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THE RELEVANCE OF RT-PCR TEST TO THE INFECTION WITH SARS-COV-2 VIRUS.

Clinical Science

(Prof.) Dr. Sujan Narayan Agrawal. MS, FAIS, DH& HM. MAHA, professor (Surgery), Department of Surgery. BRKM Government Medical College, Jagdalpur, Bastar. (Chhattisgarh) PIN 494 001. India.

ABSTRACT

It is now a fact that the disease COVID-19 is caused by the virus SARC-CoV-2. This virus is a member of the Coronavridae family and Coronavirinae subfamily. It is an RNA virus. The outer surface of the virus has characteristic projections which are club-shaped or spiked. This gives virion a typical look like the solar corona hence the name coronavirus. These viruses primarily cause respiratory tract infections which may range from mild disease to lethal one. The recent outbreak caused by this virus has posed a great threat to global public health and is now declared a pandemic. It is of vital importance to have a rapid and accurate identification of the pathogenic virus. This will help in selecting appropriate treatment, saving people's lives, and preventing its spread. The RT-PCR is regarded as the gold standard test for the molecular diagnosis of this viral infection. It has got high sensitivity and specificity. This review summarises the characteristics of the virus and the laboratory method of its detection by RT-PCR.

KEYWORDS

Coronavirus, COVID-19, SARC-CoV-2, Pandemic, Real-time RT-PCR, False negative, false positive,

INTRODUCTION:

It is now a fact that the disease COVID-19 is caused by the virus labelled as SARC-CoV-2. This coronavirus is a member of the Coronavridae family and the Coronavirinae subfamily. [1] The name of the virus SARC-CoV-2 is given by the Coronavirus Study Group of the International Committee on Taxonomy of Viruses. The full name of the virus is "severe acute respiratory syndrome coronavirus 2" (SARC-CoV-2). [2-3] The size of the genome in coronavirus is the largest among all RNA viruses. It ranges approximately from 26 to 32 kilobases. [4] The outer surface of the virus has characteristic projections which are club-shaped or spiked. This gives the virion a typical look like the solar corona, and hence the name coronavirus. [5] This name was first coined by June Almeida and David Tyrell, who first observed and studied the human coronavirus. As published in the journal Nature an informal group of virologists designates this new family of the virus as coronavirus. It is a single-stranded RNA virus. It is an enveloped virus with a positive sense single-stranded genome and a nucleocapsid of helical symmetry. [6] These viruses primarily cause respiratory tract infections which may range from mild disease to lethal one. The World Health Organization originally labelled the illness as "Novel Coronavirus-infected Pneumonia (NCIP)' and the virus was named as "2019 novel Coronavirus (2019-nCov)" [7] On 11th February 2020, The WHO renamed the disease caused by this virus as COVID-19, (a shortening of COronaVirus Disease-19). [8] The coronavirus study group of the International Committee on Taxonomy of the Viruses renamed the virus "Severe Acute Respiratory Syndrome Coronavirus-2" on the same day, (11th February 2020). [9-11] It is the same strain of coronavirus which was previously labelled as 2019 novel Coronavirus (2019-nCoV). The current outbreak was officially recognized as a pandemic on 11th March 2020. [12]

It is a single-stranded positive-sense RNA virus of ~ 29.9 KB in size. There are 14 open reading frames (ORFs) that encode for 27 different proteins. [13] It has 5' untranslated region (UTR), replication complex (ORF1a & ORF1b), Spike (S) gene, Envelope (E) gene, Membrane (M) gene, Nucleocapsid (N) gene, 3' UTR, several unidentified non-structural ORFs and a poly (A) tail. [14] [15] The SARC-CoV-2 is a non-segmented enveloped virus with a diameter of 50-200 nm [16] It is a double-layered lipid envelop, including spike glycoprotein (S), Envelop protein (E), Membrane glycoprotein (M), and Nucleocapsid protein (N). [17-18]

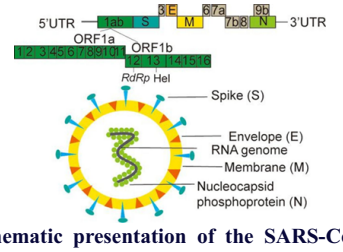

Figure:1. Schematic presentation of the SARS-CoV-2 genome Structure. [19]

[ORF: open reading frame; RdRp: RNA-dependent RNA polymerase; UTR: untranslated region].

