Chapter

Epidemiology, Pathogenesis, and Healing Strategies of COVID-19

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Abstract

In the present chapter, some notable features (epidemiology, pathogenesis, and clinical characteristics) regarding recent outbreak COVID-19 have been reviewed. Most significant features related to COVID-19 such as (i) roots of infection and disease manifestation, (ii) shape and structure of viral S-protein, (iii) genome sequence study and replication in host cell, (iv) role of environmental factors, (v) diagnosis tools and (vi) role of biosensor have been critically investigated. The biological and behavioral risk factors for pregnant women before and after child birth have been dictated clearly. Pulmonary abnormalities due to COVID-19 of the patient having diabetes, cancer etc. history have been clarified with help of CT imaging. Finally, prevention and cure strategies adopted by many health professionals based on the existing drugs are mentioned with their side effects.

Keywords: COVID-19, transmission, genome, prevention, cure strategies

1. Introduction

Emergence of COVID-19 threatens human health and economy around the globe. Possibly, world populations have ever faces such crisis and will remain the witness of such incident. Each new day experiences number of new cases with increasing death toll since its first identification. However, suggested name of corona virus comes from Latin word corona, signifies crown or halo. Electron microscopy of corona virus reveals encapsulation of crown like fringe at outer surface [1]. Novel Corona virus known as different name such as COVID-19, HCoV-19 was outbreak in Wuhan, Capital of Hubei province, China in month of December-2019 and later become pandemic by quick spreading into the major countries in the globe [2, 3]. Outbreak has very high risk with potentiality of human to human transmission. Experts around the globe suggest that, the average incubation period of COVID-19 is ~5 days with a range of 2–14 days [2]. Symptom includes high fever, dry cough to severe respiratory acute disease and death (in some cases) [2]. Average fatality rate reported to be ~1–2% [1, 2]. Scientific community and researchers are at the midst of COVID-19 pandemic and have struggling to find out how much similarity with SARS-CoV. The study reveals that, COVID-19 is similar like SARS corona virus which is believed to be originated from either bats or civet cats or raccoon dogs [2, 3]. However, due to lack of evidence many scientific communities ruled out such report. As per WHO officials, COVID-19 is ten times more infectious than the 2009 pandemic H1N1 influenza virus. There is no effective drug or vaccine against the corona virus or similar infectious agents so far and it is

still unknown how many more month require to develop. However, one needs to understand the priority and treatment protocol based on the severity of the disease.

2. Transmission and cellular mechanism of COVID-19

COVID-19 is the seventh coronavirus which infect humans like earlier reported coronavirus SARS-CoV, MERSCoV, HKU1, NL63, OC43 and 229E. [4]. For an enveloped virus, primary mode of transmission is close contact with the infected individual. Transmission is appeared to be silently enter into the host body and no immediate onset symptoms have been evident. Therefore, before infected host tested positive, he/she already transmitted virus to many others (provided infected person does not maintaining isolation/social distancing). In most cases, human to human transmission occurs, though human to human transmission has been ruled out at the very early stage of the outbreak. However, probability of getting infectious becomes higher when an infected person or person in incubation stage comes closer to the healthy person. Alternative transmission medium might be via contact surfaces i.e. skin to skin touching or touching objects having COVID-19 particles. Then direct or indirect entrance of that surface particles into one's body through mouth, nose, or eyes. The other forms of transmission possibly through inhalation of particle aerosols emanated from exhaled breathe of infected person or via droplet due to cough/sneezes [4]. A recent study reveals that, COVID-19 may survive in aerosols (size <5 μm) for at least three long hour in an open air ambient [1, 2]. Relative humidity, fomite material, and air temperature possibly are the factors for prolonging virus life. Long time survival at the outside of its host organism (surfaces such as aluminum, sterile sponges, or latex surgical gloves) will increase the opportunity to produce new host via touching or breathing [2, 4]. Facal transmission is another transmission path where COVID-19 has been found in stool specimen like aluminum, sterile sponges, or latex surgical gloves etc. [2]. The surface stability of S-protein of COVID-19 found to be more on plastic, stainless steel than the copper and card board [2]. It is worth mentioning that, some positive COVID-19 cases were also reported due to the nosocomial transmission. Recent study of Wang et al. reflects 29% health professionals and 12% hospitalized patient (associated with other disease history) becomes infectious due to nosocomial transmission [5]. It is worth to mention that, urine of infected one does not contain any COVID-19 particles and therefore does not have any role for transmission [6]. Study of Casanova et al. suggest that, corona virus may remain active even in pure water and pasteurized settled sewage for few days to one week [7]. Airborne dust particles or microorganisms or particulate matter (PM) are the potential transmitter [6]. Some study finds the virus can transmit through air up to 1 m whereas another recent study find virus particles can transmit up to 13 ft. [6]. However, COVID-19 particles combined with airborne dust particles or microorganisms or particulate matter (PM) enters into the deeper alveolar and tracheobronchial regions of the host.

3. Role of environmental factors

The transmission, survival and characteristics of COVID-19 directly influenced by environment factors like temperature, pressure, pollution level [8]. In addition, outbreak further involved with the reproduction number (R0). The reproduction number (R_0) defined as the number of healthy people getting infected from a single infectious living in a susceptible populated environment. Reproduction no (R_0)

mainly governed by the factors like (i) stage of infection, (ii) transmissible strength of the pathogen, and (ii) the number of susceptible contacts. It is meaningless to set the exact value of R_0 until otherwise the surrounding environment clearly specified. For example, Li et al. reported R_0 value for COVID-19 as 2.2 (95% confidence interval, 1.4–3.9) [9]. However, in reality R_0 for COVID-19 might be very higher than expected if one does not obey the rule of social distancing or home quarantine. Few governing factor are crucial for reproduction rate or newly infected cases in a particular area viz.; (i) isolation of infected person from the day of infection, (ii) availability of general needs for ones to remain in isolation like food and other necessities and (iii) availability of sufficient diagnosis tools in the area. Based on the above facts, a mathematical model proposed by Tang et al. [10] determines the reproduction rate or spreading rate by individual infected host per day. If the above factors favor in a particular region, then contact rate $C(t)$ (assuming reproduction rate proportional to the number of new contact) in a certain period of time follows Eq. (1) leading to decreasing reproduction rate [10].

$$C(t) = (C_0 - C_b)e^{-r_1 t} + C_b \tag{1}$$

Where C_0 initial contact rate, C_b is minimum contact rate under current control strategies, r_1 is coefficient of contact factor, t is investigated time period. However, reproduction rate further depends on the diagnosis rate as shown in Eq. (2) [10].

$$\frac{1}{\delta_I(t)} = \left(\frac{1}{\delta_{I0}} - \frac{1}{\delta_{If}}\right)e^{r_2 t} + \frac{1}{\delta_{If}} \tag{2}$$

