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MECHANISM OF VACCINATION AND DIAGNOSTIC TREATMENT AGAINST CORONAVIRUS (COVID-19): A REVIEW

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ABSTRACT
From the past decade it has been noticed that human health is highly affected by numerous existing life forms, which results in illness and disease. Certain medications are used to cure the disease conditions. In order to provide immunity to health, vaccines are invented and introduced. As said, prevention is better than cure, the vaccines are highly used medication which protects the body by networking an immune system prior to the attack of infectious agents. Vaccine is a type of biological preparation against a particular infectious disease, providing active acquired immunity to an individual. Based on the presence of active component of antigen that generates a specific immune response, the vaccine can be broadly classified as a whole pathogen vaccine, live attenuated vaccine, inactivated vaccine, subunit vaccine, recombinant protein vaccine, conjugate vaccine, outer membrane vesicles vaccine, nucleic acid vaccine, RNA vaccine, DNA vaccine etc. Before coming in use, the vaccine undergoes many stages of research and testing. Under regular circumstances, the process takes an average of 15-20 years, which is a long period of time. In the case of pandemics, numerous strategies are made which possible the process to move from each stages as quickly as possible. Being testable does not mean that vaccine will be successful. The samples which are regarded as safe and easily replicable can proceed to clinical testing; while other working laboratories remain continue to explore more alternatives.

KEYWORDS: Coronavirus, Diagnosis RT-PCR, True Nat, Antigen test, and Victimization.

INTRODUCTION
The novel COVID19 which is caused by the Corona virus is an infectious viral disease, popularly known for the recent pandemic. Basically, Corona viruses are a group of RNA virus that has a wide range of host, mainly the mammals and aves. The name 'Corona' is derived from Latin, which means 'crown', because a typical Corona virus has spike projection structure on their surface which looks similar to a crowni. Due to a large family of Coronavirus (Coronaviridae), the disease causes can range from mild illness to lethal respiratory conditions. According to the discovery and research of Coronavirus, they are grouped into three categories as Alfa (α) [e.g. 229E, NL63], Beta (β) [e.g. OC43; HKU1], Gamma (γ) [e.g. IBV], Delta (δ).[1] Although the origin of Coronavirus is still unknown and exists as a controversial issue among the nations, but the scientist consensus declared it zoonotic because wild animals, especially avian species (bats) and other more animals are an ideal nature reservoir for coronavirus. Covid19 is highly contagious and can be transmitted directly from droplets or aerosols of an infected person released during coughing and sneezing and by coming in contact with contaminated surface and can spread efficiently in the human race.[2] The individuals infected with Covid19 Coronavirus usually shows symptoms within 2 to 14 days after the encounter of virus, which includes the early common mild symptoms like cold, fever, chills, sore throat, congestion, nausea etc, which can sometimes get better on its own, but if not, then it can become more severe symptomatic as trouble breathing, constant pain in chest, sometimes coughing up blood etc, which can result in loss of life, if not reported before the fatality.[3]

HISTORY
- Corona virus is a type of viruses which cause disease in the respiratory system and result in abnormalities and uncomfortableness in functional activates.
- The successful discovery of the corona virus family was achieved in the 1960s. However, the new type of virus that infects the upper respiratory system in chicken was first reported in 1930 in Northern Dakota US, which came to know as IBV (Infectious Bronchitis Virus), officially renamed as Avian Coronavirus.
- Later, in 1947 at the Harvard Medical School of Boston, Marine encephalomyelitis, a disease of mice, which not only primary targets the brain but also other

organs, causing brain damage, necrosis inflammation of liver tissue caused by Picornavirus, this virus then titled as JHM (John Howard Mueller).

