmission occurs when a person is in close contact (within 1m) with someone who has been infected with virus and having risk of exposed to infective respiratory droplets via mucosae (mouth and nose) or conjunctiva (eyes). Transmission may also occur through fomites in immediate environment around the infected person. So, transmission of COVID-19 virus can occur by direct contact with infected people and indirect contact with surface or with objects by an infected person. Airborne transmission is different from droplet transmission as it refers to the presence of microbes within droplet nuclei. There is some evidences that COVID-19 infection may lead to intestinal infection and be present in faeces. However, to date only one study has cultured the COVID-19 virus from a single stool specimen. There have been no reports of a faecal-oral transmission of COVID-19 virus to date. [11-13]

### Life cycle of Corona virus

Steps include :-

1. Attachment and Entry
2. Uncoating
3. Replication and Transcription
4. Packaging and Assembly
5. Release of multiple copies of virus.



**Fig. 3: Life cycle of Corona virus**

Firstly virus enters in upper respiratory tract than enter in lower respiratory tract → Lower respiratory tract → Right and Left bronchi → Primary, secondary and tertiary bronchi → Terminal bronchioles → Respiratory bronchioles → Alveolar sacs (air sacs).

**Attachement and Entry:-** There are two types of cell present in alveoli Type I (helps in air exchange between alveoli and pulmonary capillary) and Type II pneumocyte (responsible for the production and secretion of surfactant). When virus enters in alveoli, it triggers Type II pneumocytes where ACE-2 receptors are present. Spike proteins and HE glycoprotein of corona virus helps in attachement with ACE-2 receptor of host cell and after attachement virus enters inside the cell via fusion.

**Uncoating:-** Virus releases its genome (+ssRNA) that hijacks ER of host cell.

**Replication and transcription:-** Here, Viral RNA acts as mRNA Endoplasmic Reticulum produces poly proteins and these poly proteins are divided into non structural proteins by the help of proteases and also forms RNA dependent RNA polymerase enzyme (RdRp), that decodes RNA strand and it changes +ssRNA into -ssRNA. Again this -ssRNA uses RdRp and changes into +ssRNA and many copies of progeny virus are produced. Ribosomes of host cell reads formed progeny RNA virus and forms multiple structural and accessory protein.

**Packaging and Assembly:-** Formed progeny RNA virus has been transferred to golgi bodies where packaging of structural proteins are carried out and packed into secretory vesicle.

**Release:-** By the process of exocytosis multiple copies of RNA virus are released from the cell.

By this whole process Type II pneumocyte are damaged so that inflammatory responsible are initiated. These damaged cells release interferon and cytokinin and due to inflammatory responses alveolar macrophages identify

cell injury and release cytokinin, such as TNF- $\alpha$ , IL-1, IL-6, IL-8. These intrferons alerts Type I and Type II cell to response Antiviral property. [14-16]

### Symptoms

Cold or flu like symptoms usually in 2-14 days after a Corona virus infection and are typically mild.

Primary symptoms include:-

1. Sneezing
2. Coughing
3. Runny nose
4. Sore throat
5. Fever
6. Fatigue and body ache.

Severe symptoms include:-

1. Pneumonia
2. Kidney failure
3. Watery diarrhea
4. High fever
5. Shortness of breath. [17]

### Corona virus risk factors

Anyone can infected with COVID-19 and most infection are usually mild, especially in children and young adults. But if you are not in area where COVID-19 is spreading and you haven't travel history of an area where COVID-19 cases are confirmed and haven't been in contact with someone who has been affected by COVID-19. Peoples over 65 are most likely to yet a serious illness, those who live in nursing homes or long term care facilities, who have weakened immune system, or who have medical condition including high BP, Heart disease, Kidney disease, Lung disease, Diabetes and Cancer etc. [18]

