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A Review on Diagnostic Development for Controlling COVID-19

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ABSTRACT

SARS-CoV-2 also known as COVID-19 first recognized in Wuhan district of China immediately become a pandemic wreaking havoc across globe, which mostly is quite significant. Initial cases of this disease are assumed to be acquired from zoonotic origin and the virus is found to have a recombinant genetic material made of Bat CoV-2 and unknown CoV-2. ACE-2 receptors are principle receptors for interaction with human nasal epithelium via which they enter and invade the respiratory airways and finally lung tissues causing respiratory symptoms like cough, breathlessness and in severe cases ARDS. It is mainly transmitted by via aerosols released in air while coughing, sneezing and through objects in contact of the diseased like door handles, pen and other formites. Elderly population and middle age groups with comorbidities like DM, CKD for the most part are the most targeted groups. The high infectivity and rapid spread of disease specifically has warranted development of fast, really effective and accurate diagnostic test. Specimen usually collected for various tests include blood, nasopharyngeal, oropharangeal swab, serum. Basically the diagnostic tools available are (a) Molecular methods like RT-PCR, (b) Antibody detection by serological sort of methods, (c) Radiological methods to really detect organ changes, (d) Rapid antigen tests, (e) Various non-specific tests. Novel methods have been also devised like LAMP and SHERLOCK based on CRISPER technology in order to make diagnosis of disease much less tedious and much more accessible to population. Development of these novel methods is still under progress. In this review articles above enumerated diagnostic methods essentially have been summarized highlighting the most of effective tools for early identification and detection of COVID-19.

Key words: Diagnostic, COVID-19, SARS-CoV-2, RT-PCR, Serological, CRISPER, LAMP

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INTRODUCTION

Cases of novel SARS-CoV-2 were first observed in Wuhan district of China which later became a major public health concern spreading globally. WHO declared COVID-19 outbreak as a health emergency crossing international borders on 30 January 2020 and then developing into a pandemic by 30 March 2020 (WHO). Coronaviruses are a part of order nidovirales. Their genetic material consists of positive sense Single Stranded RNA (+ssRNA). Major five structural proteins construct the virus namely Nucleocaspid (N), Envelope (E), Spike (S), Membrane (M) and Haemagglutinin Esterase (HE). The N protein facilitates transcription and manufacturing of virus. The S protein binds the virus to the host cell and also produces counteracting antibodies responsible for immunity in a vaccine. During virus duplication E protein is seen in high concentration in contaminated cells. Receptor interaction and host selectivity are controlled by the HE protein. Preliminary cases of COVID-19 in Wuhan, China, are assumed to be acquires from a zoonotic origin at a Huainan commercial seafood bazaar that marketed poultry, snakes, bats and other farm animals. According to the findings of a detailed genetic research to determine the host of virus among different animals, 2019 nCov is considered as recombinant virus made up of a bat CoV-2 and a CoV-2 of uncertain source [1]. SARS-CoV-2 enters the nose and throat through inhalation of aerosols and attaches to nasal epithelium. ACE-2 receptor of host form principal interface for entrance of virus into cells. In adults ACE-2 receptors are extensively present in nasal epithelium. Regional multiplication and spread occurs and it infects the ciliated epithelium of the conducting air pathway. Little immunological response is seen during this stage. Even though patients have low viremia still they are very contagious and nasal swabs tests are positive. The virus then proceeds towards the upper respiratory airways and invades it and patient present with various symptoms of COVID-19. In severe disease the virus penetrates the alveolar pneumocystis type 2 through

ACE-2 receptors and proliferates. Various cytokines and interleukins are released from this virus infected cells which attracts neutrophils and lymphocytes to fight against the virus but ultimately gets trapped in lung tissue further causing harm leading to ARDS. COVID-19 mostly affects elderly and middle age groups. Individuals present with pyrexia, body pains, malaise and respiratory complaints ranging from dry cough to breathlessness and even ARDS depending upon the severity of disease. Stomach pain, diarrhea, vomiting are other GI complaints. It takes 5-6 days for an individual to develop symptoms after contact with an infected person and one can spread the disease during this period. The major mode of transfer is *via* aerosols released in atmosphere while sneezing or coughing. Moreover these aerosols may be conveyed on objects used by infected persons that act as another source of infection [2].

LITERATURE REVIEW

Various methods have been devised to detect COVID-19 depending on different biomarkers. It includes detection of genes of responsible microorganism by molecular methods; antibodies against the antigen in blood accounting for serological methods. One can additionally directly asses the function of affected organs like chest abnormalities, kidney and liver function for diagnosis of disease. Detection of different biomarkers in bio fluids, plasmonic sensing and Field Effect Transistor (FET) predicated sensing are other technologies that are developed [3]. In this review we will elaborate main diagnostic methods for diagnosis of SARS-CoV-2.