Where $\delta_I(t)$ diagnosis rate and, δ_{I0} is initial diagnosis rate with $\delta_I(0) = \delta_{I0}$, δ_{If} is fastest diagnosis rate with $\lim_{t\to\infty}\delta_I(0) = \delta_{I0}$, and r_2 is exponential decreasing contact rate factor and the value depends the recourses available in the infected region. However, if the aforementioned factors are not favorable in the investigated region, then it is expected that, the new infected cases (induced by individual infected host) will begin to rise. A time dependent (30 days lockdown period) contact rate $C(t)$ and diagnosis rate $\delta_I(t)$ simulation study (from somewhere in Wuhan) have been reported based on adaptive Metropolis-Hastings (M-H) algorithm and Markov Chain Monte Carlo (MCMC) procedure and the result is shown

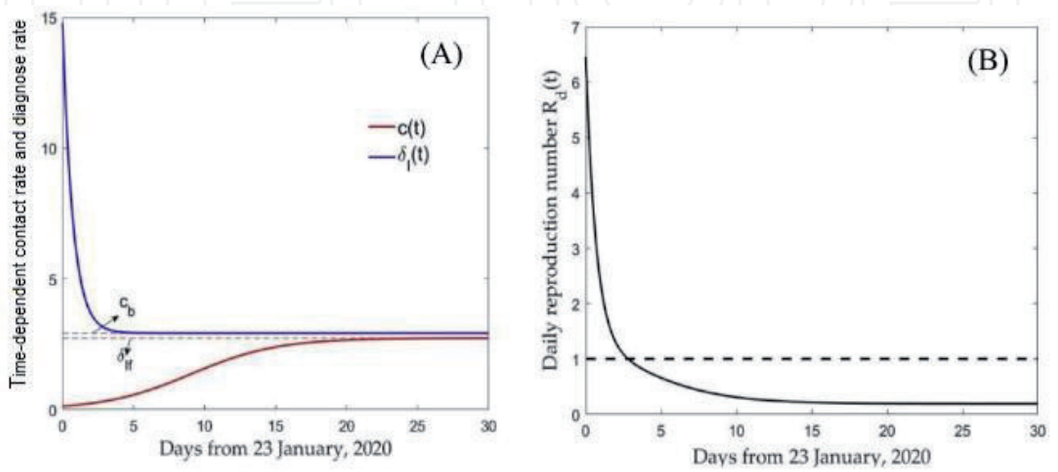

Figure 1.
Showing simulation data of (A) contact rate C (t) and diagnosis rate (B) effective daily reproduction ratio for the period of 30 days somewhere in Wuhan [10].

in **Figure 1** [10]. The model shows how self isolation (decrease in contact rate) and increase in diagnosis rate in a region can stop community spreading significantly.

4. Symptoms

Many infectious remains asymptomatic and silently affected so many. Most of the mild cases symptoms such as, fever (83–100%), myalgia (11–35%), diarrhea (2–10%), fatigue, headache (7–8%) and cough (59–82%), and dyspnoea have been found predominantly [11]. However, severe infection outcome includes drastic reduction in average circulating lymphocyte and platelet counts etc. Other abnormalities are on chest radiographic imaging, lymphopenia, leukopenia, and thrombocytopenia [2]. Respiratory system is highly affected. Though age is still not be a proven critical risk factor for COVID-19 infection, but it was observed that, mortality rate were prominent for elderly having some previous disorder like hypertension, chronic obstructive pulmonary disease, diabetes, cardiovascular disease. Such prior disorder with COVID-19 particles in body quickly developed some dangerous malfunction like coagulation dysfunction, septic shock, metabolic acidosis and acute respiratory distress syndrome which are hard to correct eventually leading to the death. Further, few other malfunction like decrease in neutrophil count, D-dimer, blood urea, and creatinine levels etc. were prominent in severe infected patient [2]. However, all effects (including inhaled particulate matter combined with an immune response or cytokine storm induced by COVID-19 infection) together exacerbate severe ill effect on respiratory system and increase the risk of patient life. In order to investigate the different organ disorder due to COVID-19, human protein database and distribution of antigiotensin converting enzyme 2 (ACE2) has been correlated. It would be appropriate to mention that, an ACE2 is a transmembrane enzyme, act as a receptor function in host body and help to enter COVID-19 in host cell [8–11]. **Figure 2(b)** and **(c)** shows the detection of ACE2 receptors over neurons and glial cells, further, how COVID-19 binds to ACE2 receptor in brain cell. **Figure 2(a)** and **(e)**, ensure presence of COVID-19 in general blood circulation with abundant number of virus in cerebral circulation. The presence of such virus possibly is the reason of slowing the blood circulation mechanism in capillary endothelium which results in higher interaction probability of COVID-19 spike protein with the ACE2 [3]. Hence, there is a possibility of neuronal damage or endothelial rupture of cerebral capillaries in association with bleeding in cerebral tissue which increases the life risk of patient infected with COVID-19. Few evidence of neurotropic mortality caused by COVID-19 has been reported but proper explanation is yet to be established [12]. A study of 218 patients from recent outbreak reveals 78 patient (36.4%) having neurological malfunctions due to the COVID-19. Rest of the patients is either losing control over breathing or suffering from acute respiratory failure [3, 12]. However, evidence of virus in cerebrospinal fluid is still under debate. Apart from blood circulation, entry of COVID-19 through cribriform plate close to the olfactory bulb can be an alternative pathway to the brain. Further, indirect consequences of multi organ failure (pulmonary, renal, cardiac, and circulatory damage) caused in patient having COVID-19 appears to be more dangerous than expected. The study reveals that COVID-19 severely damages leucocytes which possibly are the reason of multi organ failure [13]. Older infected people having diabetes mellitus-2 are at more risk to mortality due to the uncontrolled glycaemia [14]. COVID-19 infection in diabetes patient raises the stress level and hence blood glucose levels and abnormal glucose variability. Increase in blood glucose possibly due to the release of hyperglycemic hormones (glucocorticoids and catecholamines) [14].