- In 1950, during the outbreak of fatal hepatitis among laboratory mice due to P-strain (Parkers) at the National Institute of Medicine Research, London. Alan Watson Gledhill and Christopher Howard Andrews named it as Mouse Hepatitis Virus (MHV).
- On comparison of JHM and MHV with H747 (mouse virus discovered in 1959 in Japan) using a serological test, it was identified that they were antigenically similar, therefore commonly called the 'hepatoencephalitis group of murine virus.
- In 1961, a virus which causes common cold in human beings was first reported in Epsom England. It was designated as B814 and confirmed as a novel virus in 1965.
- By using a transmission electron microscope, the IBV MHV, B184 and 229E (alpha corona virus) are structurally studied and analyzed, which leads to their structural similarities. Hence, it proved that viruses belong to a similar group.
- In 1964, June Almeida and David Tyrell studied the viruses and identified their characteristics such as the presence of spikes like projection (or solar corona) on their surface. Therefore, invented a collective name coronavirus.[4]
- At the beginning of the early 20s, several other forms of corona virus were discovered, and these are considered zonotic species. SARS CoV which is known for severe respiratory syndrome related to corona virus and MERS Cov, i.e., the coronavirus causing Middle East respiratory syndrome also called Camel flu.
- The onset of SARS Cov became noticeable in southern China in 2002 and widely spread to other 28 countries, where as the MERS-Cov came to light in Saudi Arabia in 2012, which was less contagious than SARS and infected the Middle East region in a wide range.
- Other than these, more human corona virus was discovered which was less contagious with less fatality rate. In 2003, the Human Corona Virus NL63 (HCov-N463) was discovered from 7 month old infant suffering from bronchiolitis coryza, Conjunctivitis and fever in Amsterdam, Netherlands.
- In 2004, Human Corona HKU1 (HCov-HKU1) was discovered from a 71-year-old man, citizen of Hong Kong China. Among 2004-2005 the same virus was subsequently reported from the US, Europe and Australia.[5]
- On 6 January 2020, level 2 emergency was launched by China, the casual agent was unidentified, and after 5 days it was considered SARS-CoV2 and their genomic sequence was released by China. The difference of this new agent as compared to the older version is that it is undetectable in normal testing. A typical characteristic of SARS-CoV2 is that it causes pneumonia that can be demonstrated by a computer tomographic (CT) scan or a chest X ray. Therefore, it mainly attacks the lungs of an individual.
- Recently, at the end of 2020, there are 39 species that are described and discovered from many animals. E.g. pig, dog, cat, rodents, cows, horse, camel, beluga whales, bats and other birds. In all these, species of bats are found to be the reservoir of different species of corona virus.[6]

DIAGNOSIS OF CORONAVIRUS

Like other viral diseases, the coronavirus also has minor complications that may get better on their own, whereas the appropriate diagnosis of the disease can prevent from major complications that may get better on their own, whereas the appropriate diagnosis of the disease can prevent from major complications which can be life-threatening. The diagnosis can improve the effectiveness and also aid in the treatment of the disease as well as impede the infection to transmit before it takes a form of outbreak. So, the organs showing symptoms of disease are performed under diagnosis. In coronavirus, due to pathogenic viral invasion the respiratory tract system is affected. For this body response with its defense mechanism, this is maybe cellular mechanism.

The Diagnosis is related to (COVID-19) Coronavirus.

1. Nucleic Acid Amplification Tests
   - Real time RT-PCR
   - TRUE NAT
   - CBNAAT (Gene expert)
2. Antigen Test
3. Antibody Test

For the diagnosis of COVID19 virus, molecular base testing is considered as the confirmatory primary method of the suspected case. For laboratory diagnosis, nucleic acid testing is main technique for identification. Other methods for diagnosis like virus antigen/ serological testing are also valuable assays with a short turnaround time for detection of a novel virus. As with other promising viruses, amplify of methods for detection of antigens and antibodies is started after the identification of the viral genome.[7]

1. Real time RT-PCR

- The RT-PCR stands for reverse transcription polymer chain reaction. RT-PCR is said to be a golden standard test for detection of COVID19 as it is fast to result and also low cost detection of HCoV.
- RT-PCR based on detection of viral RNA of SARS-CoV2 collected from the respiratory tract that is nasopharynx for standard detection.
- The RNA from the sample collected by swab is extracted and isolated. The RNA then undergoes PCR which allows amplifying and detecting a specific DNA molecule in the viral cDNA.
- RT-PCR is three-step processes which are repeated at a minimum 40 times.