### Diagnosis

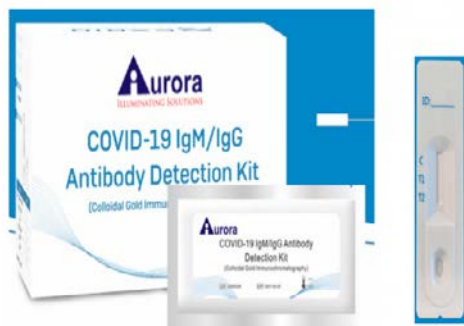
If you have been exposed to virus and symptoms of Corona virus disease 2019 are developed contact to doctor. In most cases of self limited infection, diagnosis of Corona virus is unnecessary as the diseases will naturally run its course. COVID-19 can be diagnosed similarly to other conditions caused by viral infection using a blood, saliva or tissue sample. However most tests by using a cotton swab to retrieve a Nasopharyngeal and Oropharyngeal specimen. RT PCR has become the method of choice for diagnosis of Human CoV, as multiplex Real time RT PCR assay have been developed and are able to detect all four respiratory HCoV. On April 4,

The ICMR announced the introduction of rapid antibody testing for COVID-19, which involves conducting blood test on peoples displaying influenza like illness symptoms in contaminated zones. A rapid antibody test is conducted to determine the presence of antibodies IgM and IgG, which can be detected from the seventh day of onset of COVID-19 symptoms.

Two main diagnostic technique for Human Corona virus:-

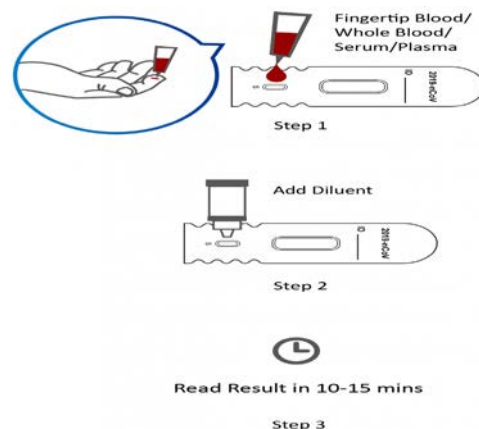
1. COVID-19 IgM/IgG Antibody test or Serological test.
2. RT PCR.

1. Antibody test:-Antibody test or Serological test detects antibodies or proteins present in blood after exposure to an antigen. Bodies develop these antibodies in order to fight against antigen. This rapid test kit is suitable for qualitative detection of SARS-CoV-2 IgM/IgG antibodies in human serum, plasma or whole blood.



**Fig. 4: COVID-19 Antibody Test Kit**

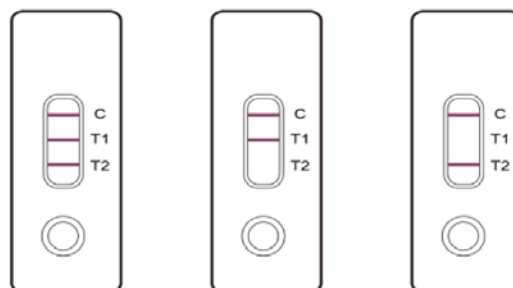
**Working:-** Test for COVID-19, IgM and IgG antibodies is a rapid and effective method for the diagnosis of COVID-19 infection. The IgM and IgG antibodies are provide information on the stage of infection. Both IgM and IgG antibodies are produced during the primary immune response. As, the body's largest antibody, IgM is the first antibody to appear in response to an initial exposure to antigen. IgM produces the first line of defense during viral infection and IgG responses for long term immunity and immunological memory. IgG is usually detectable about 7 days after the IgM appears.