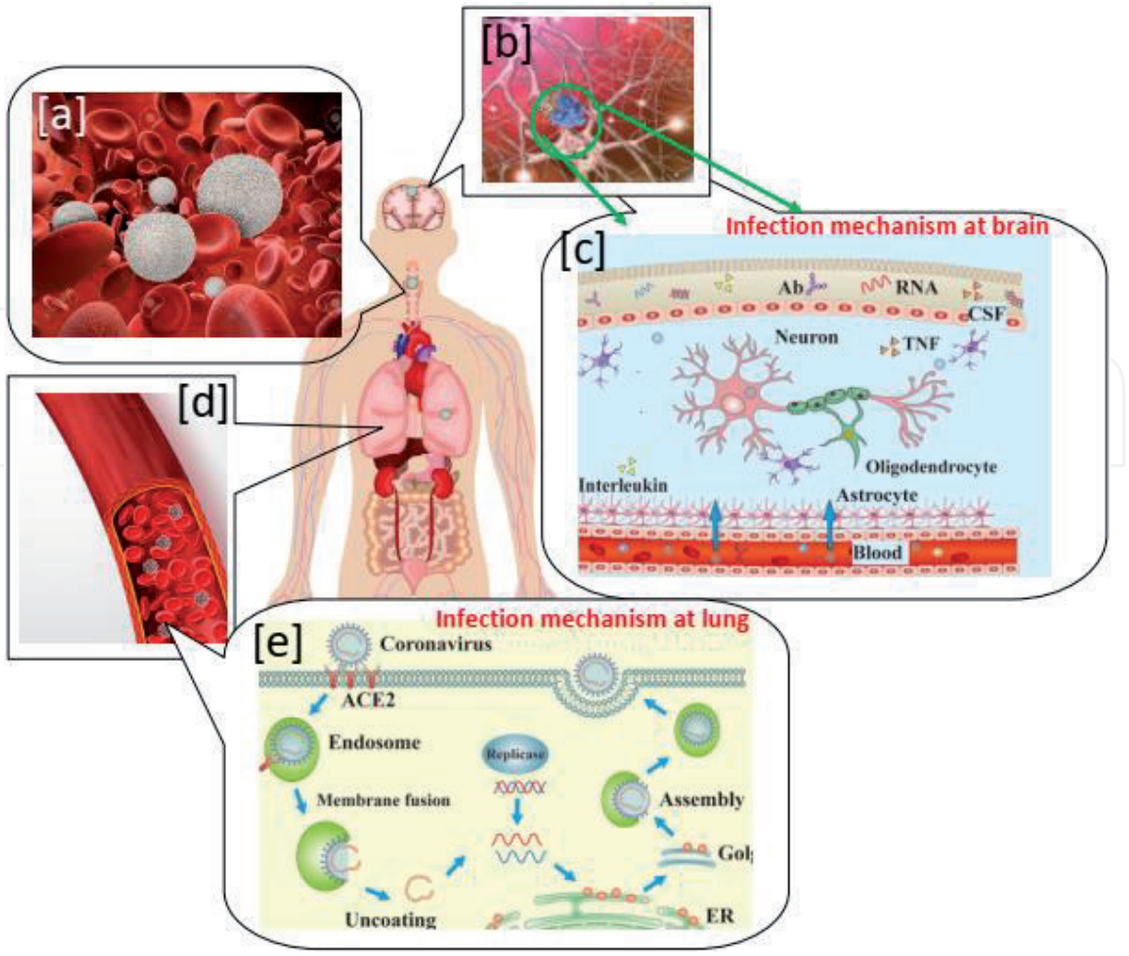

Figure 2.
Showing possible targets of COVID-19 (lungs, heart, kidneys, intestines, brain, and testicles); (a) COVID-19 distribution and ACE2 receptor in human, (b) COVID-19 transmission to brain through upper nasal trancribrial path (c) inset image shows binding mechanism of spike protein at the site of neuron (d) showing COVID-19 distribution through blood circulation at lungs and (e) inset image showing bind of COVID-19 with ACE2 receptor at lungs cell (reproduced with permission ref. [3, 12]).

5. Protein structure of COVID 19

The COVID-19 in the infected human body consists of critical virion which is a spike type glycoprotein known as S-protein [15]. The characteristics of spike protein or S-protein solely determine whether host cell is infected by corona virus or not. The spike protein has two subunit referred as S1 and S2 respectively. S1 is responsible for virus-host range and cellular tropism with the help of receptor binding domain (RBD) whereas S2 expedite the virus-cell membrane fusion with help of heptad repeats 1 (HR1) and heptad repeats 2 HR2 [16]. However, a polybasic cleavage site (RRAR) in between S1 and S2 influence the viral infectivity and host range with the effect of furin and other proteases [4]. O-linked glycans created by the proline, which possibly flank the cleavage site and shields epitopes or key residues on the SARS-CoV-2 spike protein [4]. However, the outermost part of critical virion cell is full of spike protein which helps to binding and subsequent fusion of antigiotensin converting enzyme 2 (ACE2) membranes in host cell. ACE2 is a transmembrane enzyme, act as a receptor function in human body and help to enter COVID-19 in host cell [1–9]. ACE2 exist at almost each organ of the body including arterial smooth muscle cells in the lungs, lymph nodes, stomach, colon, skin, liver bile ducts small intestine, kidney parietal epithelial cells, and the brain [14, 15].

The genome sequence of COVID-19 contains ~ 27 no of protein and almost ~30000 nucleotides in length as shown in **Figure 3(a)** [17]. Open reading frames (ORFs) are found to be variable in COVID-19 gene. In first ORFs (ORF1a/b), almost 2/3 viral RNA have been found which encodes 16 non-structural protein (NPS) and translates two polyproteins (pp1a and pp1ab). Accessory and structural proteins encoded by remaining 1/3 ORFs. Most essential proteins are RNA dependent polymerase (RdRP) and four structural proteins viz.; matrix protein (M), nucleocapsid protein (N), small envelope protein (E) and spike surface glycoprotein (S) [17]. The function of S protein is to binding and fusion of ACE2 membrane in host cell. On the other hand, M, N and E protein helps to budding, envelope formation, assembled, pathogenesis and RNA encasing in host cell [15–17]. Upper part of the respiratory tract has lower ACE2 results in less infection by S-protein whereas lower parts of the lungs have more amount of ACE2 consequently higher tendency of getting infected by S-protein as confirmed by higher opacity in CT image as shown in **Figure 4**. ACE2 has high binding capability with COVID-19 spike protein and its initiates the infection process as shown in **Figure 3(b)** [5]. The interaction of S-protein and ACE2 in the host cell is as follows; COVID-19 genome encodes many structural protein (glycosylated spike (S) protein) and non-structural protein (RNA-dependent RNA polymerase (RdRp), protease (3CLpro), and papain-like protease (PLpro)) for inducing host immune response [19]. 3CLpro and PLpro are responsible for COVID-19 genome replication in host cell by proteolytic processing of non-structural proteins. As per National Center for Biotechnology1 (NCBI) database, with ID NC_045512, the COVID-19 genome structure is 29,903 bp single-stranded RNA (+ss-RNA) coronavirus [3]. COVID-19 genome at the host cell releases it outer encapsulation and remain as single-stranded positive RNA (having 5′-cap structure and 3′-poly-A tail). RNA translated into viral polyproteins with help of host antigiotensin converting enzyme 2 (ACE2). Cleaving of polyproteins turns it to an effector protein by viral proteinases 3CLpro and PLpro [19]. Such mechanism reduces the host immune response drastically. Monte Carlo simulations by convolution contact maps suggest, receptor binding domain (RBD) area of spike protein shows various conformations with respect to the remaining portion of the protein structure [20]. The identified RBD area were then reassembled using pipeline method which produces a complex structure of spike trimer and the extracellular domain of human ACE2. Cryo-EM

