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Evolution of Experimental Techniques & Control Measures in COVID-19 Reservoir Studies: A Systematic Review

Sumit Moharana[1], Pallavi Khuntia[1], Sarthak Suvojit Das[1], Rituparna Das[1], Priyanka Samantaray[2], Subhasmita Mohanty[3]
Vellore Institute of Technology[1],Vellore, Tamil Nadu, India; MITS School of Biotechnology [2],Bhubaneswar, Odisha, India; Ravenshaw University[3], Cuttack, Odisha, India

Abstract:- COVID-19- A pandemic disease which has threatened mankind and has aborted normal human activity is spreading at a faster rate. Just like it's ancestors SARS-CoV and MERS-CoV, this SARS-CoV-19 also has proved to be fatal for everyone. Social distancing, hand sanitizing, and following the norms issued by WHO (World Health Organization) has somewhat helped in controlling the pandemic situation worldwide. Even though scientific technologies and medical facilities are much more advanced in countries like Italy and America, they have failed to save numerous lives. Vaccines are yet to be developed for their successful implementation in patients suffering from COVID-19. During such a high time, health workers, doctors, nurses and peoples engaged in sanitizing work are playing a vital role by putting their life in danger. Stress has been given to control the pandemic because the ultimate cure is yet to be found. The disease is transmitted by cough droplets, inhalation of the infected air containing virus and by social gathering. The symptoms are seen after 10-12 days in infected individuals but people having underlying disease conditions like Diabetes, heart problems etc. are more prone to be infected by this virus. This paper will give a bird's eye view about the new virus, it's transmission, severity and vaccines undergoing trial. Since new facts are still evolving everyday about the virus, readers are urged to keep themselves updated at regular intervals.

Keywords:- *COVID-19, Protein Spikes, Epidemic, Replication, Homologous Recombination, Antibody, Convalescent Plasma, Mutate, Vaccine.*

I. INTRODUCTION

Viruses are the most simple structure and very much complex in nature .They replicate in the host cell by transcribing and translating, very easily. In December 2019, a cluster pneumonia case caused in Wuhan city of China. It was found to be an unknown virus but now it was named to be Novel Coronavirus or COVID-19.

These viruses are the large group of viruses which consists of a core genetic material that is surrounded by an envelope with a "protein spike" which gives a crown shape to the virus and in Latin the meaning of crown is *"corona"* . There are various types of coronaviruses which causes various symptoms like gastrointestinal disease, respiratory diseases which can be ranged from common cold to pneumonia and it tends to be mild in most of the people. In 2002-03 the Severe Acute Respiratory Syndrome (SARS CoV-2) was identified which was mainly transmitted from Civet cats. The molecular mechanisms of replication as well as the pathogenesis of several coronaviruses have been actively studied since the 1970s. Some of the animal viruses, such as porcine transmissible gastroenteritis virus (TGEV), bovine coronavirus (BCoV), and avian infectious bronchitis viruses (IBV), (MHV) is studied as a model for human disease. This family of viruses remained relatively obscure, probably because there were no severe human diseases that could definitely be attributed to coronaviruses. Then in 2012 the Middle-East Respiratory Syndrome (MERS CoV) was identified in Saudi Arabia which was mainly transmitted from the Desert animals like camel to the people in that region. Strains of MERS-CoV that are identical to human strains have been isolated from dromedaries in several countries, including Egypt, Oman, Qatar, and Saudi Arabia. In general, respiratory viruses are usually transmitted through droplets from coughing or sneezing or something that contaminated through virus. Various biological and analytical methods are done for detection, prevention, and control from novel coronaviruses. On 9th January 2020, Chinese researchers described about the full genetic sequence of the novel coronavirus, Since the novel coronavirus was recognized, the disease it caused was termed coronavirus disease 2019 (COVID-19), and several reports on the clinical presentation, epidemiology, and treatment strategies have been published. On 30 January 2020, the World Health Organization (WHO) declared the COVID-19 outbreak to be a global public health emergency, sixth after H1N1 (2009), polio (2014), Ebola in West Africa (2014), Zika (2016) and Ebola in the Democratic Republic of Congo (2019), and on 11 March 2020, the WHO characterized COVID-19 as a pandemic.

- Origin of the Virus

Based on their genomic sequencing analysis, the most likely origins for SARS-CoV-2 followed one of two possible scenarios.[1] In one scenario, the virus evolved to its current pathogenic state through natural selection in a non-human host and then jumped to humans. This is how previous coronavirus outbreaks have emerged, with humans contracting the virus after direct exposure to civets (SARS)

and camels (MERS). The researchers proposed bats as the most likely reservoir for SARS-CoV-2 as it is very similar to a bat coronavirus. A coronavirus from a pangolin could possibly have been transmitted to a human, either directly or through an intermediary host such as civets or ferrets.Then the other distinct spike protein characteristic of SARS-CoV-2, the cleavage site, could have evolved within a human host, possibly via limited undetected circulation in the human population prior to the beginning of the epidemic.

➢ Taxonomy

The term coronavirus was coined in the year 1968, which is derived from the "corona"-like or crown-like morphology observed for these viruses in the electron microscope.[2] In 1975,the *Coronaviridae* was discovered by the International Committee on the Taxonomy of Viruses.In June 2005 it was proposed that the *Coronaviridae* was divided into 2 subgroups *coronaviruses* and the *toroviruses* , the latter of which cause enteric diseases in cattle and possibly in humans.

Coronaviruses are divided into 3 genera (I to III), usually it referred to as groups and it is based upon the serological cross reactivity.

Group	Virus	Host	Disease(s) caused	Cellular receptor
I	229E	Human	Respiratory infection	Human APN
	TGEV	Pig	Respiratory and enteric infection	Porcine APN
	PRCoV	Pig	Respiratory infection	Porcine APN
	Canine coronavirus		Enteric infection	Canine APN
	FeCoV		Enteric infection	Feline APN
	FIPV	Cat	Respiratory, enteric, and neurologic infection, and hepatitis	Feline APN
	NL-63	Human	Respiratory infection, croup	ACE2
II	OC43	Human	Respiratory infection and possibly enteric infection	Neu5,9Ac2-containing moiety
	MHV	Mouse	Enteric and neurologic infection and hepatitis	Murine CEACAM1
	Sialodacryoadenitis coronavirus	Rat	Neurologic infection	ND[a]
	Hemagglutinating encephalomyocarditis virus	Pig	Respiratory, enteric, and neurologic infection	Neu5,9Ac2-containing moiety
	BCoV	Cow	Enteric infection	Neu5,9Ac2-containing moiety
	HKU1	Human	Respiratory infection	
	SARS-CoV	Human	Severe acute respiratory syndrome	ACE2
III	IBV	Chicken	Respiratory infection, hepatitis, other	ND
	Turkey coronavirus	Turkey	Respiratory and enteric infection	ND

Table 1:- Coronaviruses, hosts, diseases and receptors

Group-1 CoV includes animal pathogens like TGEV from pigs, PEDV and FIPV as well as human coronaviruses (HCoV) and HKU1, which causes respiratory infections.Group-2 includes pathogens of veterinary specimens like BCoV, porcine hemagglutinating encephalomyelitis virus and equine CoV, HCoV also included like OC43 and NL63 which are most similar to HCoV-229E and leads to cause respiratory diseases as well as chronic demyelination. Rat sialodacryoadenitis CoV also belongs to the group 2 coronaviruses. Group-3 includes only avian coronaviruses such as IBV, Turkey CoV and Pheasant Cov. In 2008 the bats in the Philippines were tested by the method of reverse – transcription PCR to detect the presence of coronaviruses in them and 55.8% of RNA was found in 2 groups of sequences Group-1(genus *Alphacoronavirus*) and Group-2 (genus *Betacoronavirus*). To propagate Group-2 CoVs are obtained from the fruit bats (*Cyanopterus brachyotis*) then the intestine samples provided orally to Leschenault Rousette bats (*Rousettus leschenaulti).* After the replication in bats were confirmed , an additional passage was made in Leschenault Rouserre bats, and the pathogenesis was investigated in it and it shows that the fruit bats that infected with virus does not shows any clinical signs of infections.