(a) The first step involves the denaturation of double-stranded DNA molecules i.e., two strands are separated.
(b) The second step involves annealing process in which the pair of primer or probe with specifically targets the

gene of SARS CoV2 were used. The first open reading frame (ORF 1a and 1b) RNA depends on RNA polymerase (Rdrp), Envelope (E) and Nucleocapsid (N) have given a mark for the diagnostic identification. The specificity of the primer ensures that they only bind to the viral gene and not to any other gene present in the sample.

(c) In the third step which is called elongation, an enzyme polymerase is used which adds nucleotide to the end of the primer by taking the original DNA strand as a template which results in one double-stranded molecule to two double-stranded molecules.

These three processes are repeated, resulting in a doubling in DNA amount in every cycle. Therefore, if the diagnosis started with only one cDNA molecule, then after just 35 cycles these would have 235 or over 34 billion identical molecules. In RT-PCR, a probe is added during the process which gives fluorescence whenever a new DNA molecule is formed. The increase in viral cDNA can be followed in real time by tracking the increase in fluorescence signals when the level of fluorescence exceeds a certain threshold. The CT or cycle threshold is the number of cycles required for the fluorescence signals to exceed that specific threshold value. The lower the CT values, the more RNA is present in the original sample indicating high viral load. For SARS CoV2, a CT value of less than 40 is considered a positive test. If the result is positive then it is important to report the CT value as the indicator infection level for several studies. It shows that viral load as measured by CT value can help predict diseases progress. The initial cycle threshold value of a positive test by RT-PCR plays an essential role as a patient with a cycle threshold value of 10 has 1 million times as viral particles in the throat, as compared to a patient with a cycle threshold of 30. The remarkable bonus point about PCR is that it can test upto 90 samples at once, and the duration is about 4 to 5 hours approximately upto the result of the test. The sensitivity of the PCR test is about 60 to 80 percent with specificity of 90 to 95 percent. Therefore, it is a standard technique for detection of COVID-19.[8]

2. True Nat/CBNAAT (Gene expert)

The CBNAAT stands for Cartridge based Nucleic Acid Amplification Test. This is the test which is also called as Gene Expert test. The Cartridge based Nucleic Acid Amplification Test offers an alternative method to the RT-PCR test for COVID19. Generally, CBNAAT was designed for the purpose of the National Tuberculosis Control Program. For Tuberculosis, Cartridge base Nucleic Acid Amplification detects Mycobacterium Tuberculosis and Rifampicin resistance within 2 hours. Based on the Cartridge System, the Covid Cartridge was launched in 2020 and now adapted across the world. CBNAAT works on RT- PCR methodology. True Nat is the modified Indian version of CBNAAT system, employs a chip-based technology.

In October 2020, ICMR validated True Nat (CBNAAT) which is used for COVID19 diagnosis and Cepheid Xpert Xpress SARS-CoV has been approved by US FDA. Recently, a more advanced and modified version of CBNAAT has been launched by a US health care company Abbott called 'IB now' known as the fastest diagnostic machine and can produce results in 15 minutes. It is based on isothermal nucleic acid amplification. Abbott ID now has higher analytic sensitivity than the conventional RT-PCR and minimum the steps involved in the test.

True Nat is a chip based on a point-of-care RT-PCR platform that decentralized the golden standard test. Its working is a combination of a portable, automatic or battery/mains operated machine and room-stable RT-PCR reagents. It employs minimum temperature with peripheral laboratory to perform Covid19 test and report the result within less than 1 hour from the sample collection. True Nat is developed by a Goa-based company Molecular biology diagnostic and the WHO approved it for testing TB bacteria which showed a 99 percent accurate result. As discussed, the True Nat device is also approved by ICMR for performing a test for Covid19 infections. True Nat can be considered a miniature version of the PCR test. The device is mainly comprises of the following parts: True prep auto device for RNA extraction, True lab analyses (micro PCR) for RNA amplification, fixed value micro liter pipette for sample loading, True lab micro printer for instant printing of test report.

Working

A Collection of Swab

Throat or nasal swab should be collected in the viral transport medium (VTM) for swab specimen. In this, the viruses remain inactive, thus making the sample safe to handle for further processing.