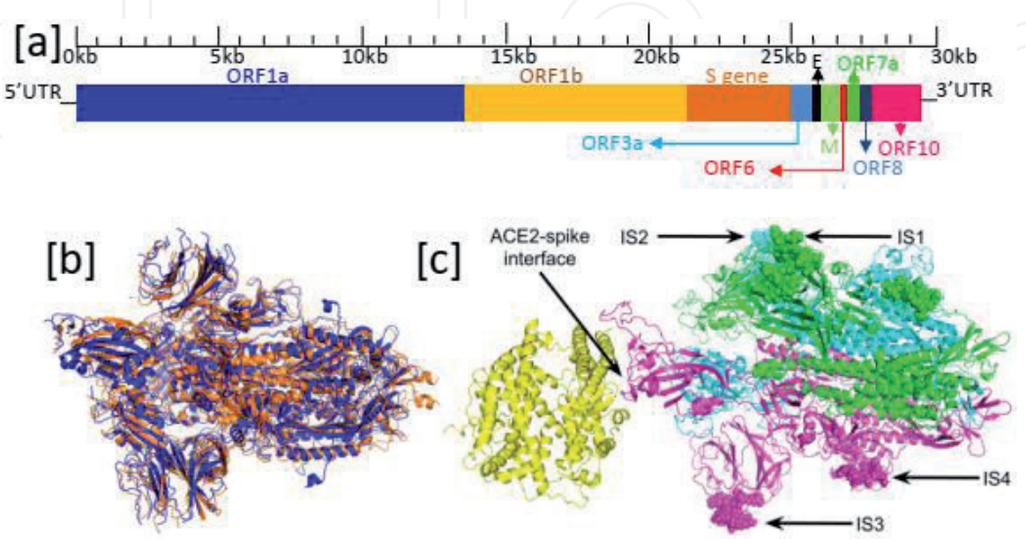

Figure 3.
(a) Genome structure of COVID-19, (b) spike protein structure of COVID-19 constructed from C-I-TASSER, and (c) human antigiotensin converting enzyme 2 (ACE2) (yellow color) and spike protein trimmer (right side multicolor (magenta, cyan and blue)).(reproduced with permission [1]).

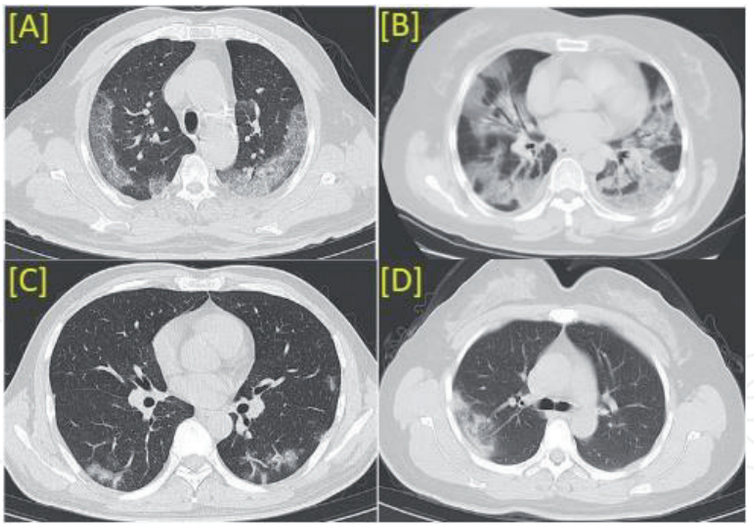

Figure 4.
CT image of (A) 75 year male patient having fever and cough since 5 days (B) 55 year female patient having fever and cough since 7 days (C) 43 year male patient having fever and cough since 7 days [D] 43 year female patient having fever and cough since 5 days; the abnormalities in axial CT were [a] bilateral subpleural CGO [B] extensive CGO with consolidations [C] small bilateral areas of peripheral CGO with minimal consolidation [d] peripheral consolidation in right lung (reproduced with permission from ref. [18]).

structure analysis reveals that, the binding affinity of ACE2 with the S protein of COVID-19 spike protein is 10–20 times higher than that of the SARS CoV spike protein [3]. However, corona virus genome sequence study suggests that, RBD in spike protein is the most variable part and determines probability of getting infected based on the binding efficiency with host receptor. From reports of Wan et al. six RBD amino acid of COVID-19 viz; L455, F486, Q493, S494, N501 and Y505 found to have high binding affinity with ACE2 receptor of human [21]. Possibly high binding capability of RBD with human ACE2 results in high rate of natural infection in human species.

6. Diagnostic tools

Diagnostics measure can play a major role for the screening of COVID-19 patient from the healthy ones. Initial identification of the COVID-19 have been carried out through molecular diagnostic approach viz.; metagenomic next generation sequencing (m-NGS), reverse-transcription PCR (RT-PCR) procedure and CRISPR. Rapid DNA alteration/genome structure of COVID-19 makes it difficult to detect by any specific method. Therefore, specific DNA sequence must be developed for early stage detection and subsequent alarming. A paper-based colorimetric assay for DNA detection based on pyrrolidinyl peptide nucleic acid (acpcPNA)-induced nanoparticle aggregation has been reported by Teengam et al. [22]. The oligonucleotide targets were detected by investigating different color measurement of silver nanoparticles (AgNP) with detection limit down to 1.53. Chen et al. followed real-time polymerase chain reaction (RT-PCR) using nucleic acid analysis for detection of COVID-19 [4]. Measurement accuracy reported to be about 71% [4]. Pathogens from bronchoalveolar lavage (BAL) fluid analysis found to be an alternative of finding genetic sequence of corona virus. Swab test possibly have higher accuracy but insufficient kits impose to go for other techniques. In this section few diagnosis process discussed elaborately.

Protein testing: Biomarker like viral protein antigens can be used for detection of infected COVID-19. Variation in infection stages make it hard to find a particular protein antigens or antibody pattern in host cell. Study of Wang et al., confirms

viral increases in salivary in the first week of onset symptoms and then decreases gradually with progress in time [23]. Saliva testing possibly show shedding from salivary glands and the upper and lower respiratory tract [23]. Author tested posterior oropharyngeal (deep throat) saliva of 23 COVID-19 infected patients among which 13 were mild disease and 10 were severely affected. The findings reveals that, at initial stage of testing, posterior oropharyngeal saliva was 5·2 $\log_{10}$ copies per mL (IQR 4·1–7·0). The viral load decline to slope − 0·15, 95% CI –0·19 to −0·11; R^2 = 0·71. Further, study finds relatively older aged patients having higher viral load of Spearman's ρ = 0·48, 95% CI 0·074–0·75; p = 0·020. Due to non-invasive and painless procedure, posterior oropharyngeal saliva testing is more acceptable to the patients. Similar study of nasopharyngeal swab or bronchoalveolar lavage fluid testing by CRISPR-nCoV method carried out by Hou et al. [24]. The finding reveals better accuracy of testing with lower turn-around time about ~40 minutes and does not require thermal cyclers. CRISPR-nCoV method composes recombinase polymerase amplification (RPA) step followed by T7 transcription and Cas13 detection step. In a typical process, a mixture of 2.5 µl of tested sample, primer (0.4 µM), reaction buffer, magnesium acetate (14 mM of) and the RT-RPA enzyme have been prepared [25]. The prepared sample was incubated at 42 °C for half an hour. Again mixture of amplification product, 166 nM of ssRNA, 66.7 nM of Cas13, 1 µl T7 RNA polymerase, 5 mM of each NTP, and 33.3 nM of gRNA allow to reacts in CRISPR. The temperature during reaction was maintained about ~42 °C. Finally, Fluorescent signals were collected. The sensitivity of CRISPR were found to be 100%. Results of 52 known infected sample showed positive COVID-19 with FC value ranging from 5–66.3 [25].