As per the World Health Organization as of 26th March 2021, there have been 124,871,140 confirmed cases of COVID-19 globally, including 2,744,543 deaths, reported to WHO. As of 24 March 2021, a total of 456,822,613 vaccine doses have been administered.

In this pandemic, the detection of SARC-CoV-2 RNA is useful in the early diagnosis of COVID-19 disease. The advances in molecular biology techniques have led to rapid and economical methods for the detection of this infection. The polymerase chain reaction (PCR) is characterized by rapid detection, high sensitivity, and specificity.

Background:

The gene expression in the cells of any organism is regulated by the turnover of gene transcripts (single-stranded RNA). The number of the expressed gene can be measured by the number of copies of an RNA transcript of that gene present in the sample. This RNA *per se* has a very small amount of genetic material. To detect and quantify the gene expression, amplification of gene expression is required. The PCR is a common method of amplifying the genetic material. For RNA-based PCR the RNA sample is first reverse-transcribed to complementary DNA (cDNA) with reverse transcription.

The real-time polymerase chain reaction (real-time PCR) is also known as quantitative polymerase chain reaction (qPCR). It is a laboratory technique of molecular biology. It monitors the amplification of the targeted DNA molecule when the process of PCR is still going on (i.e., in real-time) and not at the end of the process as in conventional PCR. This real-time PCR can be used quantitatively (quantitative real-time PCR) and semi-quantitatively, which is above/ below a certain amount of DNA molecule.

The detection of PCR product in real-time is done by using two types of dye., and they are: -

1. Non-specific fluorescent dye that intercalates with any double-stranded DNA.
2. Sequence-specific DNA probes containing oligonucleotides that are labelled with a fluorescent reporter, which permits detection only after hybridization of the probe with its complementary sequence.

The PCR method produces numerous copies of genetic material, after separating the two strands of DNA that contain the gene segment. Its location is marked by a primer. Then a DNA polymerase is used to assemble a copy alongside each segment and it continuously copies the copy. This laboratory technique is widely used to amplify minute quantities of biological material, so as provide an adequate specimen for scientific studies. The PCR-based method has become a routine and reliable technique to detect coronavirus in a given specimen. It is highly sensitive and high sequence-specific. [20-21]

The method of PCR uses at least one pair of specific primers, deoxyribonucleotides, a suitable buffer solution, and a thermostable DNA polymerase. A substance marked with a fluorophore is added to this mixture in a thermal cycle that contains sensors for measuring the fluorescence of the fluorophore after it has been excited at the required wavelength. It allows the generation rate to be measured for one or more specific products. Thus, the rate of generation of the amplified product can be measured at each PCR cycle. The data thus generated is analysed by computer software, which calculates the relative gene expression or mRNA copy number. Quantitative PCR can also be applied to the detection and quantification of DNA in samples to determine the presence and abundance of a particular DNA sequence in these samples. [22]

The real-time RT–PCR detects the presence of specific genetic material in any pathogen, including a virus. Initially, radioactive isotope markers were used to detect the targeted genetic material, but now it is replaced by a fluorescent dye as a marker. This technique allows scientists to see the result almost immediately while the process is still ongoing that's why it is called real-time (RT-PCR). In the conventional RT-PCR, the results are seen only at the end of the process. Now, this has become one of the most widely used laboratory methods to detect the COVID-19 virus. The coronavirus RNA is transferred into cDNA by reverse transcription. After that, the PCR is performed. It is followed by the detection of PCR products through specific detection methods and instruments. This real-time reverse transcriptase-PCR (RT-PCR) is a very specific and simple quantitative assay and it helps detection of infection at an early stage. [23-24] The real-time RT-PCR is the predominant method applied for the detection of all kinds of coronavirus. [25]

SARC-CoV-2 testing aims to diagnose the acute infection in symptomatic patients and screen and diagnose infection in asymptomatic patients. The detection is done by assays which include nucleic acid amplification tests (NAATs), antigen-based tests, and antibody detection tests. The NAATs are aimed to identify the presence of SARC-CoV-2 RNA in the given sample. RT-PCR is the gold standard NAAT used for the diagnosis. The actual process consists of repeated and automated thermocycling's which amplifies the specific segments of the SARV-CoV-2 genome. The amount of the virus in the sample is then quantified in real-time, by using fluorescently labelled dye, which acts as the probe. The positive test is shown by the fluorescence going above a certain threshold. The number of cycles required to achieve this threshold level is called the cycle threshold (Ct) value. This Ct value is a measure and quantification of viral load. A lower Ct value shows higher viral genetic material and vice-versa. This is used as a proxy for viral load and infectivity. In practice, a Ct value of <35 indicates active infection and strongly correlates with high viral load. A Ct value less than >35 shows a low level of positivity and may suggest a past infection, early infection, or a false-positive result.