➢ Pathogenesis

Coronaviruses are single-stranded, zoonotic RNA viruses that cause various symptoms like common cold and various respiratory, enteric, hepatic, and neurological symptoms. The severe symptoms of COVID-19 are associated with an increasing numbers and rate of fatalities specially in the epidemic region of China. On January 22, 2020, the China National Health Commission reported the details of the first 17 deaths and on January 25, 2020 the death cases increased to 56 deaths.**[3]**The studies also proved that COVID-19 S-protein is strongly interacted with human ACE2 molecules(Fig:1) in spite of dissimilarity of its sequence with that of SARS-CoV. Significantly high blood levels of cytokines and chemokines were noted in patients with COVID-19 infection that included IL1-β, IL1RA, IL7, IL8, IL9, IL10, basic FGF2, GCSF, GMCSF, IFNγ, IP10, MCP1, MIP1α, MIP1β, PDGFB, TNFα, and VEGFA. Some of the severe cases that were admitted to the intensive care unit showed high levels of pro-inflammatory cytokines including IL2, IL7, IL10, GCSF, IP10, MCP1, MIP1α, and TNFα that are reasoned to promote disease severity**.[4]**

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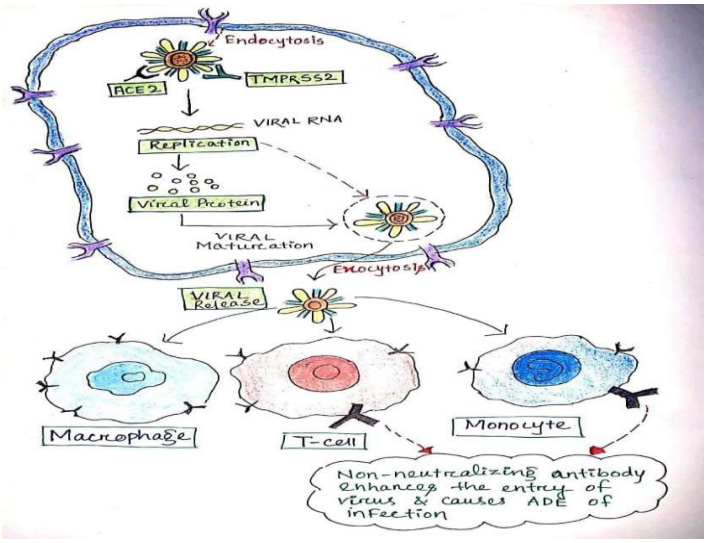

Fig 1:- Pathogenesis of COVID-19

➢ *Mechanism and transmission of COVID-19*

All coronaviruses contain precise genes in orf1 downstream regions that encode proteins for viral replication, nucleocapsid and spikes formation the glycoprotein spikes at the outerfloor of coronaviruses are responsible for the attachment and access of the virus to host cells the receptor-binding area (rbd) is loosely connected among virus, therefore, the virus may additionally infect multiple hosts different coronaviruses in most cases apprehend aminopeptidases or carbohydrates as a key receptor for entry to human cells even as sars-cov and mers-cov apprehend exopeptidases **[5]** . The entry mechanism of a coronavirus depends upon cell proteases which encompass, human airway trypsin-like protease (hat), cathepsins and transmembrane protease serine 2 (tmprss2) that break up the spike protein and establish further penetration changes. MERS-coronavirus employs dipeptidyl peptidase 4 (dpp4), HCoV-NL63 and SARS-coronavirus require angiotensin-converting enzyme 2 (ace2) as a key receptor.SARS-CoV-2 has the ordinary coronavirus structure with spike protein and furthermore communicated different polyproteins, nucleoproteins, and film proteins, for example, RNA polymerase, 3-chymotrypsin-like protease, papain-like protease, helicase, glycoprotein, and frill proteins .The spike protein of SARS-CoV-2 contains a 3-D structure in the RBD locale to keep up the van der Waals powers . The 394 glutamine buildup in the RBD locale of SARS-CoV-2 is perceived by the basic lysine 31 buildup on the human ACE2 receptor . The whole system of pathogenicity of SARS-CoV-2. The life cycle of SARS-CoV-2 in host cells; begins its life cycle when S protein binds to the cellular receptor ACE2. After receptor binding, the conformation change in the S protein facilitates viral envelope fusion with the cell membrane through the endosomal pathway. Then SARS-CoV-2 releases RNA into the host cell. Genome RNA is translated into viral replicase polyproteins pp1a and 1ab, which are then cleaved into small products by viral proteinases. The polymerase produces a series of subgenomic mRNAs by discontinuous transcription and finally translated into relevant viral proteins. Viral proteins and genome RNA are subsequently assembled into virions in the ER and Golgi and then transported via vesicles and released out of the cell. ACE2, angiotensin-converting enzyme 2; ER, endoplasmic reticulum; ERGIC, ER–Golgi intermediate compartment. Genomic varieties in SARS-CoV-2.

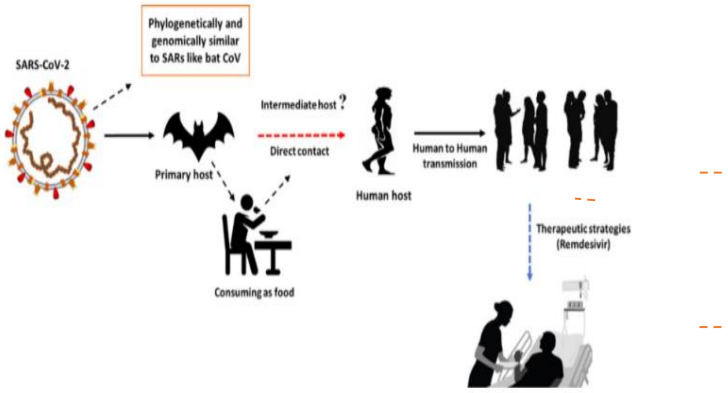

Fig 2:- Transmission of SARS-CoV 2

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➢ *Genetic variations in COVID-19*

The genome of the SARS-CoV-2 has been accounted for over 80% indistinguishable from the past human coronavirus (SARS-like bat CoV). **[5]** The Structural proteins are encoded by the four basic qualities, including spike (S), envelope (E), layer (M) and nucleocapsid (N) qualities. The orf1ab is the biggest quality in SARS-CoV-2 which encodes the pp1ab protein and 15 nsps. The orf1a quality encodes for pp1a protein which additionally contains 10 nsps . As indicated by the developmental tree, SARS-CoV-2 lies near the gathering of SARS-coronaviruses.

Ongoing investigations have demonstrated outstanding varieties in SARS-CoV and SARS-CoV-2, for example, the nonattendance of 8a protein and change in the quantity of amino acids in 8b and 3c protein in SARSCoV-2 . It is additionally announced that Spike glycoprotein of the Wuhan coronavirus is altered by means of homologous recombination. The spike glycoprotein of SARS-CoV-2 is the blend of bat SARS-CoV and a not known Beta-CoV . In a fluorescent report, it was affirmed that the SARS-CoV-2 likewise utilizes the equivalent ACE2 (angiotensin-changing over catalyst 2) cell receptor and system for the section to have cell which is recently utilized by the SARS-CoV (The single N501T transformation in SARS-CoV-2's Spike protein may have essentially upgraded its restricting partiality for ACE2 .Betacoronaviruses genome organization; The Betacoronavirus for human (SARS-CoV2, SARS-CoV and MERS-CoV) genome comprises of the 5'-untranslated region (5'-UTR), open reading frame (orf) 1a/b encoding non-structural proteins (nsp) for replication, structural proteins including spike envelop , membrane (pink box), and nucleocapsid (cyan box) proteins,

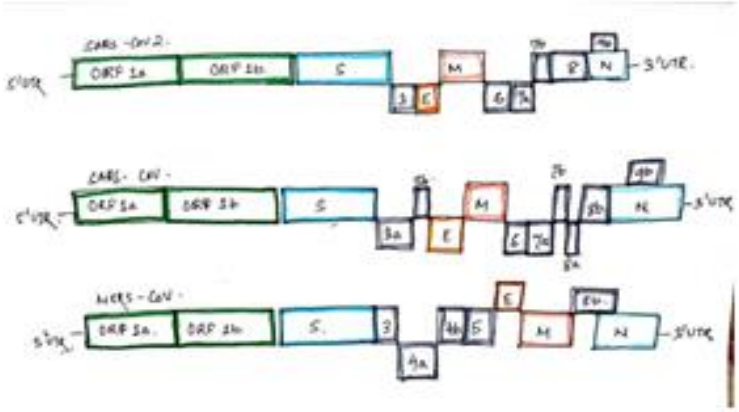

Fig 3:- Betacoronaviruses genome organization

Accessory proteins in the SARS-CoV-2 genome, and the 3'-untranslated region (3'-UTR). The doted underlined in red are the protein which shows key variation between SARS-CoV-2 and SARS-CoV(Fig-3).

II. MATERIALS AND METHODS

➢ *Employing of Analytical techniques in detection of COVID-19*

- ***ELISA test:*** It is an enzyme-linked immunosorbent assay which is commonly used as the analytical biochemical assay.It was first described by Engvall and Perlmann in 1971.It mainly uses a solid-phase enzyme immunoassays which detect the presence of ligand in a liquid sample using antibodies against the protein.