Metagenomic next-generation sequencing (mNGS): Study of Hou et al. demonstrated 52 confirm positive COVID-19 infected cases among 61 patients by mNGS method [25]. The rest are found to be negative. In this method, Qubit Fluorometer (Thermo Fisher Scientific, 99 Carlsbad, CA, USA) can be used to measure RNA concentrations and then transposase-based methodology with ribosomal RNA depletion approach might be useful for creating sequence libraries. 10 million single-end 75 bp reads must be generated for each sample followed by removal of read derived from host genome. The sequence libraries were generated by reverse-transcribed of RNA into cDNA. However, taxonomic classification and identification of sequence read may be performed by comparing with existing database of plasmid, bacteria, fungi, human, protozoa, univec, and virus sequences. It is worth mentioning that, a simultaneous testing of negative sample and its sequence generation must be carried out with each above sequence run for controlling contamination. Further, genetic similarity of all positive cases confirms Orf1ab and N gene are two potential sequences for identification of COVID-19 infection. The turnaround time ~ 20 hours (library preparation (8 hours), sequencing (10 hours) and bioinformatic analysis (2 hours)) and high cost makes this method limited use for COVID-19 detection.

Reverse-transcription PCR (RT-PCR): RT-PCR is widely used method using upper respiratory tract samples (including nasopharyngeal swabs, nasopharyngeal washes, nasal aspirates and oropharyngeal swabs) for COVID-19 testing. Probable test sample from lower respiratory tract might be sputum, BAL fluid and tracheal aspirates. However, BAL fluid and tracheal aspirates in general not used as sample for RT-PCR testing, because of aerosol formation from these samples. Mainly two steps followed in RT-PCR process; reverse transcription of viral RNA into cDNA and subsequent amplification of cDNA [26, 27]. Throughout RT-PCR diagnosis, crucial steps followed are sequence alignment, primer selection, optimization of assay (like reagent conditions, incubation times, and temperatures)

and finally PCR testing. RNA-dependent RNA polymerase (RdRp) sequence and the open reading frame 1ab (ORF1ab) sequence has been used as gene target for COVID-19 detection. One step real time RT-PCR, where swab of the infected patient were mixed with following ingredients; reverse transcriptase, polymerase, magnesium, nucleotides, nuclease-free water, primers, a fluorophore-quencher probe [26, 27]. Whole mixture then transferred into PCR thermocycler which generates a fluorescent signal with help of fluorophore-quencher probe. Corman et al., uses three types of assay structure (RdRp gene, N gene and E gene) for testing 297 samples [27]. Result reveals that, detection probability ~97% for both assay RdRp gene and N-gene with 3.8 and 5.2 copies per reaction, respectively. The process turnaround time is about ~1.5 hours possibly due to the producing capability of many copies of specific gene sequence. Need of thermo cycler and use of sophisticated instruments hinders this method to use as diagnostic tool in limited resource setting. Accuracy of the method depends on sampling location, quality of RNA extraction and training of operators etc. However, still this method finds its application due to the faster nucleic acid amplification. Lower accuracy in RT-PCR results (negative result in infected sample sometimes) possibly due to the insufficient cellular material and improper extraction of nucleic acid from the specimen [27].

Computed Tomography (CT): Imaging technique like computed tomography offers easy capture of the cross sectional surface of the lung from many angles in non-invasive way. Analysis of such image modality by radiologist can offer insight about abnormal features that may come from COVID-19 infection. However, it should be noted that, abnormalities of pulmonary involvement may arise due to other viral disease as well. Note that, in some asymptomatic patients whose RT-PCR shows negative even he/she have travel history or closure contact to infected person, in that case CT imaging could be good approach of screening. Pattern change in peripheral ground-glass opacification (areas of hazy opacity), consolidations (i.e. fluid or solid material in compressible lung tissue), bilateral involvement, peripheral and diffuse distribution are found to be the responsible factor for abnormalities in pulmonary due to COVID-19 infection [28]. Further, it should be noted that, such marker may vary depending on the infected stages of COVID-19. For example, Bernheim et al. reported 56% pulmonary involvement after 2 days of onset symptoms whereas it researches to peak involvement on 10th day [29]. A CT imaging of asymptomatic patients reveals that, at the early stage of no symptoms only lesions in lungs and it gradually bilateral diffuse disease become prominent and then consolidation found on day of first or second week from onset of the symptoms [28]. Xie et al. reported five patients having negative RT-PCR result but their CT imaging confirms positive COVID-19 viral infection [30]. All the positive confirm cases does not had any prior abnormalities in pulmonary. However, same author also demonstrated 155 patients having positive RT-PCR which was found again positive by CT imaging method and 7 other patient who were positive in RT-PCR testing showed negative in CT imaging [30]. CT imaging of the five patients (who were tested RT-PCR negative) showed abnormalities in pulmonary like ground glass opacity (5 patients) and/or mixed CGO and mixed consolidations (2 patients) [30]. A series of 51 patients test by CT imaging and verification of the same by RT-PCR method resulted sensitivity about ~98% for CT and ~ 71% for RT-PCR study, respectively [18]. A CT image of four patients is shown in **Figure 4(a)–(d)** [18]. The CT investigation of 21 patients (among which 6 male 15 were male) from the onset of initial symptoms to recovery period were carried out by Pan et al. [28]. On an average, CT image capture and analysis of all the patients were carried out after every four days interval and all have been discharge after

17 ± 4 days. In most of the patients, maximum abnormalities were noted on or after 10th day since onset symptoms with R2 = 0.25 and p < 0.001. Based on the progress of infection level during complete hospital stay (~21 day) until recovery has been categorized into four stages viz.; stage 1(0–4 days), stage 2 (5–8 days), stage 3 (9–13 days), and stage 4 (>14 days). The most pulmonary abnormalities reported are (i) CGO in stage 1, in 17 among 24 (75%) patients (b) increased crazy paving pattern in stage 2, in 9 out of 17 (53%) patients (iii) consolidation in stage 3, in 19 out of 21 (91%) patients and (iv) gradual resolution of consolidation with decreased crazy paving pattern in stage 4, in 15 among 20 (75%) patients [28]. The governing factor (ground-glass opacification, bilateral involvement etc.) for detection of COVID-19 employing CT imaging, sometimes became imperceivable due to the low severity or few symptoms in patients making this method more challenging. Use of artificial intelligence for screening the infected patient by CT imaging possibly can increase the sensitivity of the method.