**Fig. 5: Instruction for Use**

**Interpretation of Test Result:-**

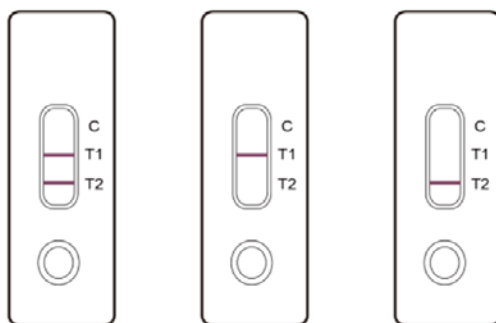
1. IgM and IgG positive:- Both the lines IgM, IgG and the quality control line (C) are coloured in COVID-19 IgM/IgG antibody test kit, it means virus affects a lot earlier (2-3 weeks are passed).
2. IgM positive:- Two lines appears on COVID-19 test kit IgM and C, it means result is positive for SARS-CoV-2 specific IgM antibody and virus affects very recently (1-2week).
3. IgG positive:- IgG and control line (C) appears coloured on COVID-19 antibody test kit. This results is positive for SARS-CoV-2 specific IgG antibody and virus affects for last 20-25 days.



**Fig. 6: Interpretation of positive result**

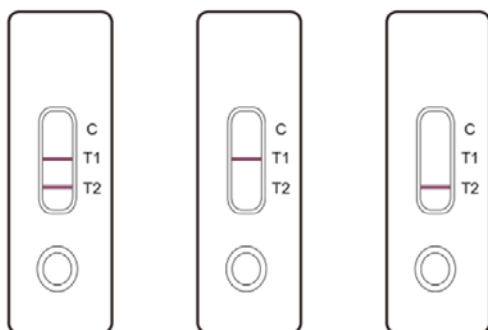
4. Negative:- One coloured line appears in control region (C). No other coloured line appears in IgM/IgG test region, it

means the result is negative for SARS-CoV-2.



**Fig. 7: Interpretation of negative result**

5. Invalid:- Control line fails to appear, it means insufficient sample or incorrect procedure techniques are reason for control line failure.



**Fig. 8: Interpretation of invalid result**

#### Limitations of COVID-19 IgM/IgG rapid test:-

1. The product is designed only for use Human serum, plasma or whole blood samples for detection of SARS-CoV-2 IgM and IgG antibody.
2. A false negative may occur if the amount of SARS-CoV-2 IgM or IgG antibody is below the detection level of kit.
3. If the product gets wet prior to use, or is stored improperly, it may cause incorrect results.
4. The test is for qualitative detection of SARS-CoV-2 IgM or IgG antibody in Human serum, plasma or blood samples, it does not indicate the quantity of antibodies present.

#### • **Specification:-**

IgG Sensitivity: 96.52%,  
Specificity: 98.52%  
IgM Sensitivity: 94.07%,  
Specificity: 97.7%  
Sample Volume: 20µl finger trip blood and whole blood sample, or 10µl serum and plasma.  
Detection Method: Colloidal Gold  
Detection Time: 10-15 min. [19-25]

#### 2. Real Time RT PCR (Reverse Transcription polymerase chain reaction):-

- **PCR:-** It is an amplification process by which a single double stranded DNA molecule is copied into multiple copies. It is in vitro technique and based on DNA Replication. Thermal cycler, is PCR machine used to run Amplification process.
- **Component Used:-** Target DNA molecule, Taq polymerase, specific primer that binds to DNA, mixture of free nucleotide (A, T, G, C), reaction buffer and mineral oil.

- **Steps involved in PCR:-** fill the samples and other component in PCR tube.

- A. Denaturation:- Heat the ingredient at 96°C for 1min, this breaks all H-bond between two structure of DNA molecule so that they can separate out.
- B. Annealing:- Cool the sample to 55°C so that primers forms H-bonds with its complimentary sequence of that target DNA.
- C. Extension:- Heat the mixture to 72°C for 2 min. Taq polymerase extend the new DNA strand by using free Nucleotides. Taq polymerase begins to polymerize- adding Nucleotide to 3'prime end of each primer and attach to DNA strand.