Pre Treatment

The swab in VIM is then pipette out to lyses buffer solution in a buffer bottle.

RNA Extraction

Cartridge based on sample Pre kit is used for the extraction of RNA. The entire content of lyses buffer is transferred to the sample chamber of the cartridge using a pipette. The sample loaded in the chamber of the cartridge then process under true prep RNA extraction device. After 20 minutes the device will give 100 to 150 micro liter of elute containing purified RNA, collected in the elute chamber of the cartridge. The elute sample is then transferred to elute collector tube (ECT).

PCR Analysis

The True Nat is a computed device which works on a chip based on micro PCR test. The ECT sample is moved to the master mix tube to stand for 30 seconds until it becomes a clear solution, then it is transferred to the reaction well of True Nat Chip. The real-time

amplification curve and running state can be viewed during the test on the screen. The PCR reaction will be completed in 30 to 40 minutes. Once the reaction is completed, the report can be viewed directly from the main screen. The report can be printed out by True Nat micro PCR printer. After 25 extractions, the True prep auto device will notify the user to change the reagents pack and reset the bottle count. True Nat is a mini-fastest diagnostic machine that takes 60 minutes to get the result. Therefore, it has an efficient mode of test with sensitivity between 50 to 80 percent and specificity of 90 to 95 percent.[9]

3. Rapid Antigen Test

It is an antigen detecting test. It mainly detects an antibody that is formed against SARS-CoV2 antigen, typically N protein. It is the simplest test and can be performed easily and reliable on many people with Covid having mild or even no symptoms but can still spread the virus. With regular self-testing, the spread can be prevented or the rate of spreading can be slowed helping in protecting the most vulnerable in communities. It is necessary to study the instruction before taking the test. And the antigen test reveals whether the person is currently infected with the pathogen, e.g., SARS-CoV2 virus. Once the infection has gone, the antigen disappears. Basically, antigen tests detects protein or glycan, like spike protein present on the surface of SARS-CoV virus.

Antigen tests are authorized to be performed on Nasopharyngeal or nasal swab specimens. For the testing process only the swab is collected from the nose and not from the or pharyngeal. The sample is prepared by mixing the swab in an extraction buffer tube. The solution of the buffer tube is then placed drop wise on the specimen well marked as 'S' on the test strip and then let it settle for 3 minutes. In the Rapid antigen kit there are two marks on the strip, C which stands for constant and T which stands for test. The possibility of the result by observation can be positive, negative and invalid. After 3 minutes, if only one line is visible next to C then the test will be negative, if only one line is visible just at the bottom next to T then the test will be invalid, and if the lines are visible next to both C and T i.e., two lines are visible, then the test will be positive. Therefore, it is important to make sure that instructions are performed carefully.[10]

4. Ig antibody test

The antibody test is a lateral flow immunoassay employed for qualitative detection and differentiation of IgG and IgM antibody. It identifies individuals with adaptive immune response to SARS-CoV2 indication recent or prior infection. In order to defend against the antigen, is the body response to build an antibody which attacks the virus and eliminates the antigen? If an individual contact with Covid19 but shows no symptoms then serological could be used in 3 to 10 days to determine whether they have been infected. There has been first rise in IgM following that, usually around 7 to 14 days later, depending on the virus, the IgG rises by immune response, which is a later and long-lasting response. In a healthy individual, the IgM levels rise before the IgG level. At the initial stage of infection, IgM is high. Therefore, infected individual with active Covid19, towards the middle and at the end of that, the IgM starts to rise. IgM clears the body quicker than IgG. Therefore IgM indicates the recently infected and sometimes using IgM one might be able to say that someone is still infected. IgG starts with speaking, especially as IgM starts coming down. IgG indicates long-lasting immunity or mounted a long-lasting immune response. So an individual who was asymptomatic and had Covid19 might show that they may have antibody IgG, which means that they were infected with Covid and have mounted an appropriate immune response to combat the virus in the system. When we talk about Immunity to a virus or disease, it is more about IgG, that's the antibody which lasts longer. It shows that an individual has been infected, and then they have recovered.[11]

Drawbacks of techniques

The diagnosis of Covid-19 diseases caused by SARS-CoV, which are highly infectious, involves the collection of a specimen sample from the patient at the correct time from upper and lower respiratory sources including throat, nasal nasopharyngeal (NP), sputum, and bronchial fluid. During the Covid-19 outbreak, it was noticed that nasopharyngeal (NP) swab were more frequent as compared to or- pharyngeal (OP), because the SARS-CoV mRNA was more detected in upper respiratory nasopharyngeal. However, the duration of collection of specimen from the patient to the testing in a laboratory also plays an important role, as the long delay between the duration of collection and testing can lead to a false result.