Nucleic Acid Testing. This testing does not require sophisticated laboratory instruments but dyes (malachite green, calcein and hydroxynaphthol blue) that utilize inherent by-products of the extensive DNA synthesis [31]. The testing based on the isothermal amplification at particular temperature for nucleic acid testing. The different isothermal techniques like polymerase amplification, helicase-dependent amplification, and loop-mediated isothermal amplification (LAMP) have been followed in nucleic testing [32]. Reverse transcription LAMP (RT-LAMP) is one of major techniques for detection of COVID-19 based on one-step nucleic acid amplification method [32]. In this method, few primer and DNA polymerase are essential to obtain insight about viral genome sequence. Yu et al. uses six primers to amplify the ORF1ab gene fragment [33]. The primer are as follows; forward inner primer (FIP), outer forward primer (F3), outer backward primer (B3), backward inner primer (BIP), loop forward primer (LF), and loop backward primer (LB) [34, 35]. In a typical process, the mixture of isothermic amplification buffer, dNTPs, manganese sulfate, FIP/BIP, F3/B3, FL/BL primers, Bst 2·0, antarctic thermolabile UDG, and Warm Start Reverse Transcriptase in ddH_20 were transferred in ice bath. The ice bath then kept in enclosed room and allows incubation at 63 °C for half an hour. RNA detection started with simultaneous occurrence of reverse transcription and amplification process. The detection can be confirmed by several identification like color change from orange to yellow or laddering pattern of bands after electrophoresis on a gel or by fluorescent light in response to UV excitation [32]. Loop mediated isothermal study of respiratory swabs employing pH-sensitive dyes and five primers for visual and colorimetric detection has been reported by Zhang et al. [31]. In conventional method, patient swabs were mixed with BSA (1%), amphotericin (15 μg/mL), penicillin G (100 units/mL), and streptomycin (50 μg/mL). The mixture sample then deactivated at 56 °C and finally COVID-19 RNA was extracted from the deactivated sample. Average detection sensitivity was ~100 copies in each five primer. All the samples were further confirmed through RT-PCR testing. Similar study by Yang et al. [36] reported detection of ORF1ab gene, E gene and N gene employing RT-LAMP method. Testing of 208 samples reveals sensitivity similar to RT-PCR method whereas specificity was 100%. RT-LAMP technique has high sensitivity and specificity. The turnaround time less than one hour and have flexibility to work at various pH level and temperature level. The cost of testing is relatively low compared to other techniques. However, optimum primer selection and producing suitable reaction environment are two major difficulties technician faces during sample testing.

Point-of-Care Testing: In point of care testing sample does not require to send in laboratory rather one can test with smaller device with turnaround time is less than one hour. One can either detect virus genetic content by nucleic acid based probes or by detection of toxin produced by pathogen or by epitopes of pathogen

membrane [37]. In later two approaches, antibodies or antibody derivatives can be used for easy diagnosis. However, specificity of the later two (antibody based) approaches is lower than the former (nucleic acid based) approach. Some of reported point of care techniques are (i) biosensors, (ii) gold nanoparticles as antibody for binding virus protein (lateral flow assay), (iii) mirofluidic devices, (iii) electrochemical sensors, (iv) paper based systems, and (v) and surface-enhanced Raman scattering based systems [37]. Lab on chip point of care diagnosis offer portability, rapid detection time, and miniaturization. Further, testing require small sample volume [36–38]. The smart phone dongle attached with devices like mirofluidic device, electrochemical sensor or lab on chip can also be a point of care strategies for COVID-19 detection. Xiang et al. compared COVID detection by (i) ELISA test with IgG and IgM antibodies for 63 patients and (ii) colloidal gold-immunochromatographic assay (GICA) for 91 patients and the sensitivity were found to be 87.3% and 82.4% [37].

7. Influence of COVID-19 on pregnant women

COVID-19 outbreak converges existing reproductive health and economic stability of the women's and girl's. The crisis reduced the access of family planning, and increase the unsafe abortion, miscarriage, unintended pregnancies, post traumatic stress disorder, intimate partner violence etc. [39]. Limited resources available for illness prediction of COVID-19 infected pregnant women but provide some insight based on the effects one encounter from similar type of corona virus infection (SARS) and MERS). COVID-19 can increase the rate mortality for the case of pregnant women and enhances the chance of transmission to new born baby via vertical transmission. A study of 33 new born baby from infected mother reveals vertical COVID-19 transmission in 3 babies [39]. US Centers for Disease Control and Prevention (CDC) sets few rule and regulation for women having new born babies are (a) sanitize the hands before touching the baby, (b) wash feeding bottles before and after use, (c) women are allowed to breast feed until evidence suggest otherwise, (d) use mask during breast feeding, (e) use of dexamethasone as an alternative to betamethasone for fetal lung maturation etc. [24]. Study of Liu et al., from January 20, 2020, to February 10, 2020 gives a clear picture of different symptoms and subsequent treatment of infected women having different stage of pregnancies [40]. The entire clinical study reviewed by three radiologist for 15 pregnant infected women (diagnosis with reverse transcription–polymerase chain reaction (RT-PCR) at the time of admission) reveals that, 11 patient gave successful deliver of new born and 4 patient are still under observation (three are in second trimester and one in third trimester). They were not facing any natal asphyxia, neonatal death or abortion up to the end of the study. The CT imaging was carried out for infected women before and after delivery. All patients chest CT imaging shows pulmonary abnormalities. Similar chest diagnosis by CT scan and pulmonary abnormalities of all admitted patient has also been found in the report of Rasmussen et al. [41]. CT imaging reveals ground-glass opacity (GGO) in early stage of the infection and crazy paving pattern (denser, more profuse, and confluent) in patients having more infection than the images of healthy lungs [40, 41]. The most common symptoms were found to be fever (13 among 15 patients) and cough (9 among 15 patients). Lymphocytopenia was the most common abnormality found in 12 patients. CT scanning ensures no evidence of COVID-19 provocation after delivery. Among 11 patients. All were given antibiotic treatment before and after delivery whereas 4 patient who were still pregnant till end of the study period were treated only with antibiotics. Another study by Zhu et al., reported nine

pregnant women with 10 new born (one twin) babies [42]. The report says onset symptoms of COVID-19 were evident in four patients before 1 to 6 day of delivery, in two patients on the same day of delivery and in three patients after 1to 3 day of delivery. Two among the nine mothers had intrauterine fetal distress and 6 babies were born preterm. No mortality was reported [42]. As far as respiratory acute failure is concern, 40% pregnant women were given mechanical ventilation whereas it was 13% for non-pregnant women [31]. However, what treatment is actually applied is still unknown. Pregnant women's are more likely to be affected due to the physical changes like diaphragm elevation, edema of respiratory tract mucosa, increased oxygen consumption etc. drive them to more complicated cases. What about the new born babies? Whether there is any vertical transmission of COVID-19 to new born or not? If transmission takes place, then in what mode and is it when fetal is in the mother womb or during delivery time (by means of surface contact)?. This question mark is still in dilemma. Because some evidence proofs that, there is no vertical transmission takes place [41]. On the other hand, few study ensure positive cases in new born babies [43]. Vertical transmission case study of four mother and their new born have been investigated by Chen et al. with different parameters variation in mother as well as in the new born babies [44]. All four mothers were admitted in hospital at their trimester with positive COVID-19. Initial health counseling of mothers was as follows; three among four have fever, two among four have myalgia or fatigue, two among four have cough. The fetal movement was normal except one mother who have dyspnea. Lymphocytes count (<1.1 × 109/L) found to be lower than in normal case and C-response protein was found to be significantly increased in level for all four mothers. Chest CT imaging before delivery confirms abnormalities. However, after antiviral treatment, COVID-19 test found negative in three mother and they were released after 3–5 days. One who suffer with dyspnea takes more time to recover from COVID-19. The states of babies are as follows; all four babies were isolated upon birth from their mother. For prevention of COVID-19 perinatal and postnatal transmission, three mothers opted cesarean section and remaining one had vaginal delivery due to the sudden labor pain. The RT-PCR testing were carried out after 72 hour of their birth and only three babies were tested since one among four were not given consent for testing.

