International Journal of Medical and Allied Health Sciences

ISSN: 2583-1879
Available at http://www.ijmahs.org/

Review Article

Emerging Spectroscopy Techniques for Detection of Coronavirus (COVID-19): A Review

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Article Info: Received on: 06/06/2021, Revised on: 30/06/2021, Accepted on: 07/07/2021, Available online: 08/07/2021

Abstract

With the coronavirus pandemic (COVID-19) global outbreak in December 2019 and its unprecedented impact on society, health, and economy, it has become imperative to detect it earliest and prevent further escalation. At present, there have already been several detection techniques, including rapid antigen or antibody tests, immunoenzymatically serological tests, and molecular qRT-PCR tests, are widely used. Although these are exceptionally reliable diagnostic methods for the diagnosis of COVID- 19 effectively and accurately, all these techniques have some limitations. Therefore, intensive research is going on for the instant, specific and sensitive detection of COVID-19 using a varied approach. There has been a growing interest in spectroscopic techniques to use as a biomedical tool for the early diagnosis and monitoring of COVID-19. In this review article, apart from these conventional methods, we have summarized recently developed spectroscopy techniques to detect COVID-19.

Keywords: *SARS-CoV-2, COVID-19, RT-PCR, NAATs, Spectroscopy, Antigen*

1. Introduction

The commencement of people getting infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in late 2019 [1], lead to its widespread transmission amongst humans across the globe [2] and eventually was declared a global pandemic by World Health Organization (WHO), in March 2020 [3]. Till date approximately 168 million positive cases with 3.5 million mortalities have been reported [4]. This large scale spread of the virus has made it mandatory to diagnose its presence in the body at the earliest, as there are no particular drugs available for the treatment of the disease. Real time reverse transcription-polymerase chain reaction (rRT-PCR) being the gold-standard [1,5,6] to diagnose coronavirus disease (COVID-19) is used conventionally to detect the presence of viral ribonucleic acid (RNA) [7], but requires a great deal of time, sophisticated handling, capital and transportation [1].

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https://doi.org/10.54618/IJMAHS.2021112

Despite all these laborious tasks, the efficacy of rRT-PCR is not 100%, as it sometime shows erroneous results owing to virus mutation, sample contamination or sample damaging. In addition to this, nucleic acid amplification test (NAATs) is also one of the conventionally used procedure to detect the presence of SARS-CoV-2 virus detect the presence of SARS-CoV-2 virus in the body, with greater efficacy [15]. [9] As the number of infected people is soaring day-by-day, meagre availability of testing kits restricts the number of testings done [10].

To circumvent the limitations of rRT-PCR and NAATs, a number of efforts have been made to develop and find other ways for carrying out the detection of COVID-19 more efficiently, to consume less time, handling, cost and transportation. Some of these ways are also vigilant of the fact that more sample handling will lead to greater peril of spreading, as the virus is airborne, hence minimizing the sample handling and safe incineration of the testing kit after the testing is over [1] In this review article we are going to discuss about, electrochemical sensors [1], biosensors [11], computed tomography (CT) [12], Surface Enhanced Raman Scattering (SERS) [6,13], Paper spray mass spectrometry (PS-MS) [14] and Real-time Accurate

Portable Impedimetric Detection Prototype (RAPID) 1.0 [11] for efficient detection of COVID-19.

2. Detection of Covid-19 Using Different Devices

The growing demand of COVID-19 detection has compelled the researchers across the globe to come up with devices that are cheap and less time taking to detect the presence of SARS-CoV-2 virus in the body, with greater efficacy [15].

2.1 Electrochemical Sensor

Owing to the above mentioned growing demand, Yokah et al. [1] has come up with a paper based electrochemical immunosensor which works on the principle of subsided current response in the redox indicator, due to interruption caused in redox conversion ([Fe(CN)6]3-/4-) by the immunoglobulins (antibodies) of SARS-CoV-2 [16]. After four to seven days of entry of SARS-CoV-2 in the body, the body starts forming immunoglobulins corresponding to it. The antibodies produced in defence of SARS-CoV-2 are IgG, IgM or IgA (IgM formed on day four or beyond and IgG formed on day seven or beyond) [17,18,19]. Lateral flow immunoassay (LFA) using this paper based electrochemical sensors has the advantage of detection of SARS-CoV-2 even when multiple types of antibodies are present at once, requiring no labelling of any of the antibody. Besides, the sensitivity of paper based electrochemical sensor (1ng/mL of detection limit) is greater (in the orders of magnitude of 3) than the sensitivity of colorimetry 1. As this electrochemical sensor is paper-based, it also has many advantages over other devices, as paper is highly abundant, less expensive, easy to carry and easy to incinerate [20].