Methodology

Comprehensive searches were carried out in PubMed without any restrictions on time frame or language. "Diagnostic," "test," "assay," "COVID-19," "SARS-CoV-2" and other keywords were joined with the logical operators and or in the search strategy. Systematic searches of included studies' citations lists and literature (e.g. googlescholar) were also conducted. The titles and abstracts of the papers that were found were checked for eligibility. Suitable papers were reviewed in their entirety and information from those that met the criteria for inclusion was collected. Studies that met all of the following parameters were used in this review:

- Analysis of any diagnostic test.
- Directed at SARS-CoV-2 (COVID-19) detection.
- Utilizing any human specimen.
- Collecting information on the test's accuracy (e.g. sensitivity and/or specificity).

Factors influencing the quality of the results

Limit of detection of an assay is determined by its framework and method of detection for correct diagnosis of a disease adequate sample acquisition is required which can be explained by the fact that inability to cough out saliva from back of throat may reduce the test sensitivity of posterior oropharyngeal saliva. The precision of the result can also be affected by improper specimen handling, delivery and processing hence specimen should be maintained and evaluated in accordance with CDC recommendations for clinical samples. It is also critical to have a sufficient supply of detection materials as varying clinical samples have diverse RT-PCR detection rates of which broncho alveolar lavage had the greatest rate followed by, nasal swab, pharyngeal swab, stool, whole blood and urine. Moreover the amount of viral load in different detection materials decreases as the disease progresses due to which throat swabs initially show increased viral load but later on deemed not reliable but blood tests and anal swabs show positive results in later part of disease. Antibody development also follows the same pattern. These findings indicates that the test results obtained must be read in conjunction with the patient's medical history and other diagnostic data in order to ascertain the patient's infection condition [4].

Molecular methods

Reverse transcriptase real time polymerase chain reaction is gold standard for diagnosis of COVID-19. It is a Nucleic Acid Amplification Technique (NAAT) which detects various viral genes for Nucleocaspid (N), Envelope (E) and RNA Dependent RNA Polymerase (RDRP) as well as sequences like ORF1b or ORF8. For screening of SARS-CoV-2 RT-PCR assay for identifying E gene followed by RdRp gene for conformation is advocated by WHO although CDC recommendations include using the RT-qPCR assay, which was made up of two nucleocapsid protein genes (N_1,N_2) [5]. The assay is performed by extracting RNA from the input and transferring it to the stock solution, which includes forward and reverse primers, nuclease free water and the reaction mixture (reverse transcriptase, polymerase, nucleotides, magnesium and other additives). The extracted RNA and stock solution are fed into a PCR thermo cycler and the temperature is adjusted to begin the PCR reaction. Throughout this process, the fragmentation of a fluorophore quencher probe provides a fluorescence response that is captured by the thermo cycler and thus the amplification status is documented. When doing any RT-PCR experiment, positive and negative references must be incorporated, making analysis of findings simple and precise. A nasopharangeal and oropharangeal swab is adequate as sample. The amassment of sample should be done under all safety precautions utilizing PPE kits, masks, gloves, goggles and the sample should be kept in convey media. It takes 24 hours for reporting which integrates to patient's apprehensiveness. Further with incrementing number of cases the desideratum for adroit personnel, extravagant RT-PCR kits and well equipped laboratories may pose quandary. Point of care or bed side testing has emerged as a possible solution for above quandaries which includes methods like CRISPR COVID-19 and SHERLOCK (Specific High Sensitivity Enzymatic Herald unlocking) [6].

Serological methods

These methods employ humoral immunity or antibody mediated immunity for identifying COVID-19 cases. Patients who present with clinical manifestations of the disease with supporting evidences from laboratory and radiological findings but yields unfavourable RT-PCR response then a conclusive immunological test can help establish diagnosis. Immunological test have become pertinent in pandemic for recognizing individuals, either infected or immunized, with immunity against the virus. Immunoglobulin A (IgA), IgM, IgG, and total antibodies can be detected using these. IgG antibodies the richest antibodies in blood and made by host immune system for developing immunological memory against the disease which play significant role in later phases of disease whereas IgM antibodies are generated by host immune system in early phase of the disease. Despite all these facts IgA is used for identifying disease because it is readily available in mucosa of host and also play an important role in course of disease [7]. Broadly Chemiluminescence Immunoassay (CLIA), Enzyme Linked Immune Sorbent Assay (ELISA), Lateral Flow Immunoassay (LFIA) and Immunofluorescence Assay (IFA) are serological tests used. The fundamental process of ELISA involves covering the virus antigen with either serum antibodies or antibodies which produces an enzymatic reaction on binding with viral protein. Indirect ELISA is mainly used for assays detecting SARS-CoV-2 viral antigens. CIF and IFA utilize similar principle but instead use a chemical reaction and fluorescence microscopy for visualization of results. LFIA is an effortless and expeditious method and most suitable for detecting COVID-19 in masses within 3-30 min and a diversity of samples can be used serum, plasma, whole blood, urine, saliva, tears and other liquids but have shown high rates of false negative and positives. Colloidal gold tagged viral antigen is used in LFIA. Upon detecting the appropriate antibody SARS-CoV-2 antibody can trap viral antigen in serum or whole blood. CLIA is the most sensitive and specific of all serological tests. ELISA is more sensitive than LFIA but lacks specificity. IFA being more personalized and tedious is not widely used. Albeit the ELISA and CLIA have shown comparatively better precision, they are still inhibited to laboratories and clinics due to the intricate testing process, high price value and dependence on complex instruments [8].