8. Adopted cure strategy

Worldwide scientist and physicians started major campaign to understand the emergence of the disease and its possible antiviral treatment by drug development or therapeutic agents or developing vaccines. As of now there is no specific therapeutics agent or vaccine approved to cure COVID-19 patient in clinical procedure. Due to limited clinical and basic research information, most of the clinical trial/manifestation follows basic symptomatic treatment protocol and supportive care which was followed for curing SARS and MERS patients [45]. The strategies of SARS-CoV and MERS-CoV therapy or antiviral drug have been extrapolated for the treatment of COVID-19 (**Table 1**). Most of the hospitalized infected patient have following status; (i) among the admitted patients, 23%–32% enters into ICU, (ii) 17%–29% feels critical respiratory failure (iii) ~7–8% were discharged and (iii) ~1% reported death. S-protein of COVID-19 has much similarity (in structural as well as replication procedure) with SARS and MERS protein and hence most of the articles reported broad spectrum antiviral activity of remdesivir, baricitinib, and chloroquine as the clinical trial antiviral drug [19] (**Figure 5**). Remdesivir demonstrated effectiveness for curing COVID-19 in USA [46]. Nucleotide type of remdesivir drug

Sr No	Target Protein	Possible Drug	Ref
1	Angiotensin-converting enzyme 2 (ACE2)	Arbidol	[34]
2	Viral spike glycoprotein (S-protein)	Arbidol	[34]
3	Transmembrane protease, serine 2 (TMPRSS2)	camostat mesylate	[50]
4	Coronavirus main protease 3CLpro (3CLpro)	lopinavir	[46]
5	Papain-like protease PLpro (PLpro)	lopinavir	[48]
6	RNA-dependent RNA polymerase (RdRp)	remdesivir, ribavirin, favipiravir	[47]
7	JAK kinas	baricitinib	[51]
8	Endosome/ACE2	Chloroquine, Hydroxychloroquine	[47]
8	RNA-dependent RNA polymerase (RdRp)	IDX-184	[35]

Table 1.
Target protein related to nCoV-19, SARS-CoV and MERS-CoV and possible drug proposed for prevention (data taken from reference [34–51]).

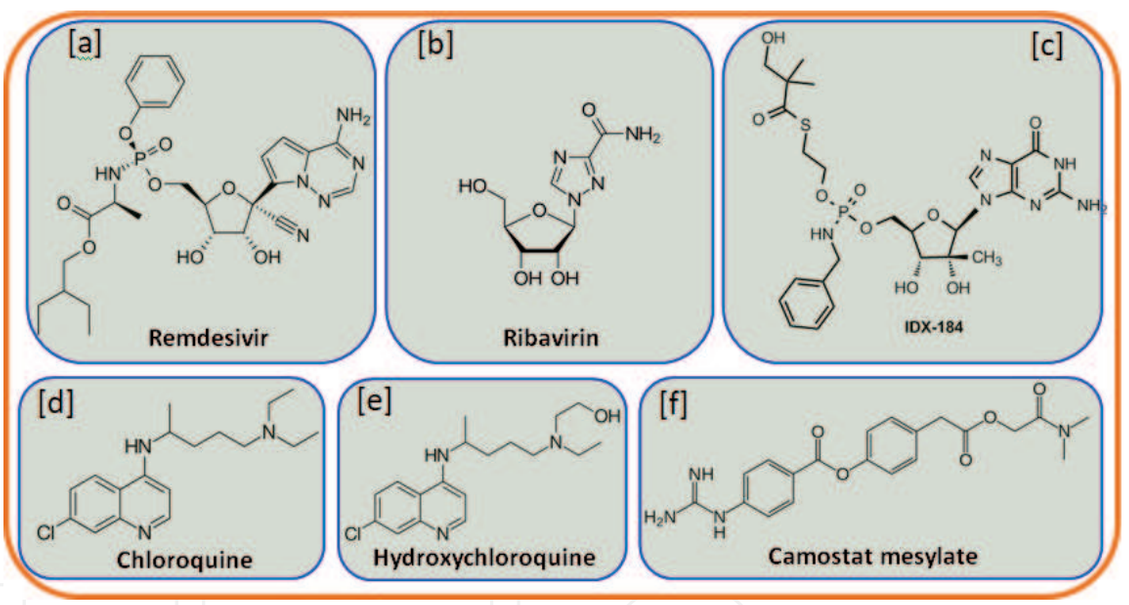

Figure 5.
structure of viral entry inhibitor (a) remdesivir, (b) ribavirin, (c) IDX-184, (d) chloroquine, (e) hydroxycholoroquine, and (f) camostat mesylate.

assisted to premature termination of RNA chain in host cell. On the other hand, ribavirin is a guanosine analogue mostly used for treating chronic hepatitis C [47]. However, study finds suitable dosage of ribavirin might stop the replication spike protein RNA [47]. Lopinavir (a viral protease inhibitor) with its pharmacological booster ritonavir (LPV/R) initially proved to be useful for HIV, SARS-CoV, MERS-CoV treatment with the action of protease inhibitors. Recently study in South Korea reveals significant decrease in COVID-19 viral load after treating with (LPV/R) [46, 48]. Similar reduction in viral loading (associated with pneumonia related symptoms) was also observed after treating with arbidol [34]. Chloroquine and its hydroxy-analogue hydroxychloroquine demonstrated to be relevant for patient having diabetes with infected COVID-19 [14]. Researcher and scientific community stress more on 3CLpro, PLpro and RdRp protein target than other target possibly due to the most responsible proteases for COVID-19 replication and hence attractive

targets for antiviral therapies. Further, one needs to understand, the possible action mechanism of existing drug on COVID-19 before being used. For example, arbidol can be used for fusion of virus-host cells to prevent virus entry into the host. The clinical trial of arbidol is in process [34]. Clinically approved camostat mesylate a possible inhibitor used (to reduce activity of TMPRSS2) for blocking the COVID-19 entry into human body [35]. Combination of tocilizumab and hydroxychloroquine found to be very effective for curing COVID-19 patient underwent kidney transplant surgery [49].