This electrochemical sensor is a paper-based device comprising of three major parts: a working ePAD, a counter ePAD, and a closing ePAD. Following the procedure (Carrilho et al., 2009, Yakoh et al. 2021), COVID-19 ePAD is prepared [1,21]. Spike protein receptor-binding domain (SP RBD) is immobilized, to bind with immunoglobulins (IgG and IgM both) of SARS-CoV-2, on the hydrophilic portion of the working Epad [20]. For the testing purpose, human serum sample (10 µL) carrying aimed antibodies is placed on the test zone and incubated at room temperature, followed by washing with 0.01M Phosphate -buffer saline (PBS) to expel the unbound antibodies. It was then prepped up in such a way as to prune the possibilities of direct contact with biohazardous fluid and restrict environmental exposure. Further for diagnosing purpose, the electrochemical outcome for the redox indicator Fe (CN)6]3-/4- is monitored using square-wave voltammetry (SWV). The output of SWV waned in response to immunocomplex formation. This was performed on a number of SARS-CoV-2 positive as well as negative patients and the result was tallied with that of the commercial standard enzyme-linked immunosorbent assay (ELISA) method to illustrate the effectiveness of sample testing in real scenario [1]. This proposed mechanism was further extended to detect antigen i.e. the spike protein of SARS-CoV-2, to unfold more possibilities for detection of COVID-19.

The fabricated COVID-19 ePAD was further utilized for the detection of spike protein of SARS-CoV-2. SARS-CoV-2 encodes four proteins, namely spike, matrix, nucleocapsid and envelop proteins. Out of these four proteins, spike being an abundant transmembrane protein of the virus has high immunogenicity [22]. Moreover, spike protein holds diversity from other coronaviruses owing to its amino acid sequencing further deeming it apt antigen for the purpose of selective identification of SARS-CoV-2 [23]. The procedure of detection of spike protein is same as that of antibody testing, besides a bit of change in incubation time. In this also, waning of current due to presence of spike proteins was the indication for presence of SARS-CoV-2 virus in the body [1]. Being easy to handle, this electrochemical sensor presented promising results for the detection of COVID-19. Additionally, its applications can be expanded in future as point-of-care (PoC) diagnosis using different analytes

2.2 CT Images Using Deep Learning

Distinguishing patients on the basis of severity of the disease is also an important task, as the treatment given will differ accordingly. Quiblawey et al. [5] has come up with a cascade study including lungs segmenting, detection, localization, and quantification of COVID-19 using computed tomography (CT) images. The severity classification [24] is done by the system can be categorized as mild, moderate, severe or critically severe [25], depending upon the percentage of infection caused in the lungs. But following the conventional medical procedure could be time taking. Alternatively, this can be achieved administrating Artificial intelligence (AI) [26,27,28,29] based solutions, to supplement the already existing medical procedures. A large number of experiments were done employing state-of-the-art deep Encoder-Decoder Convolutional Neural Networks (ED-CNNs), Feature Pyramid Network (FPN) [30] and UNet with different encoder using the alteration of DanseNet and ResNet.

This procedure is inclusive of Lung segmentation [31,32], Lesion segmentation [33], and severity classification of COVID-19. Here lung segmentation refers to computer-based probe done on lungs to analyze the boundaries of the lungs on computed tomographic (CT) images [34]. Similarly lesion segmentation refers to boundary delineation of lesion using CT images and severity scale was measured MosMedData Dataset [35].

The input computed tomography volumes are considered slice-by-slice. First the lung is segmented using generated binary lung mask for insertion in CT slice by the usage of first ED-CNN. This lung segmentation was then fed to the second ED-CNN to identify the infected regions of the lung [36,37,38,39].

The infection mask generated was then analyzed for the detection of COVID-19 slices from the common slices.

Using generated infection and lung masks, COVID-19 pneumonia lesions are localised. Furthermore, the percentage of infection in the lungs was allocated to designate the severity of the disease at four levels depending on the percentage of infection. At the end, using a visualisation tool, the infection areas of the lungs of the patient are visualised for further treatment.