The accuracy of any test is determined by measuring two things: sensitivity and specificity. A sensitive test will identify people with the disease. Sensitivity measures correct positive results. for example: if a test is 90% sensitive it will correctly identify 90% of people who are infected called a true positive. However, 10% of people who are infected and tested would get a false negative result- they have the virus but the test said they don't. Specificity: the specificity of the test will identify the people without the disease. Thus, specificity measures correct negative. For example, if a test is 90% specific it will correctly identify 90% of people who are not infected and register a true negative test. However, 10% of people who are not infected will test positive for the virus and receive a false-positive result. The RT-PCR is quick, supersensitive, and specific.

Clinical relevance:

Whether a positive test for SARC-CoV-2 PCR means that person is suffering from COVID-19?

The positive result only shows the presence of the virus in the given sample. It does not differentiate between the replicating (viable) and non-replicating (non-Viable) virus. So, it is crucial to correlate the Ct value with the clinical picture. A positive result with a low Ct value strongly suggests acute infection and a high risk of transmission. On the contrary, a high Ct value represents an early, resolving, or past infection. It may be interpreted as a false positive result or a poor sampling technique. To know the trajectory of the disease repeat testing along with Ct value is more helpful.

Another scenario is: if a person is having a negative SARC-CoV-2 PCR test whether it signifies the absence of COVID-19 infection?

This can be due to the absence of viral genetic material in the sample or a too low viral load to be detected i.e., a false-negative result. In a systemic review of 34 studies enrolling over 12000 patients with COVID-19, the false-negative PCR rates were found to range from 1.8% to 58.0%. [26]

The false-negative saga:

It has been found that many suspected cases having the typical clinical picture and computerized tomography findings of the chest are not reported positive with RT-PCR. [27]Thus, a negative result does not always exclude the possibility of the disease. So, the lab result must be combined with the clinical feature for reaching a diagnosis. It is a well-known fact that the result of RT-PCR is affected by the primer used, genetic diversity, and rapid evolution of mutation in the virus. [28-29] The mutation in the primer and probe-target region in the SARC-CoV-2 genome can very well give rise to a false-negative result. The mismatch between primer and probe and the target sequence can lead to a potential false–negative result and decreased assay performance. It must be understood that the sensitivity and specificity of the real-time RT-PCR are not 100% accurate. It also depends upon the sampling procedure. Because the viral load may be different from the different anatomical sites of the patient. It has been found that that the most accurate results are by samples: the sputum, followed by nasal swabs, while the throat swabs were not recommended for the diagnosis. [30]

It is also suggested that bronchoalveolar lavage fluid (BALF) can be used for diagnosis and monitoring. but is not practical to obtain BALF for routine lab diagnosis and monitoring. The false-negative results may also occur due to amplification inhibitors in the sample or low viral load. Inappropriate methods of sample collection, transport, and poor handling all contribute to false-negative results. The sampling time and period of disease development also play an important role in the result of real-time RT-PCR.

Because of these fallacies, all clinical laboratories must follow the standard operative procedures (SOP) and reporting guidelines based on their appropriate public health authorities. The results of RT-PCR must be interpreted with the given clinical picture and CT scan findings. The inaccurate results can be reduced by proper sampling procedure, good laboratory practice standard, and use of high-quality extraction and real-time RT-PCR kits. It is no way minimizes the importance of clinical picture correlation.

The massage:

As of date, there is no specific treatment for COVID-19. There is a high rate of transmission among the human host and the disease is now pandemic. Thus, it becomes very important to identify the basis of its replication, structure, and its pathogenicity. It is all the more important for finding out the specific treatment and to prevent its spread. The RT-PCR goes a long way in the identification of disease in time. However, the test results should not be interpreted in isolation. The clinical picture, timing of taking the sample, sample quality, and performance of the diagnostic platform all contribute to the true result. A liaison with the clinical virologist and infection control team is necessary to generate an appropriate result.

Declarations:
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