DISCUSSION

Radiological methods

COVID-19 chiefly acts on respiratory system hence chest CT is recommended as screening test because CT changes in lungs can be appreciated even before the symptoms develop. Chest x ray has little investigative value at early stage of disease although they may detect ARDS on complicated cases [9]. One can also assess the severity of disease by cheat X ray using RALE scoring system wherein the extent of consolidation of a particular lung lobe is giver a scoring between 0-4. The sum of scores for all the lobes provides the final severity score [10]. Chest CT is shown to have superior sensitivity than RT-PCR (98% *vs.* 71% respectively, $p<0.001$) and should be used for screening of patients especially of the present with suggestive symptoms but negative RT-PCR values [11]. Typical CT findings of COVID-19 include areas of higher densities visible peripherally called as Ground Glass Opacities (GGO). Consolidation occurs due replacement of air in alveoli with inflammatory fluids which also visible as areas of increased density but the differentiating point is that in consolidation the bronchial and vascular marking are obscured well. Another finding second to GGO and consolidation is thickened septa between lobes in lung interstitium due to lymphocytic invasion which appears as a number of small straight opacities forming reticular patterns [12].

Antigen tests

The various structural proteins that build the SARS-CoV-2 are detected using rapid antigen assays which incorporate immunological techniques like lateral flow sandwich immunoassays, microfluidic immunofluorescence assays and chromatographic digital immunoassays. Nucleocaspid protein is highly preferred because of its abundance in clinical sample. Specimen from nasal cavity and nasopharynx is utilized and 15-20 min is required for detection of viral antigen hence they are referred as rapid diagnostic tests. These tests can be utilized as point of care test because of they are simple to use and do not require any additional materials. Rapid antigen test present with disadvantages of decreased sensitivity as compared with NAAT. This is because of inability to amplify their selected protein which hinders with detection of low levels of viral antigen. Cross reactivity with viral proteins of other strains of Coronavirus affecting humans is responsible for high specificity of these tests. WHO suggests using rapid antigen tests in areas where NAAT is inaccessible or prolongs waiting period renders it impracticable. The test should be employed within 5-7 of appearance of symptoms [13].

Novel methods

Zhang and colleagues developed SHERLOCK, which is a Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) based diagnostic test using CRISPER CAS enzyme, especially Cas13. Cas13 recognizes specific regions in SARS-CoV-2 genome namely ORF1ab and S genes under direction of pre-arranged CRISPER RNA. Cas13 also cleaves the surrounding RNAs as collateral effect. This effect is used to create fluorescence which is detected as calorimetric response in paper strips. The test results are available in less than an hour and can be utilized as bedside testing method. This technology has been implied in developing COVID-19 detection masks in which virus particles can be detected in the exhaled air using CRISPER and color change signals virus presence [14].

Loop mediated isothermal amplification is another method which is low cost alternative to detect SARS-CoV-2. A DNA polymerase and a series of four specially

engineered primers are used in this approach, which recognize a total of six different sequences on the target genome and amplify it. In addition, gel electrophoresis is used to investigate amplified objects and locate endpoints. Poon, et al. used ORF1b region as target genome for LAMP and developed an inexpensive method for COVID-19 detection [15-20].

Other methods

These tests are vague and do not help in establishing cause of disease. Blood tests show decreased WBC count especially lymphocytes and decreased platelets. Inflammatory markers like CRP and ESR are raised. CPK, D dimer, ALT, AST and LDH could also show elevated levels in severe disease. Serum urea, creatinine and cystatin C levels are seen to be significantly greater in individuals with serious diseases than in any of those with minor symptoms [20-30].

CONCLUSION

COVID-19 has spread rapidly and evolved into a pandemic in no time. Effective and rapid diagnostic test have stood the test of time. RT-PCR continue to be gold standard for diagnosing COVID-19 as it can even detect low viral load and shows less false negative results. CT scans although can diagnose disease at early stage but still is used to complement RT-PCR test. CT is mainly used for determining severity of disease using RALE scoring system. Serological antibody test are used to asses immunity in population against the disease and conduct serological surveys. Rapid antigen tests are available as kits which act as easy to use point of care testing tools but because of their low sensitivity are limited to areas which where RT-PCR is unavailable and impractical point of care or bedside testing such as SHERLOCK and LAMP can possibly replace RT-PCR as screening methods. RTPC requires extensive laboratory settings and it is time consuming all the while point of care offer better alternative in highly populated country in India because of their rapid and colorimetric/fluorescent response which can be easily visualized. Development of self-detection home kits can help us recognize the real extent of spread of disease. New testing methods with low equipment demands, quick response time and high performance are widely sought to aid in the containment of the outbreak before the procurement and launch of vaccination programs. Prevention of disease is of utmost importance and physical distancing, mandatory face masks, precautionary hand washing have found to be effective against COVID-19.

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