The results obtained for five-fold cross-validation used for lung segmentation showed that using UNet with DenseNet, 121, 161 and 201 results in best Dice Similarity Coefficient (DSC). Similarly, for lesion segmentation using DenseNet201 FPN gives the best result corresponding to 91.85% and 94.13% of Intersection over Union (IoU) and DSC respectively. These databases were taken and used to calculate the infection percentage using MosMedData Database.

Using this CT images and deep learning [40], COVID-19 can be detected with 99.64% sensitivity. And by providing the output of lung and lesion segmentation, severity level of COVID-19 patients can also be navigated [25]. Hence computer based diagnosis is more reliable owing to its accuracy and ease of handling.

2.3 Paper Spray Mass Spectroscopy (Ps-Ms)

De Silva IW et al. [14] has proposed a paper spray mass spectrometry (PS-MS) by utilizing Teslin substrate for rapidly detecting lipid metabolite changes at the time of COVID-19 infection [14]. Lipids are very much indulged in different stages of virus's lifestyle, where it is acting as receptors or co-receptors and can dictate inside the host cell, the propagation of virus. Hence the metabolomics that are connected with the lipid are helpful in getting an insight of novel coronavirus's immune response [41,42].

As already known from spectroscopic studies, that mass spectrometry is one of the techniques that is used for studying chemical reactions in the laboratory. Keeping this in view, mass spectrometry can also be used to monitor chemicals present inside the body [43]. So, the hormones, drugs, lipids and other molecules present inside the body can be detected using PS-MS[44]. Besides this, PS-MS can also be used in total tissue biopsy of the body for clinical diagnosis [45,46,47].

This PS-MS method is used with a Tensil substrate with the requirement of little sample preparation, and is an easy device to use for the purpose of diagnosing COVID-19. Tensil being monolayered, microporous polyolefin-silica matrix is befitting synthetic paper for reliable use to do analysis of sample undergoing test in PS-MS [48]. A large number of samples (by mixing oropharyngeal (OP), nasopharyngeal (NP) and sputum specimen swabs) were collected and put to analysis in PS-MS resulting in good efficacy. This is not the ultimate device for the detection of COVID-19 but can be used as a complementary testing along with RT-PCR to eliminate the occurrence of false report [49,50].

2.4 Biosensors

Biosensors also come in the line of being useful as they offer the advantage to diagnose rapidly. Torres MDT et al. [11] have devised a low-cost biosensor for rapidly detecting SARS-CoV-2 at point-of-care (PoC). The research group has fabricated Real-time Accurate Portable Impedimetric Detection Prototype 1.0 (RAPID 1.0), which is easy to handle and is highly sensitive. This biosensor is modified using angiotensin-converting enzyme-2 (ACE2) and can diagnose the presence of SARS-CoV-2 within 4 minutes, from 10μL of sample. This method uses nasal/oral swab and saliva sample for the detection SARS-CoV-2 with sensitivity and specificity of 85.3%, 100%, 100% and 86.5% respectively. RAPID 1.0 gives the output by transforming biochemical information of an event involving specific binding between SARS-CoV-2 spike protein and ACE2, into electrical signal, which can be detected easily. This device works on the phenomenon of electrochemical impedance spectroscopy (EIS), which is an electrochemical technique, to carry out the required detection [51,52]. For the diagnosis of SARS-CoV-2, it solely transduces the signal corresponding to interaction between ACE2 and spike protein [53]. The binding between these two molecules leads to alteration in interfacial electron transfer kinetics amid the redox probe. This electrochemical change then get detected by probing the charge-transfer resistance (RCT), the semi-arc diameter on the Nyquist plot that correlates the amount of targets bound to the receptive surface [51]. The signals that are obtained in response of this binding can easily be accessed and read on a computer or a smartphone.

2.5 Surface Enhanced Raman Spectroscopy

Raman spectroscopy which is already employed in detection of RNA virus present in the saliva to detect diseases like human immunodeficiency virus (HIV) [54,55] and dengue fever [56]. It can also be used for the detection of COVID -19 as this spectroscopy can give chemical fingerprints of molecules [57]. COVID-19 being an airborne infection can get transmitted through coughing, ocular routes [58], nasal opening, direct inhalation and oral routes [59]. The virus can be found in human biofluids, like in blood, tears, wound cut and saliva. These biofluids carry biomarkers, carbohydrates, lipids, hormones, nucleic acids, that show the presence of infection [60]. Biomarkers of COVID-19 are positive-sense single strand RNA (+ssRNA) and are given entry inside the host cell by S proteins. These biomarkers can be detected using Raman spectroscopy [61].