An ELISA test may be used to diagnose HIV, which causes AIDS, Lyme disease, pernicious anaemia, Rocky Mountain spotted fever, rotavirus, squamous cell carcinoma, syphilis, toxoplasmosis, varicella-zoster virus, which causes chickenpox and shingles, Zika virus.

As a heterogenous assay, ELISA separates some component of the analytical reaction mixture by adsorbing certain components onto a solid phase which is physically immobilized. In ELISA, a liquid sample is added to a stationary solid phase with special binding properties and is followed by multiple liquid reagents that are sequentially added, incubated, and washed, by following some optical changes in the final liquid from which the quantity of analytes were measured. Then the reading was taken on the base of spectrophotometric technique which involves the quantitation of transmission of some specific wavelength through the liquid, this process takes 1-5 hrs . It has the abiity to speeds up the multiple sample and it is very sensitive and suitable for the point of determination.

➢ *There are 4 types of ELISA*

Direct,Sandwich,Competitive and Reverse. But in most of the cases Direct(Fig-5) and Sandwich (Fig-4)ELISA is used.

Nowadays COVID-19 is on trend and creates a pandemic situation round a globe by keeping a sight on it researcher was going through various techniques for the testing in which ELISA test is also includes in it. It checks whether or not you have antibodies in your blood to SARS-CoV-2, the scientific name of the new coronavirus that causes COVID-19. According to the researcher, ELISA works like antibody tests for other viruses, such as hepatitis B. It will show whether your immune system -- the body's defense against germs -- made contact with SARS-CoV-2, even months before.This test was very helpful for the

scientists to fight against this pandemic situation It can give researchers a more accurate measure of how many people had the new coronavirus. It would also let health care workers who were ill with COVID-19 symptoms, but were never tested for the disease, return to work -- confident that they are now immune. The test might also help with an experimental treatment for COVID-19 called convalescent plasma. In this therapy, doctors collect blood from people who have recovered from the disease. Researchers hope antibodies in the blood can treat people with COVID-19. The ELISA test could help identify people with antibodies who might be able to donate their blood.

FDA has been decided to work on convalescent plasma. But because of the pandemic, it says some doctors may be given permission to use the method in life-threatening cases.

➢ *Assay Principle*

The COVID-19 IgG ELISA Assay is designed, developed, and produced for the qualitative measurement of the COVID-19 IgG antibody in serum samples (serology). This serological assay utilizes the microplate based enzyme immunoassay technique. **[6]**

The samples were added to the microtiter wells of a microplate that was coated with the COVID-19 peptide antigen nucleocapsid protein. After the first incubation period, the unbound protein matrix is removed with a subsequent washing step. A horseradish peroxidase is labelled with COVID-19 IgG tracer antibody which is added to each well. After an incubation period, then an immunocomplex of COVID-19 polypeptide antigen – a new coronavirus IgG antibody HRP labelled as COVID-19 IgG tracer antibody is formed if there is coronavirus IgG antibody present in the tested materials. Then after the unbound tracer antibody is removed by the subsequent washing step. HRP labeled tracer antibody bound to the well is then incubated with a substrate solution in a timed reaction and then measured in spectrophotometer. The enzymatic activity of the tracer antibody bound to the coronavirus IgG on the wall of the microtiter well is proportional to the amount of the coronavirus IgG antibody level in the tested materials.

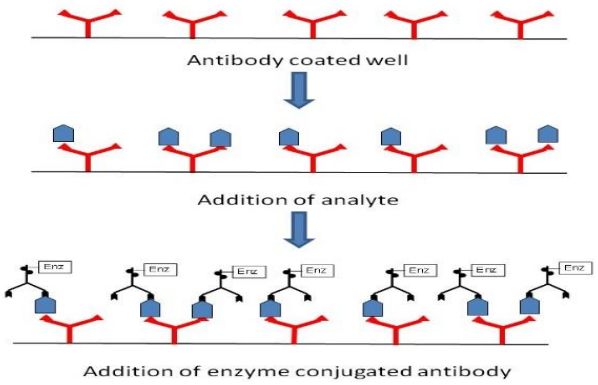

Fig 4:- Sandwich ELISA

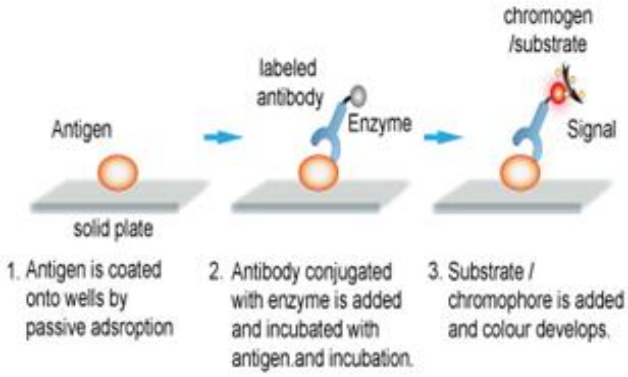

Fig 5:- Direct ELISA

➢ *Real-time Polymerase Chain Reaction :*

A real-time reverse transcription–polymerase chain reaction (RT-PCR) assay was developed to rapidly detect the severe acute respiratory syndrome–associated coronaviruses (SARS-CoV).**[7]** The assay is based upon multiple primer and probe sets located in different regions of the SARS-CoV genome which could differentiate SARS-CoV from other human and animal coronaviruses with a potential detection limit of <10 genomic copies per reaction. This assay was more sensitive than a conventional RT-PCR assay. It is the gold standard or frontline test for the detection of COVID-19. This came into effect after the rapid anti-body tests showed unreliable results. This test is done by taking a nasal/throat swab from a patient as a sample. It involves extracting ribonucleic acid or RNA, which is the genetic material of the virus. This technology

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is fairly expensive method which requires a RNA extracting machines, a laboratory and well trained technicians. A minimum of 30 samples were needed to make it economically viable. It can take up to 4 hours to test for the presence of virus from one batch. The cost of the chemicals and importing elements was required for the test which is high. One test can cost a minimum of ₹4,500.

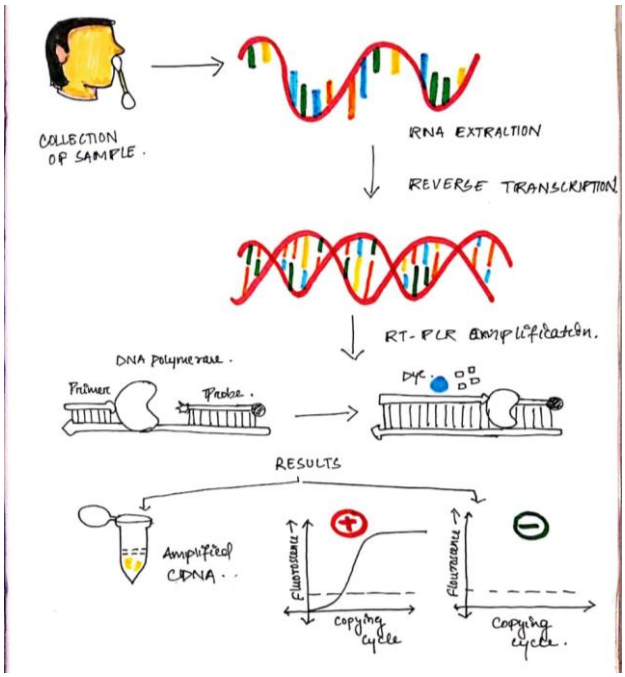

Fig 6:- RT-PCR test a)Samples were collected from the nose and throat linings b)Extraction of RNA c)Transcription of CDNA d)Degradation of DNA polymerase results in increment of fluorescent signal. e) Increase of flouroscent level shows positive decrease shows negative.

➢ *Mixture preparation for RT-PCR:*

A blend is prepared which consists of a forward primer (short oligonucleotides required to begin a DNA combination), backward primer, and a fluorescent probe, along with the enzymes Reverse Transcriptase (responsible for changing over RNA into cDNA) and DNA polymerase (liable for DNA replication). National research facilities and organizations around the globe have created various primers/probes tending to various districts of the viral genome. A few companies give these blends in a lyophilized structure for direct use. *Reagent blending and Real-Time PCR Amplification:* In a single or two-steps RT-PCR, the first RNA is changed over into reciprocal DNA, and afterward the DNA signal is intensified by an ongoing polymerase chain reaction. In this technique, the probe strand ties to a particular sequencing to COVID-19 situated between the forward and reverse primers. During the extension period of the PCR cycle, the polymerase can degrade the bound probe, causing a reporter dye to isolate from a quencher dye, resulting in an increment of fluorescent signal. The fluorescence intensity is observed at every amplification cycle. The fluorescence signal increases as more duplicates of DNA are created. If the fluorescence crosses a specific edge, set above expected background levels then the test is positive. If the virus was not present in the sample, the PCR test would not have made copies, so the fluorescence threshold is not reached the test is then negative (Figure-6).