9. Role of biosensor devices

Recently nanomaterial used for point of care diagnosis, therapeutics agent, or in vaccine development. Nanomaterial found to be the promising candidate for the modulation of viral infection cycle [4]. Especially, carbon quantum dots having size below 10 nm found to be a promising interferes for the viruses into the cells. Nanomaterial having different nanostructure offers multivalent character due to surface to volume ratio. Such multivalent properties facilitate several ligands to attach with virus. The viral-ligands interface blocks the entry of virus into the host cell [20]. Łoczechin et al. reported function of carbon quantum dot (CQD) as inhibitor for COVID-19 [52]. CQD synthesis itself from different precursor offer different level of inhibition strength to corona virus. Two different study of CQD synthesis from (i) citric acid/ethylenediamine and further conjugated by boronic acid, and (ii) 4-aminophenylboronic acid and phenylboronic acid offer 50% inhibition concentrations of EC50 = 52 ± 8 μg mL^{-1} and EC50 = 5.2 ± 0.7 μg mL^{-1}, respectively [52]. CQD inhibit growth of s-protein by fusion mechanism and stop replication process of S-protein by signal transduction mechanism or by interaction with cytosolic proteins [52]. Nanomaterial particles as therapeutic agent for stopping viral entry and subsequent replication of S-protein in host membrane may be an alternative of many existing treatment to avoid their side effects. For example, use of ribavirin and IFN as an antiviral drug for COVID-19 spike protein have many side effects including short-term memory loss, confusion, extrapyramidal effects and deficits in executive functions [20, 52].

Plasmonic biosensor working on the cumulative effect of plasmonic photothermal (PPT) and localized surface plasmon resonance (LSPR) transduction principle found to be another potential alternative diagnosis of COVID-19 [53]. Two dimensional (2D) gold nanoislands (AuNIs) functionalized with DNA receptors exploited as sensing of RNA gene sequence. Sensitivity of material can be enhanced to some order by direct thermoplasmonic heating to biosensor chip. The usable plasmonic heat to the chip has been generated by setting a particular plasmonic resonant frequency. Photon generated oscillation frequency modulated the electrons behavior on the surface of plasmon material which might be the crucial factor for detection of selective COVID-19 gene sequence from multi gene mixture. Enhanced plasmonic field at the nanostructures surfaces increases sensitivity of sensor by suppressing local variation like refractive index and molecular binding. Employing field effect transistor as biosensor for fast and accurate spike protein detection through nasopharyngeal swab has recently been reported by Seo et al. [54]. Graphene coated specific antibody has been used as sensing material for spike protein detection [54]. The spike protein directly not attached with graphene surfaces rather 1-pyrenebutyric acid N-hydroxysuccinimide ester was used as probe linker to conjugate protein structure on graphene surfaces. Such attachment of spike protein induced by 1-pyrenebutyric acid N-hydroxysuccinimide ester on graphene surface leading to changes in conductivity and subsequently in current through the FET structure

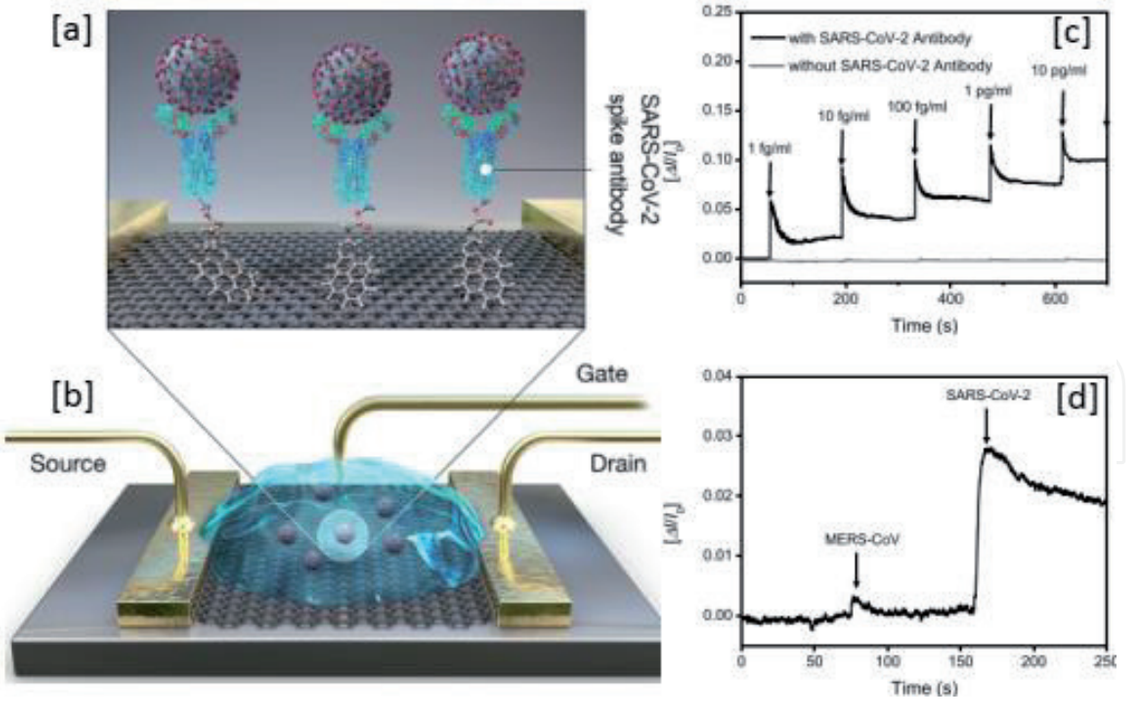

Figure 6.
(a) showing conjugation of spike protein on to the surface of graphene via 1-pyrenebutyric acid N-hydroxysuccinimide ester (b) model showing spike protein on the surface (covered with graphene) of field effect transistor (c) FET sensor sensitivity in presence of SARS-COV-2 antibody and in absence of SARS-COV-2 antibody and (d) FET sensor sensitivity in MERS-COV and SARS-COV-2 (reproduce with permission [32]).

(shown in **Figure 6 (a)** and **(b)**). The sensitivity which was measured by current fluctuation due to presence or absence of spike protein is shown in **Figure 6(c)** and **(d)**. The biosensor capable to detect down to 16 pfu/mL in cultural mode and 2.42 × 102 copies/mL in clinical sample [32]. The diagnosis method does not require sophisticated laboratory equipment and provide very high sensitivity with instantaneous measurements employing small volume of nasopharyngeal swab.

10. Conclusions

Like other epidemic, COVID-19 may also become seasonal, but at present one can only predicted about it not for sure. Meanwhile, to reduce the outbreak, it is required to have international collaboration with data sharing policies. Because, of the limited information, one can reuse the existing drugs as clinical trial for curing COVID-19 infection (based on the similarity of target protein with other coronavirus). Further, fight against COVID-19 requires the knowledge of computer science, medicine, health policy, environmental factors and risk management etc. Present situation imposes researcher and scientific community a number of research target viz.; (i) production of rapid point of care diagnosis (ii) enhancement in surveillance and monitoring (iii) design of new therapeutic agents and finally (iv) vaccine development. We can only reduce the transmission level up to certain extent but cannot be demolished completely. For complete cure one has to develop vaccine.

Conflict of interest

Author declares there is no conflict of interest.

Notes/thanks/other declarations

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