After one complete cycle there are 2 double stranded DNA molecule is formed. This cycle is continued and multiple copies of DNA molecule is formed.

**Real time PCR:-** Real time PCR is a specific type of PCR technique that explains the synthesis of target DNA fragment is Real time. We can measure the amount of DNA produced after every rounds of per cycle. This technique is also known as the quantitative PCR or quantitative Real Time PCR.

**RT PCR:-** It is a laboratory technique combining Reverse transcription of RNA into DNA and amplification of specific DNA target using PCR. It is primarily used to measure the amount of a specific RNA.

#### **Working principle of RT PCR :**

A sample is collected from parts of the body such as a person's nose or throat. To detect RNA molecule must be change into complimentary DNA sequence by RT. Than the newly synthesized DNA can be amplified by PCR technique, this is universally known as RT PCR. To perform this procedure viral RNA should be extracted. To extract the viral RNA by using commercial kit. The sample is added in a micro centrifuge tube than it is mixed with lysis buffer, this buffer is highly denaturing and consists of Phenol and Guanidine isothiocyanate. Also RNA inhibitor are also used, after that tube is mixed with vortex mixer and incubated at room temperature. Than the virus is lysed under the highly denaturing condition provided by lysis buffer. Once the sample is lysed purification procedure is carried out by using spin column. The sample is loaded into spin column, than centrifuge is performed. This procedure is solid-phase extraction method in which the stationary phase consists of silica. RNA molecule binds with silica gel membrane and at the same time protein and other contaminants are not retained. After centrifuge the spin column is placed in a clean column tube and filtrate is discarded and then wash buffer is added to it. Again the column is placed into centrifuge forcing the wash buffer through membrane, this removes any remaining impurities from the membrane leaving the only RNA bound

to silica gel. Once the sample is washed the column is placed into a clean micro centrifuge tube and elution buffer is added. Then centrifugation is carried out forcing the elution buffer through the membrane. The elution buffer removes viral RNA from the spin column and the purified viral RNA which is free of protein inhibitors and other contaminants are obtained. After that extraction of viral RNA the next step is the preparation of reaction buffer. In this step a master mixture is used, which consists of RT, dNTPs, Reverse Primer, Forward Primer, Taq man probe and RNA Polymerase. Finally to complete this reaction mixture the RNA template is added the tube is mixed with vortex mixer technique. Then the reaction mixture is loaded in a PCR plate and the plate is placed into PCR machine, which is essentially a thermocycler. Real Time RT PCR is used for detection of Corona virus disease 2019 by the amplification of target sequence of RdRp gene, E gene and N gene. The choice of target gene depends on probe sequence and primers. First step in RT PCR is Reverse Transcription. The first strand is complimentary DNA synthesis. RT starts to add nucleotides at 3' prime end of primer synthesizing cDNA of virus RNA. Second step is Denaturing, third one is Annealing and the Fourth step is Extension. Taq man polymerase consists of Reporter of fluorescence (R) at 5' end and quencher of fluorescence (Q) at 3' end. At Extension step, DNA Polymerase synthesizes new strand.



**Fig. 9: Real Time PCR machine**

When the polymers reach to Taq man polymerase, the R and Q dyes are separated out. With each cycle of PCR many dye molecules are released that results in increased fluorescence intensity. This method allows the estimation of the

amount of the given sequence present in the sample. The number of double stranded DNA is doubled within each cycle. For the measurement of fluorescence signals a tysten halogen lamp, a excitation filter and CDC camera are used. Light from lamp is reflected from excitation filter of mirror passes through condensing lens and focused into centre of each well of PCR Plate. Than light from well reflected to mirrors than fluorescence light emitted from well reflected of mirror and passed through emission filter and detected by CDC camera, which converts the light and capturing digital value. This method is known as Real Time RT PCR, which allows the monitoring of progress of PCR reaction as it occurs in real time. [26-32]

### **Treatment**

At present time, there is no any specific treatment or vaccine approved for COVID-19. Treatment and vaccine are currently under study. Instead, treatment focuses on managing symptoms as the virus runs its course. Other Corona virus like SARS and MERS are also treated by managing symptoms. In some cases, experimental treatment are tested to see how effective they are.