➢ *Rapid Tests(Antigenic and Serological) using of POC kits:*

These tests are less dependable than RT-PCR tests however can be performed at the point-of-care, or in community settings without the need of expensive equipment. **[7]**The concept of the test is similar to pregnancy tests. Normally, they depend on lateral flow assays which is a simple cellulose-based gadget expected to recognize the presence of a target analyte in a fluid. They utilize antibody-antigen recognition, utilizing monoclonal antibodies to identify viral antigens. Test strips are coated with antibodies that predicament to a viral protein. During testing, the specimen reacts with coronaviruses antigen which is coated particles in the test cassette. The mixture then migrates upward on the membrane chromatographically by capillary action and reacts with the anti-human IgG in IgG test line region, if the specimen contains IgG antibodies to 2019-nCoV. A colored line will appear in IgG test line region as a result of this it was proved it was positive. If the specimen does not contain CoV antibodies, no colored line will appear in either of the test line regions, indicating a negative result. These tests should be possible in 10-30 min and distant from huge laboratories, yet with the end goal for them to give dependable estimations, the concentration of the analyte should be higher than 10 copies/ul. Serological tests utilize a similar principle as other immunoassays, but instead of distinguishing viral antigens, the assay recognizes the presence of antibodies against the virus in the patient sample. These tests can be utilized to identify the present

and past exposure to CoV and should be possible in batches in laboratories or individually at POC settings (Fig-7).

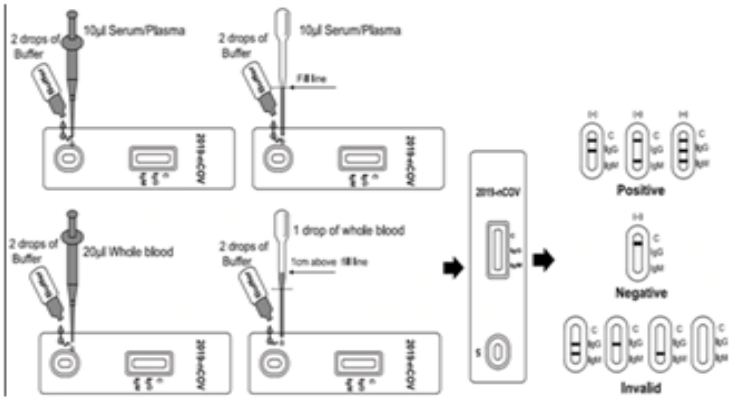

Fig 7:- Typical lateral flow assay for a serological test a) Inside the cassette a strip made of filter paper and nitrocellulose. Normally, a drop of blood is added to the cassette through one opening (sample well), and afterward a number of drops of buffer typically through another gap (buffer well).Buffer carries the sample along the length of the cassette to the outcomes window. b) Interpretation of results. c) A schematic of the COVID-19 lateral flow test from 11. The antibody first binds to an antigen conjugated to colloidal gold in the conjugation pad, and the resultant complex is caught on the strip by a band of bound antibody, forming a visible line (T-test line) in the outcomes window. A control line (C-control line) gives data on the integrity of the immune antibody gold conjugate

➢ *Epidemiology*

The first four cases of an acute respiratory syndrome of unknown disease were identified among people that present in a local seafood market in Wuhan City China on 29 December 2019 **[8,9]**. It has been proved that COVID-19 infection occurs upon exposure to virus and both normal and immunosuppressed population seem susceptible**[10]**. Most of adult patients were between 35 -55 years of age with fewer cases found among children and infants. Most probably in men it has been found but very least in women. World Death rate Reports says that Women at Less Risk of Severe COVID-19 illness than men**[11]** .Epidemiological studies have found that different sexes and age groups have a differing risk of infection and have higher variability and different mortality rates, including higher rates of death in males, especially those over 65 and those with co-morbidities. Risk of mortality appears to be both age- and gender-dependent. To understand these phenomena, extensive survey of relevant scientific literature; it shows that females infected with a variety of viruses--including HIV, varicella, Ebola, and across the spectrum of those viruses. More specifically, females of child-bearing age--between 12 years to 45 years in age—have a significantly lower risk of serious viral infection than those in younger and older age ranges. This also holds true for COVID-19.

Women have higher Angiotensin-converting Enzyme 2 (ACE2)**[12]**, this is significant because COVID-19 has been found to be utilizing ACE2 receptors to enter into the host cells. Using a publicly available (GTEx) dataset, it is observed that the expression of ACE2 is regulated by the estrogen receptor for Estradiol (E2) **[13,14]**.

The second reason is that females are at less risk so they have a better immune response due to higher levels of the immune hormone E2. Although this hormone is present in all mammals, both male and female, it is found at higher levels in females. E2 regulates a variety of immune cells and their signaling pathways. These cells include B cells, CD4+ T cells, CD8+ T cells, NK cells, plasmacytoid DCs**[15,16]**, monocytes, and monocyte-derived DCs. E2 enhances antigen-specific CD4 T cell responses and promotes the growth of IFN gamma-producing cells. E2 imbalance can lead to a wide number of health problems, including cancer, arthritis, hypertension, diabetes, bone marrow disorders, shrinkage of kidney and liver malfunction, hair loss, memory loss, impaired immune response, thyroid problems, Asthma, migraine, mental illness, infertility, and sex disorders**[17]**

E2 is at its highest level in premenopausal women aged 14 to 45 years. Its level in post-menopausal females is similar to that of males. High ACE2 expression and heightened immune response in premenopausal women gives them high protection against COVID-19; mortality amongst premenopausal women is negligible, but after age 45 risks are greatly increased due to reduced E2. In this case IgG and IgM production is low and the immune system is less able to fight against viral replication. Adequate levels of E2 greatly reduce the risk of severe illness and/or mortality.

In the situation of COVID-19 pandemic situation the pregnant women are safe but the new born baby have to be keep safe because this virus catches infants child very fast ones. E2 protects mammals against viruses and other health problems. Pregnant women's E2 levels rise 100% from 15 weeks to 40 weeks gestation.

In this period, ACE2 expression, immune response and antigen production are high. This protects mother and child from severe infection. Nursing newborns are also protected because E2 is present in breast milk. The protective effects of E2 extend from ovulation, through pregnancy, and into the nursing period for both mother and child.

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In our body COVID-19 i.e Corona Virus shows various actions in different types like flu , fever in the body . COVID-19 binds to Human Angiotensin Converting Enzyme 2 (ACE2) via CDC10-Dependent Transcription 1, also known as CDT1, via the virus spike (S) protein, which replication promotes accumulation at the end of the cycle. In licensing replication, Geminin is involved in mitosis by promoting the accumulation of CDT1 because decreasing Geminin levels inhibit CDT1 accumulation and inhibit DNA replication. In addition, Geminin is known to inhibit CDT1 function. Geminin and CDT 1 as well as MCN7 is activated by transcription factors E2F7 and E2 induce E2F7 gene expression.
In the body, Immune System Produce Antibodies Against COVID-19 . In this pandemic outbreak of COVID-19 there are three types of infection Symptomatic, Severe, Asymptomatic.

With COVID-19 immune response is particularly dependent on B cells, T cells and dendritic cells, all of which are regulated by E2. E2 also directly increases IgM and IgG, which are antibodies to the virus. IgG and IgM are generated by B cells, dendritic cells, and T cells in the presence of a signalling pathway for E2. If the signalling pathways are weak or absent, then there is no way to instruct the cells to produce IgG and IgM. In the presence of a judicious level of E2, up to 200% more IgG and IgM will be produced, but without sufficient E2, there will be insufficient IgM and IgG to generate a robust and effective immune response, leading to very poor outcomes and heightened risk of mortality. In infection ACE2 receptors are used by COVID-19 to enter host cells. E2 provides a protective effect by directly modifying the Renin–Angiotensin System (RAAS), where ACE2 converts angiotensin 2 into angiotensin 1.