In some instant, PCR can yield its result in 4 to 5 hrs or in the same day but during the pandemic test results taking up to one to two weeks have been reported. Often, RT-PCR tests are unable to detect SARS-CoV2 infection unless there is 90% viral load. Also, other serological tests like the antibody test, the body takes several days or weeks to develop antibodies after the exposure and these proteins remain in the blood for several weeks after the recovery. Mild cases of Covid 19 may not trigger the development of antibodies due to which it can show a false report. Therefore, the antibody test shows whether an individual has had an infection, making them not effective for diagnosing active coronavirus infection. Whereas, in the antigen test, the results are less sensitive to Covid19, therefore can generate a higher negative false report (they may report negative in an individual who possesses the virus), but the test is specific, so if it results in a positive test then the individual is infected. Due to this, the antigen test is used for screening and required a negative confirmatory test with the gold standard PCR. The time taken for the result by RT-PCR

may take up to 4 to 5 days, while in CBNAAT & True Nat it takes maximum 45 minutes. Therefore, a laboratory can roll out results for about 8 to 10 samples within 8 hours, and in ABBOT ID NOW it takes 13 minutes for negative results and 5 minutes for a positive result. In comparison to running a number of samples, the PCR takes 90 samples at a time and the CBNAAT takes 4 samples at a time, True Not takes up to 16 samples at a time and ABBOTT ID NOW takes only a single sample.[12]

The price of testing varies in each individual test. For PCR, the price varies from state to state between rupees 280 to rupees 2000, capped by the stated government, but the cost of the cartridge is rupees 3000. Therefore, the testing cost is more than rupees 4000 per sample.[13]

Vaccination Mechanism

Vaccination is a procedure that protects all of us from infectious diseases. Vaccination works by providing protection more commonly by promoting the generation of antigen-specific memory cells and also by utilizing pre-existing particles or components of the immune response. Immunization is of two types, active and passive immunization. The most common is active immunization where there is an injection of antigen. These often required additional components, for example, the adjuvant. The immune response is generated in vivo. It takes time to develop, but it provides long-term protection because there is a generation of memory T cells and memory B cells in response to the vaccination. There is a potential to develop both a cell-mediate and a humoral response. In contrast, passive immunization is the injection of pre-formed antibodies. This provides immediate protection, but the protection is short-term. In the circulation, the half-life of antibody is about three weeks. So the protection provided by these injected antibodies does not last for a very long time. It only provides humoral immunity. The pathogen needs to be susceptible to the antibody-mediated destruction. If cytotoxic T-cells are required, then passive immunization will not be helpful, and there is the risk of adventitious pathogen transfer.[14]

Basic mechanism of a vaccine

During a viral infection, the virus attaches itself to a cell using its spikes. Following entry to the cell, it uses the host cell machinery to replicate, producing viral proteins and genetic material. These are then assembled into the new viral particles, which are released as the host cell dies. New viral particles again infect more cells, destroying body tissues and producing symptoms. The Infected cells represent and display the piece of viral protein on their surface in order to alert the immune system. By doing so infected cells are said to present the viral antigen to certain immune cells, namely, cytotoxic T cells, and thus active them. At the same time, debris of dead cells and viral particles are picked up by professional antigen presenting cells i.e., Dendritic cells, B cells, Macrophages, Reticular cells, of which dendritic cells are the most effective.