Jadhav SA et al. [6] has come up with a technique that majorly employs the phenomenon of Surface Enhanced Raman Spectroscopy (SERS), which is a sensitive method to enhance scattering by utilization of metallic nanostructures' plasmonic properties [62] gives hope when paired with microfluidic devices

possessing integrated microchannels functionalized with carbon nanotubes coated with gold or silver [63]. When the disease commences, biomarkers are found to be low in concentration, their closeness with the metallic nanostructures enables enhancement of the scattering of particles [64]. Virus contained in the biofluids are captured according to their shape and size by the device and then identified using Raman signatures [65,66]. Owing to its high sensitivity and selectivity, the device can detect even a single molecule if traced. This is also useful due to its capability to screen symptomatic as well as asymptomatic individuals with good efficiency.

In addition to this Carlomagno et al. [13] have come up with a Raman-base assay which uses the saliva as a substrate to discriminate the resulting signals from group of healthy, COVID-19 positive and COVID-19 negative people [67,68,69]. Taking saliva has made sample collection easier for further probe. This method basically focuses on the presence of nucleic acid of virus. In this method they have used the technique of SERS to locate the presence of SARS-CoV-2 present in the body of the patient infected by COVID-19 by taking the Raman fingerprints from the saliva [66,70,71]. Presence of biochemical signature in the saliva of the infected people is a way to discriminate them from those who are healthy and those who have already recovered, with good efficacy.

In this experiment, sample collection was done by taking saliva from people and then was grouped into three categories: healthy, COVID-19 infected (CoV +ve) and COVID-19 recovered (CoV -ve). The collected samples were processed and then projected for spectral analysis which majorly dominated in peaks corresponding to CH3 rocking (1155 cm-1), C-N stretching (897 cm-1) and C-H stretching (1453 cm-1) of glycoproteins, secreted by mucines [72,73]. The peaks that were of utmost interest and gave the sight of difference can be attributed to: O-O stretching in proteins that were oxygenated, glycoproteins that were rich in mucines and proline and symmetrical stretching of tryptophan (peak present at 748 cm-1) [73]; glucose and glycogen (peak present at 922 cm-1) [74]; tryptophan and phenylalanine signal (peak present at 1048 cm-1) and stretching of C-N and C-C (peak present at 1126 cm-1) [75,76]. The prominent difference between healthy patient and that of the CoV +ve and CoV -ve patients was observed in intensities of signal corresponding 1048 cm-1, which can be attributed to aromatic amino acids, particularly tryptophan and phenylalanine. These two aromatic amino acids are present in the coronavirus family in the protein structure or else while it is interacting with physiologically expressed molecules [77]. The region rich in tryptophan will also be rich in the presence of virus and can easily be detected when the virus interact with receptor Angiotensin Converting Enzyme type 2 (ACE2) [78,79,80]. Hence the abundance of saccharide and aromatic amino acids in the saliva is the key factor to differentiate between healthy, CoV +ve and CoV -ve patients, using Raman Spectroscopy. Further the data generated are uncluttered using machine learning.

3. Conclusion

As it has already been almost one and a half year of people struggling with COVID- 19, and there is no sign of it getting subdue, it is very crucial to diagnose the disease at the earliest possible in order to stop further transmission as its transmission can lead to further evolvement and change in the genetic code of the virus because of mutation. As we look around the globe, it is already clear by this time that the coronavirus strain is not the same as it was at the onset of its emergence. Owing to it being a RNA virus, by very nature of it, it has the potential of mutating and further evolving to result into a new variant. A number of variants have already generated by now and it is very urgent for us to come up with diagnostic assays that are cheap and easy to use in day-to-day life with good efficiency. The ardent dedication of researchers across the globe has provided us with devices that have been discussed in this review. The electrochemical sensor being paper based is easy to handle and discard, preventing the spread of virus into the environment. The artificial intelligence-based CT scans have been helpful in monitoring the severity of the disease. The device employing mass spectrometry has the advantage of needing little sample preparation and giving high efficiency results. The biosensor RAPID involved has the advantage of being highly scalable and inexpensive, results of which can be easily accessed using a desktop or a smart phone. Surface enhanced Raman spectroscopy has the benefit of needing no prior preparation and the test can be conducted directly using the sample. A device with holistic advantages is the need of the hour.

4. Competing interest

The authors declare that there are no conflicts of interest.

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