Therapies used for this illness include:-

- a. Antiviral or Retroviral and Antimalarial medication.
- b. Blood Plasma transfusion. Breathing support, such as mechanical ventilation.
- c. Steroids to reduce long swelling.
- d. Breathing support, such as mechanical ventilation.

Antibiotics weren't help because they treat bacteria not virus. If you hear about people affected with COVID-19 getting antibiotics, it's for infection that came along this disease. People with severe symptoms used to cured in hospital. [33,34] Many clinical trials are under way to explore treatments used for other condition that could fight COVID-19 and to develop new one. Several studies are focused on an antiviral medication Remdesivir, which has created to fight against Ebola. Antiviral drugs such as, ribavirin, lopinavir/ritonavir has been used based on the experience with SARS and MERS.

### **Mechanism of action of remdesivir:**

Remdesivir is an antiviral drug that was developed by gilead science for a possible

treatment for Ebola Hemorrhagic Virus (EBV). It is a nucleotide analog, specifically an adenosine analog, evidenced to have broad-spectrum activity against the single stranded RNA viruses. Remdesivir enters in infected cells, it takes place of real adenine and interferes in new synthesis of virus, which blocks viral RNA polymerase from linking the newly RNA chain. So, replication of viral RNA is blocked.

An emergency FDA says let's doctors use Hydroxychloroquine and Chloroquine for people affected or hospitalized with COVID-19 and in clinical trials to study them further. This medication is approved to treat malaria and autoimmune condition like Rheumatoid Arthritis. FDA is also allowing clinical trials and hospitals use of blood plasma who have been infected with COVID-19 and recovered to help other to build immunity. The U.S. FDA issued an emergency use authorization for blood purification system that reduces the amount of cytokines and inflammatory mediators. [35-37]

### **MOA of Chloroquine and Hydroxychloroquine in viral infection like COVID-19:**

Experimental studies suggested that chloroquine is an Antimalarial medication that has capability of inhibiting replication of several intracellular microorganism including Corona virus. It has been demonstrated that chloroquine has potent Anti SARS-CoV-1 effect invitro, So SARS-CoV-1 can not bind with its specific ACE-2 receptor. Since SARS-CoV-2 also uses same receptor of host cell, So it is believed that chloroquine also interferes and prevent the attachment of SARS-CoV-2. In addition, chloroquine also inhibits Quinone Reductase-2 which involves in biosynthesis of sialic acid and this sialic acid is an acidic monosaccharide of cell transmembrane which is responsible for ligand recognition. Virus use it as receptor to enter into cell by inhibiting synthesis of sialic acid. It acts as a broad spectrum Antiviral Agent. More over chloroquine also changes pH of lysosome directly and inhibits cathepsin that leads to formation of spike protein. Spike proteins are important protein molecule present on viral cell surface that helps the virus to enter in Human cell. After entering in cell, spikes are detached and envelope is cleaved off. Furthermore chloroquine also inhibits MAPKinase. MOA of both antimalarial drugs are

exactly same. Both acts as weak base that changes pH of endosomes/lysosomes which is responsible for membrane fusion.

There is an increased amount of cytokines in general circulation which is associated with the diseases severity of SARS-CoV-2. Hydroxychloroquine significantly decreases production of pro inflammatory and cytokines. So, Hydroxychloroquine is effectiveness in controlling diseases severity.