Dendritic cells patrol body tissues and continuously sample their environment for intruders. After capturing the antigen, dendritic cells quickly leave the tissue for the nearest lymph node, where they present the antigen for another group of immune cells, known as helper T cells. Viral particles are also active B cells in lymp nodes. This group of immune cells work together to mount 2 types of immunity specific to the viral antigen: cell-mediate immunity and antibody-mediated immunity. However, the process may take up to a couple of weeks, during which time the person is sick and the virus will have destroyed a vast number of cells. Without causing disease, the vaccine delivers the viral antigen to trigger immune response. The events of the vaccine induced immune response are similar to that induced by a nature infection, although some types of vaccines may induce only antibody-mediated immunity and may therefore be less effective. For generating immunity, the process sometimes produces symptoms similar to mild infections, even though there is none. As the lymph nodes near the injection site start producing antibodies, they may become swollen and tender for a few days. Many existing vaccines contain whole virus which is weakened and inactive. Due to which these vaccines require extensive safety testing.[15]

Attenuated vaccines may still cause disease in people with compromised immune system. Inactivated vaccines (Sinovac, Covaxin) only induce antibody mediate immunity. Subunit vaccine contains only one part of the virus, usually a spike protein (EpiVacCorona). These vaccines cannot cause disease, but they may not be seen as a threat to the immune system, and therefore may not elicit the desired immune response. For this reason, certain substances are called adjuvant are usually added to stimulate the antigen presenting cells to pick up the vaccine. A subunit vaccine may also consist of empty virus shells, without genetic material. Having typical size and shape of the pathogen, this vaccine may not require adjuvant to be perceived as dangerous, but they can be difficult to produce. Instead of the antigen itself, the nucleic acid vaccine contains genetic information for making the viral antigen. DNA vaccines introduce viral DNA into the nucleus of the cell, where it is transcribed into mRNA. The mRNA is translated into viral spike protein in the cytoplasm. The protein is then displayed on the cell surface, just like with other types of vaccines. Naked DNA vaccines (Inovio) require a special delivery method to reach the nucleus of the cell. Alternatively, a harmless, unrelated virus may be used as a vehicle to drive the DNA. In this case, the vaccine is known as viral vector vaccine (Sputnik V, Johnson & Johnson's). For example, the Oxford-AstraZeneca Covid19 vaccine uses a chimpanzee adenovirus as a vector. The adenoviral genome is modified to remove viral genes, and the coronavirus gene is added. This way, the viral vector cannot replicate or because of disease, but it acts as a

vehicle to deliver the DNA. The reason a non-human adenovirus is used is because most people may have been exposed to human adenovirus and has immunity against it. The immunity could destroy the vehicle before it can deliver the DNA and thus blunt the vaccine's effectiveness. DNA vaccines have raised concerns about the possibility of viral DNA integration into the human genome. However, studies in human models shows that integration frequency is well below the frequency of nature, spontaneously gene mutations. mRNA vaccines (Pfizer, Moderna) introduce mRNA that contains information for making the viral protein. mRNA molecules are delivered within a lipid covering that will fuse with the cell membrane. Once into the cytoplasm, the mRNA is translated into viral antigen, which is then displayed on the cell surface. Unlike DNA vaccines, mRNA vaccines are extremely difficult to integrate into the human genome.[16,17]

CONCLUSION

The Coronavirus continues to spread across the countries, so rapidly such that it has changed its own rhythms to survive. The way coronavirus is surging in India, it overwhelming the health care system, causing illness and death in high rates. Researchers believe that there could be a number of factors that spill the infection, including ease in restrictions that led people to mingle, including at political assembly, rallies and religious gathering; and low vaccine coverage. India is facing the second wave of Covid-19 and the positivity rate is very high. However, the vaccination scheme for public health is implemented by government. Despite it, the coronal wave is getting higher. it has been predicted that the virus will be in its peak in month of April to May. Quarantine and social distancing can prevent its transmission but the situation will be remaining uncontrolled until the complete tender of vaccination is not fulfilled. However, it is observed that in people who had recovered from a previous SARS-CoV-2 infection, their antibody and memory B cell responses peaked after the first dose of the vaccine. Interestingly, the individuals who had experienced more side effects after vaccination such as fever, headache, fatigue, and muscle pain tended to mount stronger antibody responses for protection.

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