It acts as a vasodilator and has protective effects on the cardiovascular system and reduces hypertension. E2 is needed in our body because it acts like protective layer against the COVID-19 and other viruses **[18].** In this we can now analyze who will be seriously ill from exposure to COVID-19; the important is the calculate the E2 level in the serum.
1. Symptomatic - if serum E2 level is between 20 and 35pg/ml, there is no need to be hospitalized.
2. Severe - If serum E2 level is less than 20 pg/ml then serious illness is much more likely and hospitalization and possible invasive interventions may be required.
3. Asymptomatic - If serum E2 level is above 35pg/ml, an infected person may not show any symptoms and could infect others as a silent carrier of COVID-19. Females aged from 14 to 45 are generally most likely to be asymptomatic. **[19,20]**

- *Replication of virus*

Virus has largest genomic DNA with 5' methylated cap & 3' poly A tail .This allows the virus to attach with the host cell & use host cell ribosome for the synthesis of viral proteins. The positive ssRNA plays role as a whole genome & also as RNA template or mRNA .The genomic DNA as a template in the host cell's ribosome translate the initial overlapping open reading frame (ORFs)& form long polypeptide (1a & 1b) ,which is then cleaved by its own protease activity into many nonstructural protein(nsps). 2/3rd of the ORFs of the genome encodes nSPs**[21,22]**.

Non-structural proteins are generally not a part of viral particle but it encoded by virus .The nsps have various enzymes & transcription factors like replicase & transcriptase**[23]**. In CoV the nsps are cleaved & form multiprotein complex of Replication Transcription Factor(RTCs) protein which is mainly RNA dependent RNA polymerase (RdRp).The overall mechanism is conducted by the host ribosomes in a double membrane vessicles. NSPs also encode some exoribonuclease which conduct proofreading activity by removing nucleotides from 5' end or 3' end .RdRp direct mediates synthesis of the RNA from RNA strand & exoribonuclease correct the errors or mutation . Normally RNA Viruses have more mutation rate (viruses having upto 10kb genome) but in case of COVID -19 the largest length of the genome produce RTC Which encode exoribonuclease which maintain the genome error free.

The 1st important function of RdRp is mediates the synthesis of -ve ssRNA genome from + ve ssRNA.The the replication of + ve RNA from -be ssRNA. The 2nd important function of RTC is to transcribe viral genome. RdRp directly mediates synthesis of negative sense subgenomic RNA from positive sense RNA genome. The minus strand subgenomic RNA serve as the template for subgenomic mRNAs.

The genomic & subgenomic RNA contain 6 ORFs .Out of these the 1st ORFs (ORF1a/1b) occupy 2/3rd of genome length & encode 16NSPs (Gammacoronavirus lacks NSP1).-1 frameshift between ORF1a &ORF1b encode 2 polypeptide pp1a & pp1b. These polypeptide encodes chymotrypsin like protease (3CLpro)or the main protein & 1or 2 protein like protease .These protease activity cleaves the polypeptide to form NSPs .The function of NSPs are mentioned nsp1 cell mRNA depletion, repressing IFN signaling, nsp2 is unknown, nsp3 PLP, polypeptides separating, blocking host innate immune response, advancing cytokine expression,sp4 DMV development, nsp5 3CLpro, Mpro, polypeptides cleaving, restraining IFN flagging, nsp6 confining autophagosome extension, DMV arrangement, nsp7 cofactor with nsp8 and nsp12,nsp8 cofactor with nsp7 and nsp12, primase,nsp9 dimerization and RNA binding,nsp10 Scaffold protein for nsp14 and nsp16, nsp11 unknown, nsp12 Primer ward RdRp ,nsp13 RNA helicase, 5′ triphosphate, nsp14 Exoribonuclease, N7-MTase and nsp15. Endoribonuclease evades dsRNA sensors, nsp16 2′-O-MTase evades MDA5 recognition, contrarily managing innate immunity. The genome and subgenomes of a typical CoV contain atleast six ORFs. The first ORFs (ORF1a/b), about two-thirds of the entire genome length, encode 16 nsps (nsp1-16), with the exception of Gammacoronavirus that needs nsp1. There is a −1 frameshift somewhere in the range of ORF1a and ORF1b, prompting the production of two polypeptides: pp1a and pp1ab. These polypeptides are processed by virally encoded

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chymotrypsin-like protease (3CLpro) or fundamental protease (Mpro) and a couple papain-like protease into 16 nsps. 10, 11 other ORFs on the one-third of the genome close to the 3′-terminus encodes at any rate four main structural protein spikes (S), membrane(M), envelope (E), and nucleocapsid (N) proteins. Other than these four main structural proteins, different CoVs encodes unique structural and accessory proteins, for example, HE protein, 3a/b protein, and 4a/b protein. All the structural and accessory proteins are translated from the sgRNAs of CoVs. **[24]**

The genome arrangement of CoVs shows 58% character on the nsp-coding district and 43% identity on the structural protein-coding locate among various CoVs, with 54% at the entire genome level, recommending the nsps are increasingly conserved and the structural proteins are progressively assorted needing adjustment to new hosts. Since the transformation rates in the replication of RNA viruses are much higher than that of DNA viruses, the genomes of RNA viruses are normally under 10 kb in length. However, the CoV genome is larger, with generally 30 kb of length, the largest known RNA viruses. The maintenance of such an enormous genome of CoVs might be identified with the exceptional highlights of the CoV RTC, which contains a few RNA handling proteins, for example, the 3′-5′exoribonuclease of nsp14**[25]**. The 3′-5′ exoribonuclease is unique to CoVs among all RNA viruses, probably providing a proofreading function of the RTC. 12 , 13 , 14 Sequence analysis shows that the 2019-nCoV possesses a typical genome structure of CoV.

The genome sequence alignment of COVID-19 shows 58% identity to NSPs & 43% identity to structural proteins.The NSPs conserve in nature & structural proteins are diverse in nature which lead to the virus to cross the species barrier because it adapt new host.The rest ORFs on 1/3rd of the genome encodes many structural protein . Structural proteins are essential for virion assembly.The ORFs encode many structural proteins but among them 4 are very essential for virion assembly.The four main structural proteins are S,E,M,N besides them there are also some accessory proteins like HE protein ,3a/b protein & 4a/b protein.The S protein (homotromeric) is essential for spikes on the viral surface for the attachment on host receptor.M protein has 3 transmembrane domain essential for the membrane curvature & bonds to the nucleocapsid .E protein for envelope help in assembly ,release & viral pathogenesis. N-protein for nucleocapsid has 2 domain which can bind to virus RNA. It was reported that N protein can bind to NSP3 protein for the RTC & package genome into virions.N is also an antagonist of interferon & viral encoded repressor of RNA interferon.

➢ *Release of virus*

The progeny virus have also the replicated positive ssRNA .The mRNA translated by host ribosomes into structural & accessory protein. The viral translation occur at endoplasmic reticulum & S,E,M,N move along the secretory pathway into Golgi intermediates. M protein help in assembly of virus.The virus progeny are then released by exocytosis from the host cell.(Fig:8)

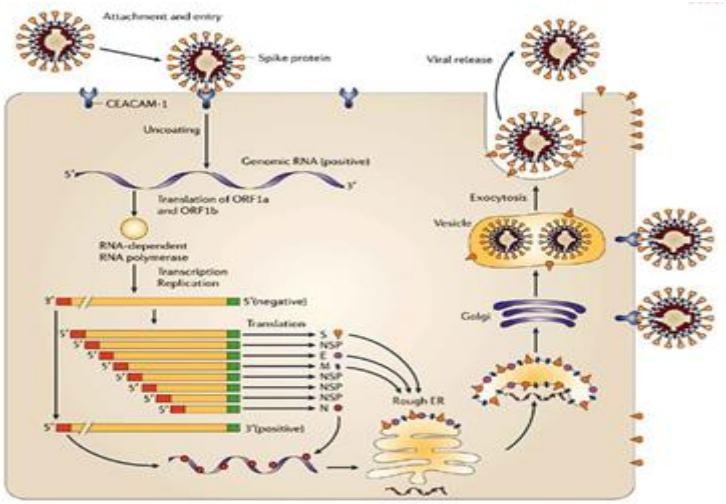

Fig 8:- replication and Release of CoV

➢ *Signalling Pathway of Virus*

There are 80% similarity between the SARS.CoV & SARS-CoV-2. So their mode of infection is almost same .They target ACE2 receptor but in case of MERS-CoV ,it target the furin like receptor.In the 1st step the recognition include the interaction between spike protein with ACE2 receptor in SARS -CoV & SARS-CoV 2 & the airway blockage takes place .The trimeric S protein cleaved into S1 & S2 during the infection & S1 bind with peptidase domain of ACE2 directly because it contains RBD & S2 protein fuse the membranes as it contain Fusion peptide (FP) region with 2 hapted repeat region HR1 & HR2 .In case of MERS-CoV it target furan protein receptor. The serine protease TMPRSS2 is require for proper processing of SARS-CoV2 spike protein.(Fig:9) **[26]**