**Contraindication:**

- a. Hypersensitivity
- b. Retinopathy
- c. Porphyria
- d. Epilepsy
- e. Recent myocardial infraction
- f. Maculopathy [38-40]

**Safety concern by FDA:-** FDA caution against use of Hydroxychloroquine for COVID-19 outside of hospital setting or a clinical trial due to risk of Heart Rhythm problem. The FDA is aware of reports of serious Heart Rhythm problems in patient with COVID-19 treated with Hydroxychloroquine, often in combination with Azithromycin and other QT prolong medicines. [41]

**Plasma Therapy:-**

Plasma is the clear, yellowish liquid component of blood. It makes up about 55% of body's total blood volume.

Convalescent plasma therapy:- When people get sick with a virus or bacteria, they recover from the illness because the body produces antibodies specifically to fight against that antigen. Vaccine also spur the body into producing these protective antibodies. But, there is no COVID-19 vaccine yet. So, plasma from recovered patient remaining doctor's last source of antibodies. Plasma from patients who have been recovered from COVID-19 with contain antibodies that their immune system have produced in order to fight against virus naturally. That plasma can be transferred to patients whose own immune system are struggling to develop their own antibodies. The plasma transfusion is so intended to provide the poorly patient with antibodies from a recovered patient to help their body to fight the COVID-19 virus.

Process of plasma therapy:- To get the plasma, people who have fully recovered from infection

must first donate blood. Donors must be free of symptoms for at least 14 days. Then the plasma is separated from blood, that process is known as Plasmapheresis and screened for toxins or infection, like hepatitis or HIV. Next, the plasma is tested to make sure it has enough antibodies to effectively treat or prevent COVID-19. After that it can be infused into the recipient through a procedure that is similar to a blood transfusion and is known to be safe.

Various trials and studies:- In Shenzhen, China doctors used convalescent plasma therapy in some patient with COVID-19. The study found that the therapy was safe and cleared the virus in seven out of ten patient. Plasma from recovered patients used in outbreak of Ebola, as well as Corona virus, like SARS and MERS. The ICMR has allowed states to start the clinical trials of plasma therapy. States like Kerala, Gujarat and Punjab have already initiated the plasma therapy for patient suffering from COVID-19. Reportedly, a 49 year old male is the first person to receive plasma therapy in MAX Hospital Saket. The treatment worked for him and now he is out of danger, reported the hospital. [42-45]

**Prevention**

For preventing transmission of Coronavirus disease 2019 maintaining the good personal hygiene, washing hands often with soap and water for at least 20 seconds, avoiding touching the eyes, nose or mouth with unwashed hands and coughing or sneezing into a tissue and put that time into a waste container. People who may already have the infection are advised to wear a surgical mask in public. Physical distancing are also best way to prevent transmission. Maintain social distancing (at least 1m distance). If you have traveled from an affected area, there may be restriction on your movements for up to 2 weeks. If symptoms of Corona virus develop, seek medical advice and call the office of your health care provider.

Isolation:- Separate sick people with a contagious disease from people who are not sick. You must away from others at least 7 days after your symptoms started and until your symptoms have gone away.

Quarantine:- Separate people and restricts their movements if they were exposed to a contagious disease. For people who are not sick, but they



have been exposed (in contact with someone who is sick). Quarantine period is 7-14 days. [46-47]

### Conclusion

The COVID-19 virus spreads primarily through droplets of saliva or discharge from the nose. When an infected person coughs or sneezes and only mild symptoms. Some reports have included that people with no symptoms can transmit the virus. This virus can infect children, adulterants and any other age group of people. Several dogs and cats in contact with infected humans have tested positive for COVID-19. Corona virus on surface can easily be cleared with common household disinfectants that will kill the virus. COVID-19 virus can survive for up to 72 hrs on plastic and stainless steel, less than 4 hrs on copper and less than 24 hrs on cardboard. As, always clean your hand with an alcohol based hand rub or wash them with soap and water. Avoid touching your eyes, mouth and nose. As per WHO and ICMR guidelines avoid to come in contact with someone who has been affected with COVID-9 and also avoid to visit public places. Maintaining social distancing is the best weapon to fight against Corona virus diseases 2019.

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