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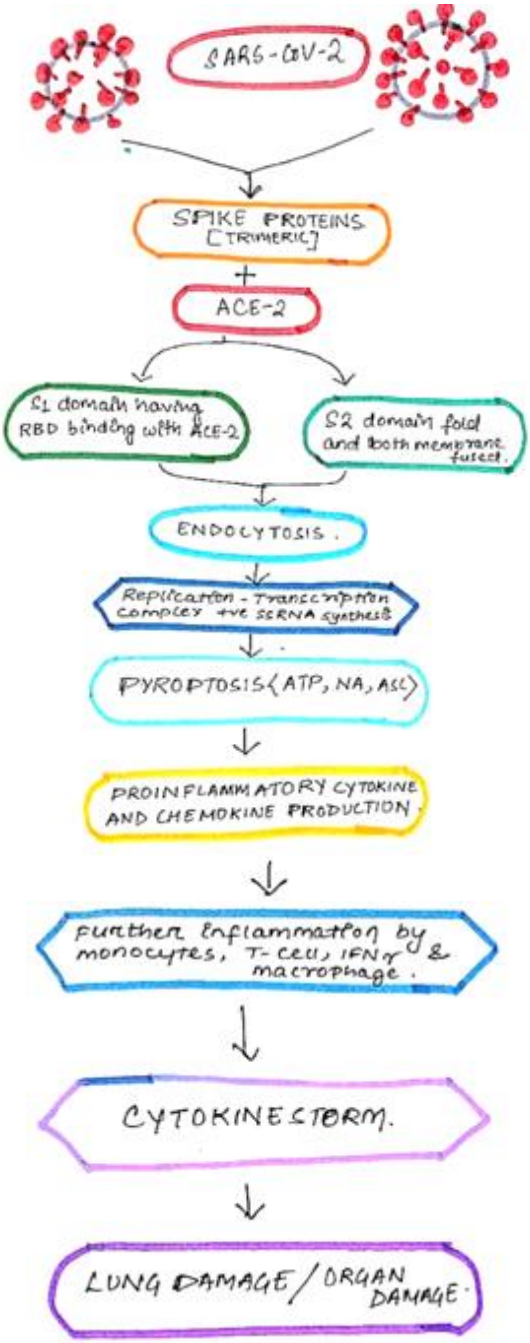

Fig 9:- Figure 6 Flowchart showing the signalling pathway of SARS-CoV-2

Respiratory coronavirus 2 (SARS-CoV-2) has entered the cells that express the surface receptors are angiotensin-converting enzyme 2 (ACE2) and TMPRSS2, active repeats and the release of the virus caused the host cell to undergo pyroptosis and release damage-the corresponding molecular patterns, including ATP, nucleic acids and ASC oligomers. This is recognized by neighboring epithelial cells, endothelial and alveolar cells macrophages, which generate the generation of pro-immune cytokines and chemicals (including IL-6, IP-10, macrophage inflammatory protein 1α (MIP1α),

MIP1β and MCP1.These proteins attract monocytes, macrophages and T cells to the site of infection, to promote continuous inflammation (with the addition of IFNγ produced by T cells) and establishes an inflammatory response loop. In response to innate immunity this can lead to increased accumulation of immune cells in the lungs, causing them excess production of pro-inflammatory cytokines, which ultimately damages the lungs infrastructure. The resulting cytokine storm circulates to other organs, leading to it multiple organ damage In addition, neutral immune cells produced by B cells are possible increase SARS-CoV-2 infection by using antibody-dependent development (ADE),increases organ damage. Alternatively, in response to healthy defenses (right side), early inflammation attracts virus-specific T cells to the infection site, where they can remove infected cells before the virus spreads. Neutral immune system in these individuals they can prevent viral infection, and then

alveolar macrophages are recognized invasive viruses and apoptotic cells that are wiped out by phagocytosis. Overall, these processes lead to viral clearance and small, destructive lung damage recovery. G-CSF, a granulocyte colony-activating factor; TNF, tumor necrosis factor.**[27]**

III. THERAPEUTICS AND RECOVERY

➢ *COVID-19, Vaccines and Drug Repurposing*

Vaccines play a very major role in cure of diseases. The understanding of genomics and structural biology is very important in developing a suitable vaccine. The vaccine for H1N1 influenza was developed rapidly due to the already developed influenza vaccine technology and the use of egg and cell based techniques which made it easy for licensing under rules used for strain change. But, vaccines for Ebola, Zika and SARS didn't follow the regular path followed by the influenza vaccine.

Vast research is still going on for successful development of COVID-19 vaccine. The dependency on DNA and RNA based vaccines has proved to be more reliable than other production techniques due to convenience and high speed. DNA or RNA based vaccine development techniques doesn't require culture or fermentation. **[28]**

Techniques employed	Characteristics			Companies involved in preclinical development		Companies involved in human trials
	Single dose	Licensed or not	Speed	Current scale		
DNA	No	No	Fast	Medium	Takis/ Applied DNA Sciences/Evvivax/Zydus Cadila	Inovio Pharmaceuticals, Phase 1(NCT04336410)
Inactivated	No	Yes	Medium	Medium to high		Sinovac, Phase 1(NCT04352608)
Live attenuated	Yes	Yes	Slow	High	Codagenix, Serum Institute Of India	
Non replicating vector	Yes	No	Medium	High	GeoVax/BravoVax/Janssen pharmaceutical companies/Greffex	University Of Oxford,Phase1/2(NCT04324606)
Protein subunit	No	Yes	Medium to fast	High	WRAIR/U.S. Army Medical Research Institute Of Infectious Diseases/Clover Biopharmaceuticals Inc./University Of Miami/University of Queensland	
Replicating viral vector	Yes	Yes	Medium	High	Zydus Cadila/Themis/Tonix Pharma	
RNA	No	No	Fast	Low to Medium	China CDC/Pfizer/BioNTech	Moderna/ NIAID(NCT04283461)
Uncertain					University Of Pittsburgh/ImmunoPrecise/Tulan university/Doherty Institute	

Table 2:- Methods employed for Vaccine production, Characteristics, Companies Involved and the status of vaccine candidates [29]

COVID-19 Vaccine is facing many challenges due to which the production rate is very slow. Some of the problems faced by the development units are:-

1) To ensure optimal response, an efficient antigen design is necessary for the virus spike like protein which acts as an effective immunogen. Targeting the full length protein or only the receptor binding unit is still a question which is yet to be solved.
2) During preclinical exposure of vaccine during SARS and MERS, it was observed that condition of patients having lung disease worsened. So, to ensure safety monitoring of patients, clinical trial is vastly needed which also involves ethical and moral issues.
3) Potential duration of vaccines is still unknown. Whether a single dose of vaccine or a multidose of vaccine can provide immunity depending on the seriousness of disease is still a big issue.
4) An antiviral drug or vaccine must only kill the virus and not human cells that the virus has occupied.
5) Viruses being highly adaptive, mutate at a faster rate due to which it can easily resist to vaccines developed for each generation. SARS-CoV-2 has about 66 binding sites, each of which can bind to multiple ligand binding sites. The structural similarity of the virus with already approved drugs for respiratory illness and other diseases can prove to be effective in the development of anti

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SARS-CoV-2 drug in the preclinical studies of drug development. [30] The general molecular mechanism and structural similarity of MERS, SARS, and SARS-CoV-2 viruses are very similar due to which the drugs used for these corona viruses can also act on a common target. Some already existing drugs that were used as anti viral agents during malaria, MERS and SARS are researched more to treat COVID-19 patients while some have already entered clinical trials.

➢ *Role of Ivermectin in the Treatment of COVID-19*

Ivermectin $C_{48}H_{74}O_{14}$ (22,23 dihydroavermectin B_{1a}) $C_{47}H_{72}O_{14}$ (22,23-dihydroavermectin B_{1b}) is an antiparasitic agent is used to treat infections in the body that are caused by the certain parasites like lymphatic filarisis,trichuriasis, head lice etc.

It is currently being used in the treatment of SARS-CoV 2 which are the root cause of the pandemic CoViD-19.The clinical trials has also shown that it reduces the no of cell-associated viral DNA by 99.8% in 24hrs.

It was first discovered in 1975 and distributed worldwide during 1980s.It is also enlisted on the WHO list of Essential medicines.

The main concern of Ivermectin is the neurotoxicity,in which most mammalian species may manifested as CNS depression and ataxia as might be expected from potentiation of inhibitory GABA synapses.

Since it inhibits the enzyme CYP3A4 it also inhibits the P-glycoprotein transport and the risk of absorption past the blood brain barrier exists when ivermectin is employed along with other CYP3A4 inhibitors. The drugs includes statins, HIV protease inhibitors, Calcium channel blockers, lidocaine, benzodiazepines, and glucocorticoids i.e. dexamethasone.

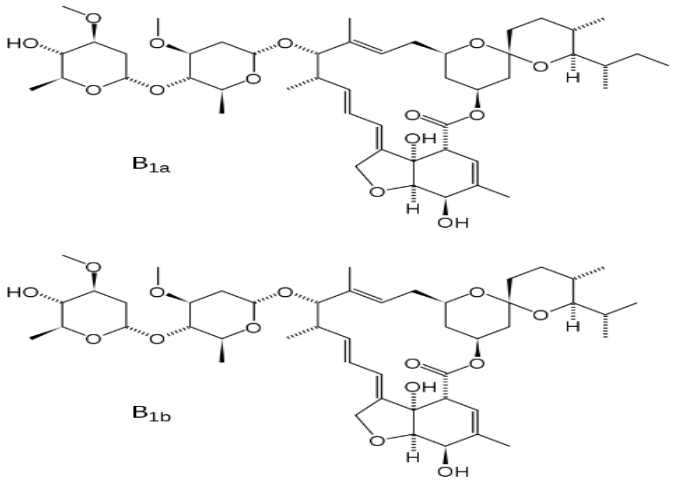

Fig 10:- Skeletal Structure of Ivermectin

According to FDA, Ivermectin is the broad spectrum anti-parasitic agent.**[31]** In recent years the anti-viral activity against a broad range of viruses in vitro. It was originally identified as the interaction between the human immunodeficiency virus-1 (HIV-1) integrase protein (IN) and the importin (IMP) α/β1 heterodimer responsible for the IN nuclear import. Since it has been confirmed that ivermectin. It has been demonstrated to limit the infection caused by RNA viruses such as DENV 1-4(dengue virus), Venezuelan equine encephalitis virus (VEEV) and influenza . It has been similarly shown to be effective against the DNA virus pseudorabies virus (PRV) both in vitro and in vivo, with ivermectin treatment shown to increase survival in PRV-infected mice. Efficacy was not observed for it against Zika virus (ZIKV) in mice, but the various authors has been acknowledged that study limitations were justified for the re-evaluation of ivermectin's anti-ZIKV activity. The causative agent of the current COVID-19 pandemic, SARS-CoV2, is a single stranded positive sense RNA virus that is closely related to severe acute respiratory syndrome coronavirus (SARS-CoV). On the various researches and studies it has been identified that a potential role for IMPα/β1 during infection in signal-dependent nucleocytoplasmic shutting of the SARS CoV 2 nucleocapsid protein which may impact in the host cell division.In addition of the SARS-CoV the accessory protein ORF6 has been shown to antagonize the antiviral activity of STAT1 transcription factor by isolating IMPα/β1 on the rough ER/Golgi membrane.

The antiviral activity of ivermectin towards SARS-CoV-2, the infected Vero/hSLAM cells with SARS-CoV-2 isolates were tested followed by the addition of 5μM ivermectin. Supernatant and cell pellets were harvested at days 0–3 and analysed by RT-PCR for the replication of SARS-CoV-2RNA(Fig.A/B).

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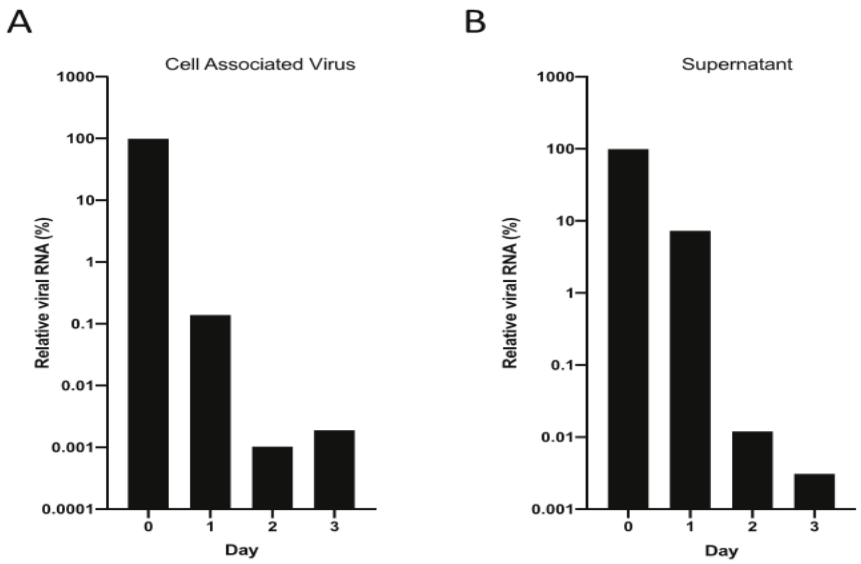
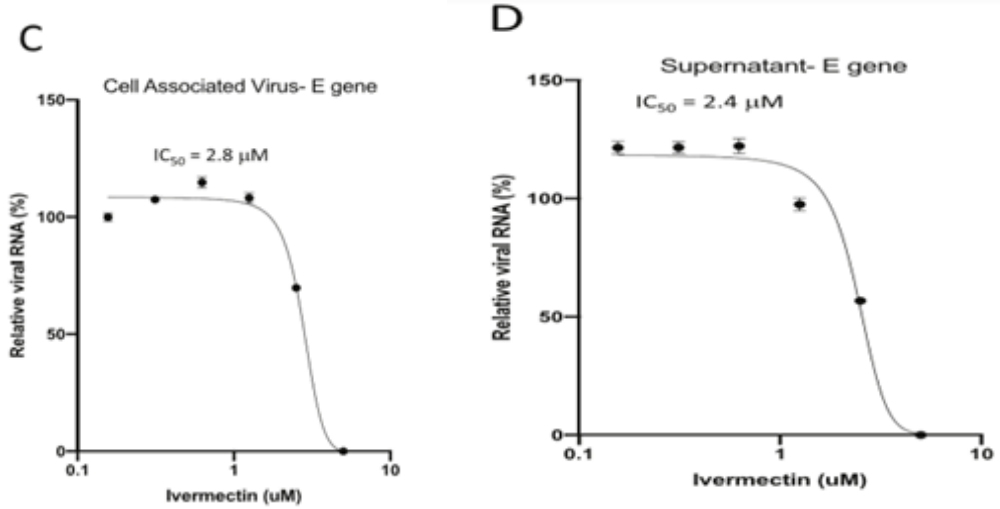
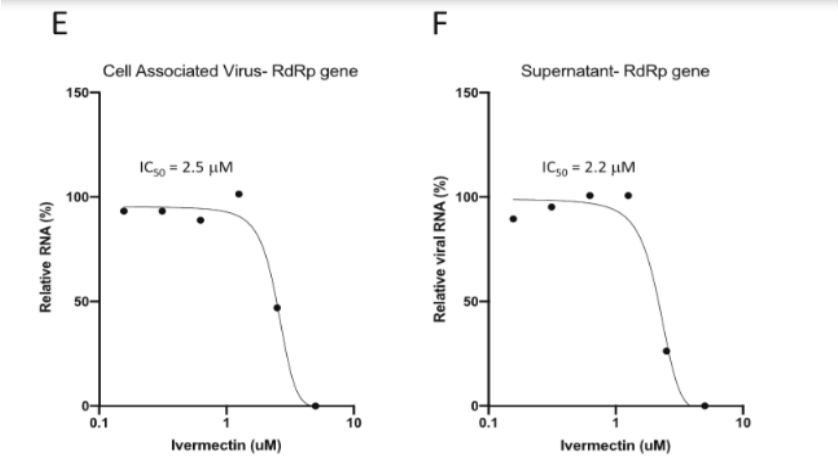


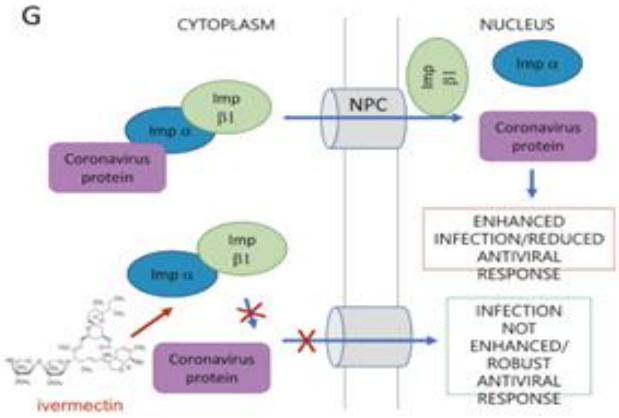
At 24 h, there was a 93% reduction in viral RNA present in the supernatant (indicative of released virions) of samples are treated with ivermectin compared to the vehicle DMSO. Similarly, a 99.8% of reduction in the cell-associated viral RNA (indicative of unreleased and unpackaged virions) was observed with ivermectin treatment. By 48 h this effect increased to an ~5000-fold reduction of viral RNA in ivermectin treated compared to control samples, indicating that ivermectin treatment resulted in the effective loss of all essential viral material. No further reduction in viral RNA was observed at 72 h. To further determine the effectiveness of ivemectin, The cells were infected with SARS-CoV-2 were treated with serial dilutions of ivermectin 2 h post infection and supernatant and cell pellets collected for real-time RT-PCR at 48 h (Fig. C/D).



As above, a>5000 reduction in viral RNA was observed in both supernatant and cell pellets from samples treated with 5 µM ivermectin at 48 h, equating to a 99.98% of reduction in viral RNA in these samples and no toxicity was observed with ivermectin at any of the concentrations tested. The IC50 of ivermectin treatment was determined to be ~2 µM under these conditions. the assay indeed specifically detected SARS-CoV-2, RT-PCR experiments were repeated using primers specific for the viral RdRp gene (Fig.E/F) rather than the E gene (above), with nearly identical results observed for both released (supernatant) and cell-associated virus.



By taking this together,the results demonstrate that ivermectin has anti viral action against the SARS-CoV 2 clinical isolate in vitro, with a single dose able to control viral replication within 24–48 h in our system. We hypothesise that this is likely through inhibiting IMPα/β1-mediated nuclear import of viral proteins (Fig.G), as shown for other RNA viruses confirmation of this mechanism in the case of SARS-CoV-2.



➢ *Mode of action [32]*

Ivermectin kills the larval Onchocerca volvulus worms – microfilariae – that live in the subcutaneous tissue of an infected person. It is believed to paralyse or kill the microfilariae gradually, so avoiding the intense inflammatory responses induced when they die naturally. The treatment with ivermectin relieves intense skin itching and halts the progression towards blindness.

It does not kill the adult worms but suppresses the production of microfilariae by adult female worms for a few months by following the treatment, so it reduces the transmission. As the adult worms can continue to produce microfilariae until it dies naturally, ivermectin has to be taken once a year for 16–18 years to break the transmission. In APOC countries, it is estimated that 65% of the total population living in an endemic area and need to take ivermectin annually to eliminate onchocerciasis as a public health problem.

➢ *Plasma Therapy*

As scientists & researchers are exploring various drugs, vaccines, for treatment to fight against covid-19. **Convalescent Plasma** therapy may be considered as a solution for a treatment.**[33]** After China, US, India has also started to perform clinical trial for convalescent plasma therapy. As it has been already used in the past for treatment of H1N1 (Swine flu) & MERS (middle east respiratory syndrome). So, it has become a ray of hope in this crucial period of time.

In this therapy antibodies are taken from the blood of recovered person & used for the treatment of infected person. Then plasma is extracted from the blood, liquid part that contain Abs. After extraction, Ab-rich plasma is injected into infected patient. This Ab will reach to the tissues, through the blood of the infected person to provide an immune response or protection against infection .

There may be some risk when blood transfusion takes place as infection might occur. This process might fail in some patient because of immune system. For this treatment more experiment & study is still further needed for the treatment.

➢ *Types of Repurposed drugs used for COVID-19*

1) ***Antiviral drugs***

- *Remdesivir-* it was originally developed to treat other viral infections caused from Ebola virus. It works by acting on an enzyme necessary for viral replication and reproduction. Thus, the spread of Coronavirus to other healthy cells can be prevented. The FDA recently approved Remdesivir developed by Gilead's Science Inc. for emergency use in COVID-19 patients. The drug when given by intravenous infusion worked better and improved the condition in COVID-19 patients
- *EIDD-2801-* An experimental antiviral agent in a pill form which can be taken orally, unlike Remdesivir which is a liquid and can be taken only intravenously. It has entered Phase 1 safety trials to ensure there's no such toxic effect on health. **[34]**
- *Avigan-* Influenza drug approved in Japan and has entered phase 2 trials in US. Birth defects are one of the side effects of this drug.

2) ***Anti Malarial drugs***

- *Chloroquine/Hydroxychloroquine-* the use of these two drugs is recommended by FDA only in those hospitals where clinical trials are not available through an Emergency Use Authorization (EUA). These two drugs are also used along with Azithromycin. They have many serious side effects such as ventricular tachycardia (dangerously rapid heart rate). Patients having kidney or lungs disease are at an increased risk of heart failure when these drugs are used.
  These drugs along with Azithromycin have been observed to block ion channels on heart muscle walls preventing flow of ions, which can cause irregular rhythm of the heartbeat and can stop beating altogether.
- *Mefloquine-* Anti malarial drug which interferes with growth of any parasite inside red blood cells. It has not been recommended for use in COVID-19 patients due to its side effects such as anxiety, depression, hallucination, and other nerve related issues. **[35]**

3) ***Interferons***

Human Type 1 Interferons (IFNs) are a large subgroup of signaling proteins which bind to a specific receptor called IFN-α receptor. SARS-CoV-2 responds better to IFN-β subtype of cytokines. IFN-1s are produced first when they encounter a viral infection. They in turn activate the Interferon Stimulated Genes (ISG) which are involved in signaling, inflammation and immune modulation.**[36]** Clinical trials are still going on to found the efficacy of these interferons in combination with other drugs. A report on these interferons said that a combination of IFNβ with lopinavir/ritonavir against MERS-CoV did not reduce the severity of lung infection or viral replication but improved pulmonary function. **[37]**

- *Lopinavir or Ritonavir:***[38]**

It was already used against viruses like "HIV". It shows similar effect like Remedesivir & perform the blocking of viral protein "protease". It has been showing its effect against SARS-cov-2 in labs as well as in infected mice. Lopinavir is tested along with an antiviral β.

- *BCG (Bacillus Calmette-Guérin)*

In the current investigation, when there is a wide reason for going down to fight against this pandemic situation.**[39]** Some investigations looked on the effect of COVID-19. With a high peak of the disease and the country with BCG revaccination policies, which provide a added protection to the population against severe COVID-19.This vaccine is a live attenuated strain derived from an isolate of *Mycobacterium bovis* and has been used widely round a globe as a vaccine for tuberculosis. A live attenuated vaccine means that it uses a pathogen whose potency as a disease producer has been artificially disabled, but whose essential identifying characters, which help the body mount an immune response to it which have been left unchanged.

IV. CONCLUSION & DISCUSSIONS

The new infection COVID-19 which causing a pandemic situation round a globe. It is caused due to the evolution of the strain of coronaviruses like SARS-CoV 2, MERS CoV (2012).All these affects the human respiratory tracts very severly which cause pneumonia and leads to death. According to various studies and research it was came to know that ACE-2 could be the most important binding site for the viral spike proteins,for the fusion of proteins with host cells.So, it has the major role of controlling blood pressure, the viral inactivation of this protein found on the surface of the lungs cells that increases heart rate. It is proved by researchers by making a CoV2 like virus by use of a lentivirus and incorporation of a viral protein into it, which shows same corona like activity, which is validated by taking positive and negative controls.Many experiments has proved the detection of this virus through various analytical devices like RT-PCR, POC Rapid tests etc. Based on various clinical trials and unexpected hope of drugs estimation the results of hydroxychloroquine, which inhibit the ACE 2 temporarily to avoid the binding spike S protein and phenotypically it observed by rapid recovery from fever and increases the rate of recovery, may be it don't have any direct effect on the virus, but it makes the environment harsh for the virus to survive and multiply. Remdsivir also proved the therapeutic expectation. Vaccination is the field where more importance is given as the only solution to eradicate this virus in future. As we know vaccine is a small non-virulence part of virus for which activate immune response (IgG,IgM). As choosing a part from virus as a thing for increasing immunity it leads to the wide spread of it which takes it a very long time to manufacture. Ivermectin is currently being used in the treatment of SARS-CoV 2 which are the root cause of the pandemic CoViD-19.The clinical trials has also shown that it reduces the no of cell-associated viral DNA by 99.8% in 24hrs. Tuberculosis vaccine (BCG), proved to be the major cause of controlled picture of pandemic in South Asian countries like India but not in western countries where targeted BCG vaccine use is very limited and rarely used. Hence, western countries have high mortality rate than South-Asian countries. The world has no doubt becoming a

global village which is riched with various kind of advanced technologies,but it is also seen as a means of that how it could be helping in curing from this pandemic situation.

Life has taken a U-turn where now we are hiding at home to cure ourselves from this invisible particles, which has also given a positive views to Mother Earth the LOCKDOWN has successful in controlling the pollution rate for which this year global warming has been reduced.Beneath, that it is the high time to stop a political blame game as done by different countriesand focus more on its eradication.Rather than panicking fight with it by taking various hygienic steps and practicing social distancing from everyone. Think twice before its got late.

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"On behalf of all the authors, I would say there is no conflict of interest for this review paper"

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